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(54) **COLD-ADAPTED, LIVE ATTENUATED SARS-COV-2 VACCINE**

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C07K 14/005	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 7/00*** (2013.01); ***A61K 39/215*** (2013.01); ***A61P 31/14*** (2018.01); ***C07K 14/005*** (2013.01); *A61K 2039/5254* (2013.01); *A61K 2039/543* (2013.01); *C12N 2770/20022* (2013.01); *C12N 2770/20034* (2013.01)

(57) **ABSTRACT**

An isolated nucleic acid comprising a recombinant coronavirus genome having a genetic modification that provides cold-adapted growth relative to a parent virus that is not cold-adapted and a vaccine comprising the recombinant genome and methods of using the vaccine are provided.

Specification includes a Sequence Listing.

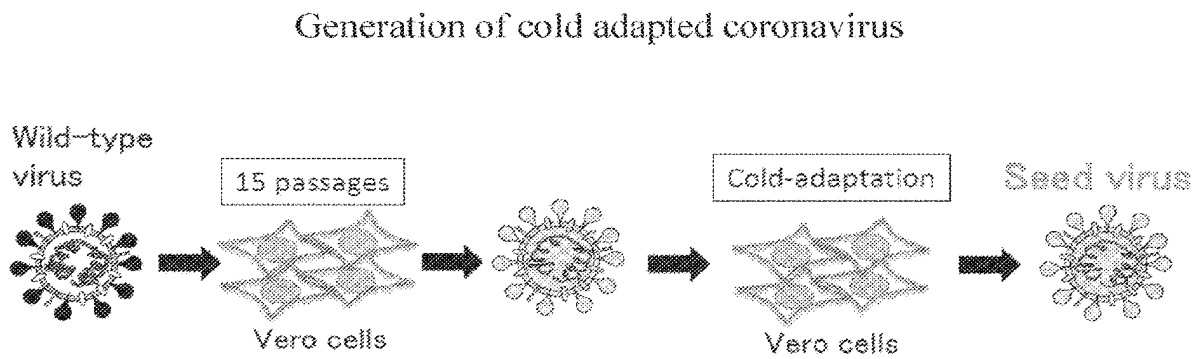


Fig. 1

Generation of cold adapted coronavirus

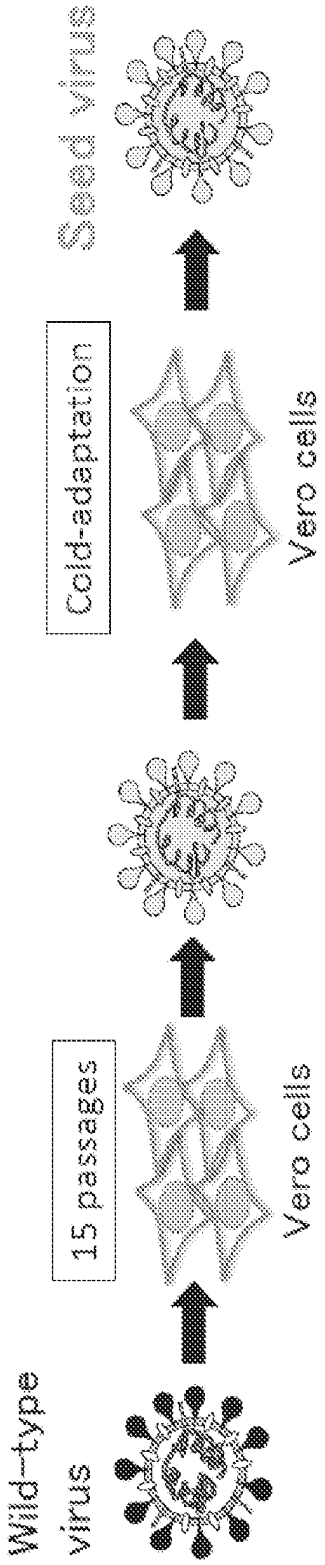


Fig. 2
Propagation of an exemplary seed (vaccine) virus in Vero cells

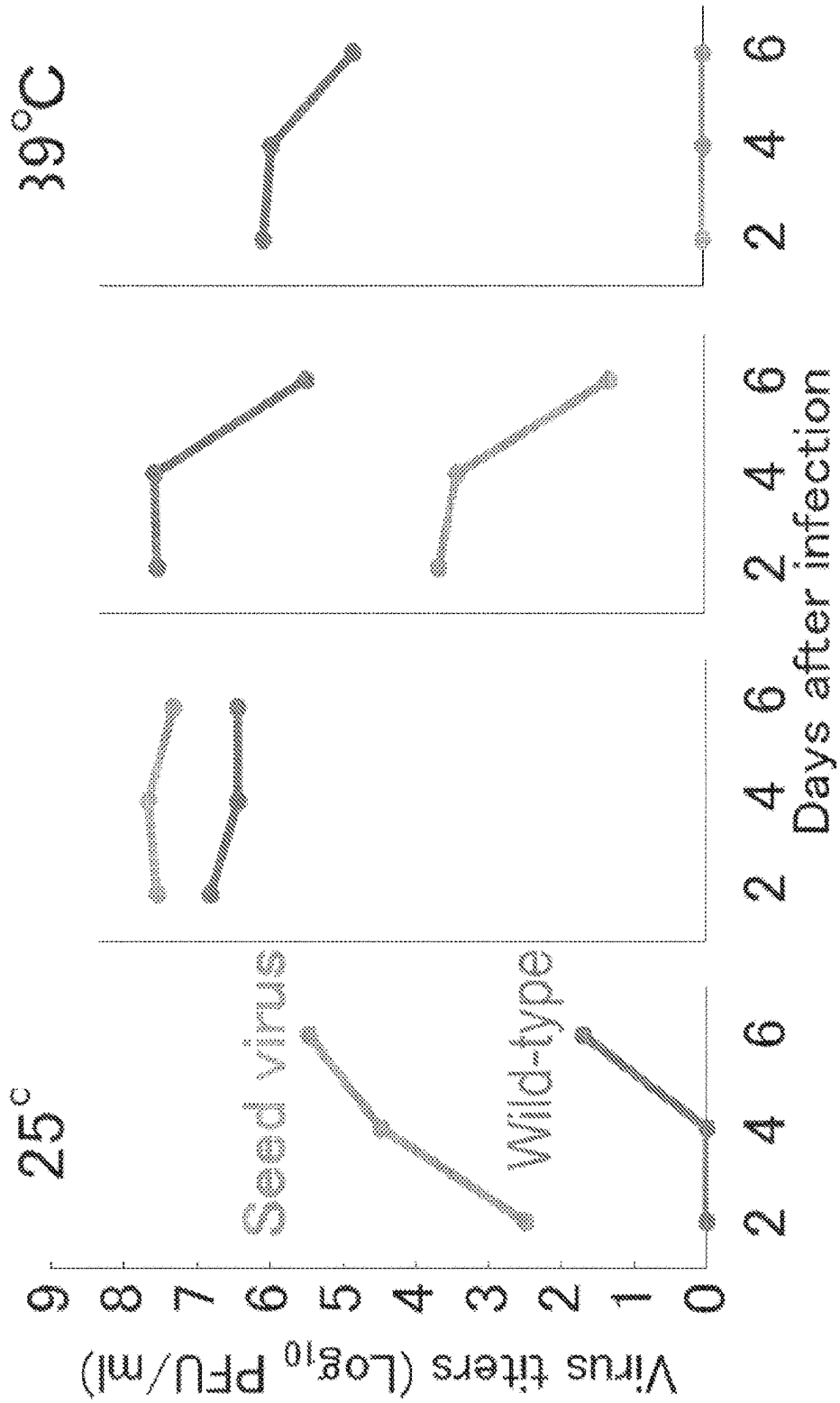


Fig. 3

Pathogenicity and immunogenicity of seed virus in hamsters

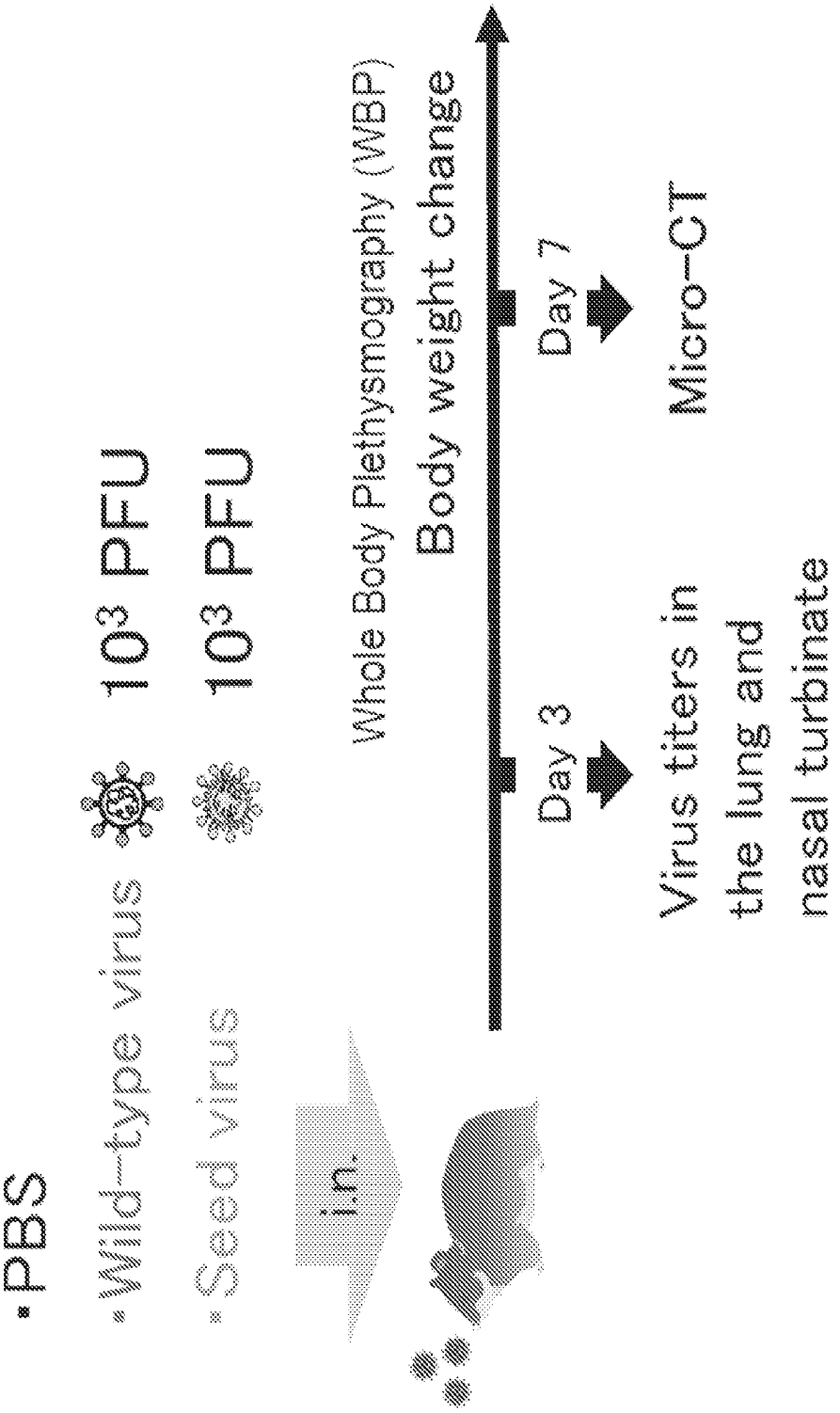


Fig. 4

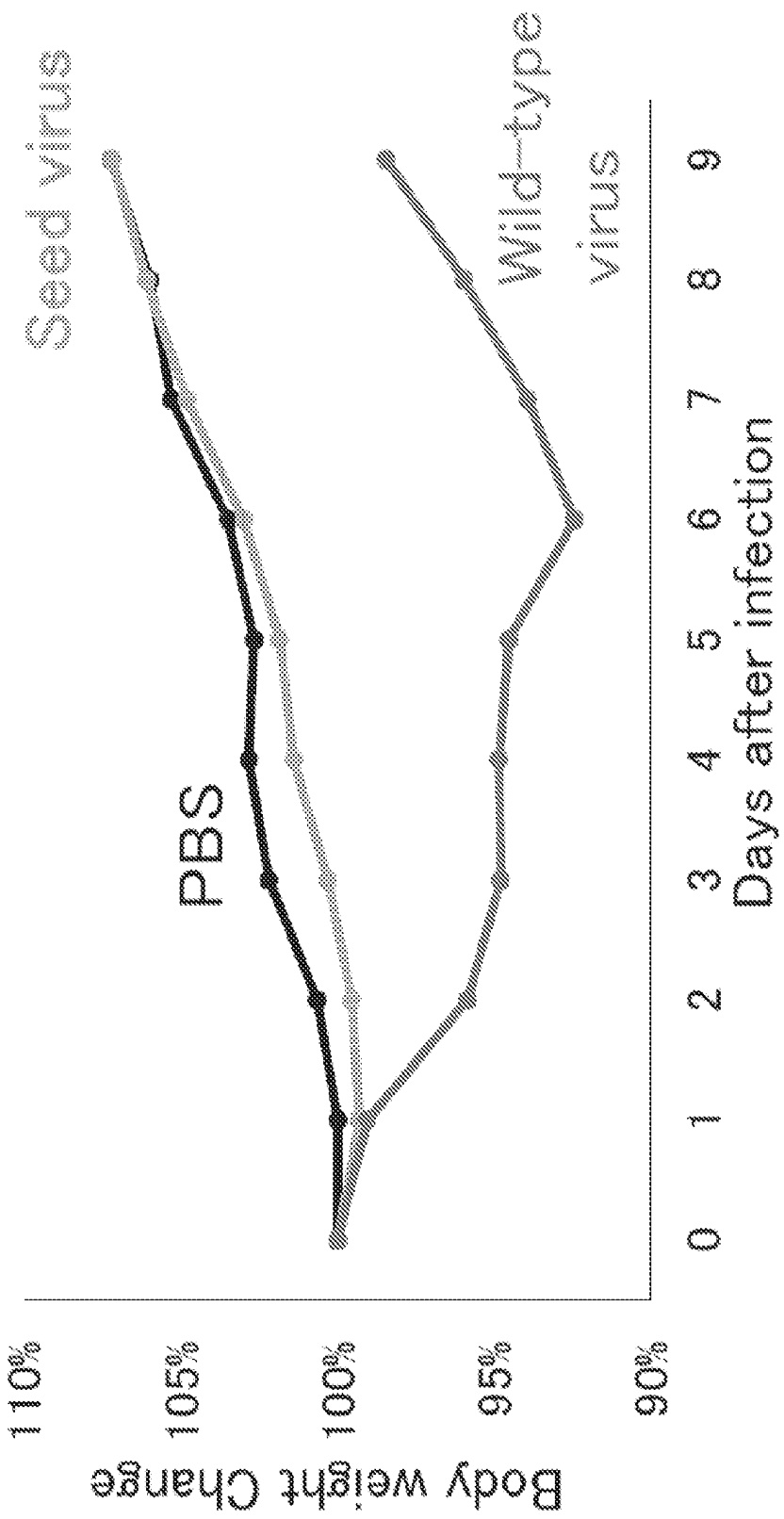
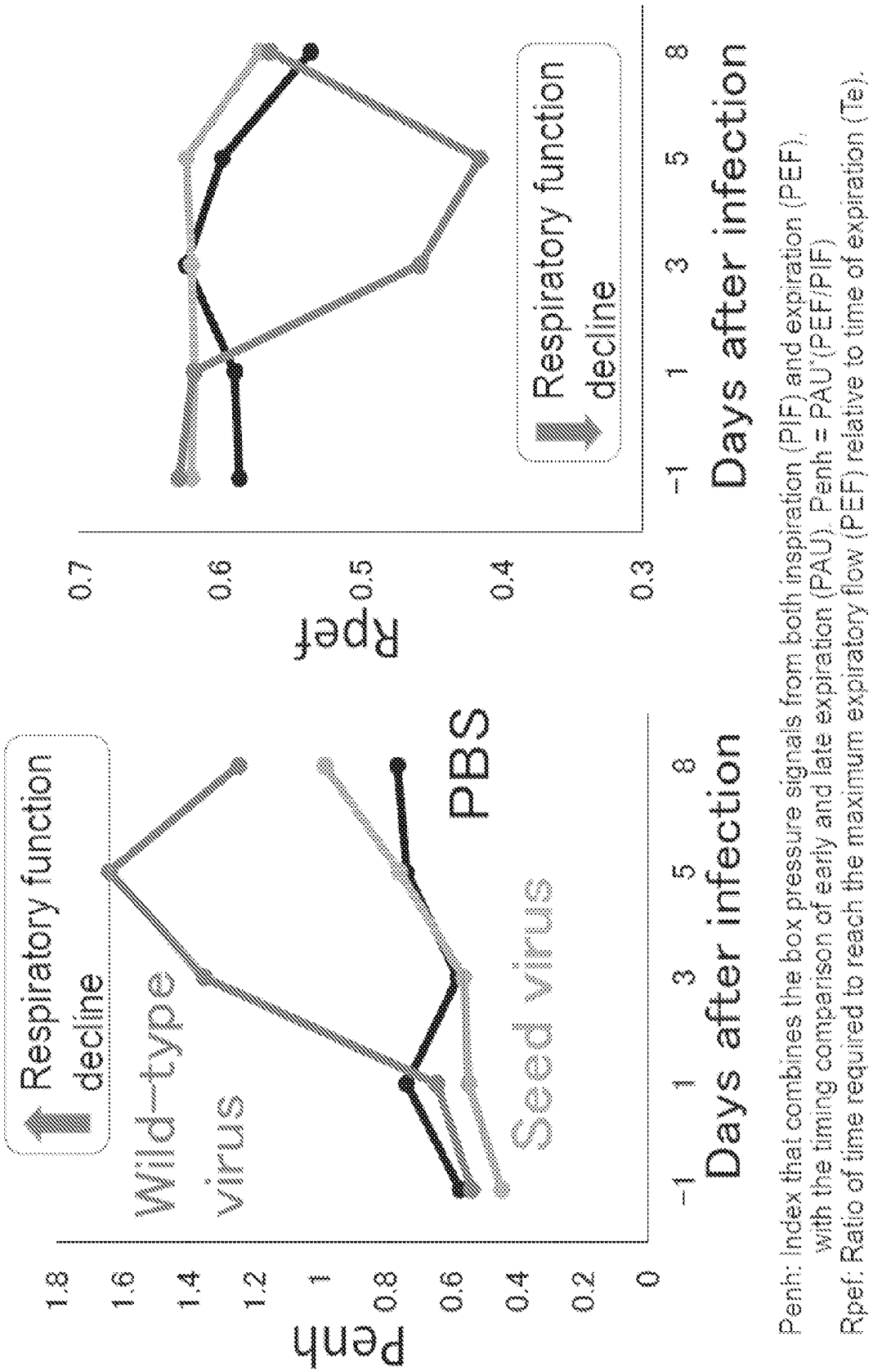

Body weight Change
110%
105%
100%
95%
90%
0
1
2
3
4
5
6
7
8
9
Days after infection
PBS
Seed virus
Wild-type virus

Fig. 5



Penh: Index that combines the box pressure signals from both inspiration (PIF) and expiration (PEF), with the timing comparison of early and late expiration (PAU). Penh = PAU*(PEF/PIF)
Rpef: Ratio of time required to reach the maximum expiratory flow (PEF) relative to time of expiration (Te).

Fig. 6

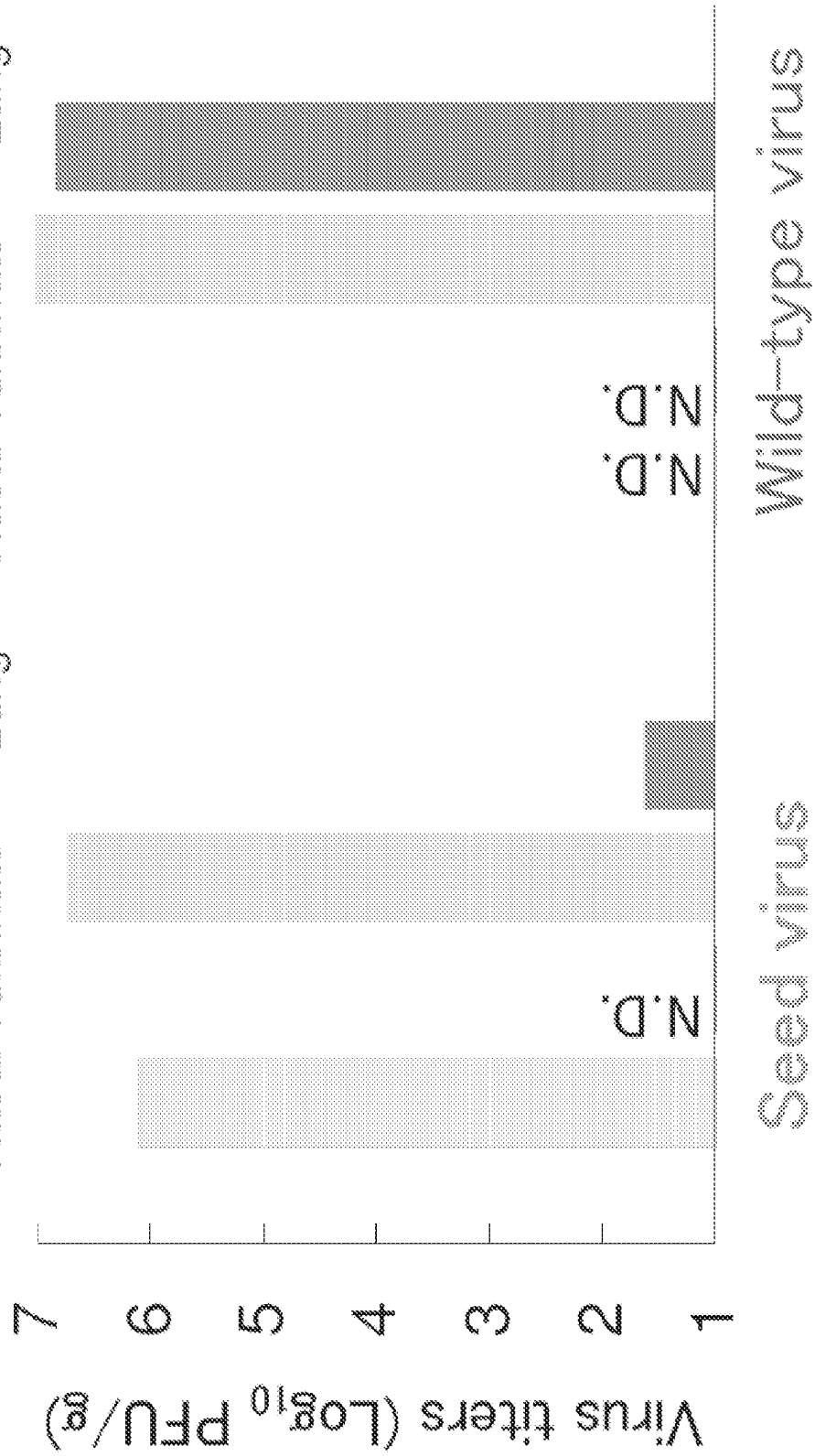

Titers at 25°C
Nasal Turbinate
Lung
Titers at 37°C
Nasal Turbinate
Lung
Virus titers (Log10 PFU/g)
7
6
5
4
3
2
1
N.D.
N.D.
N.D.
Seed virus
Wild-type virus

Fig. 7

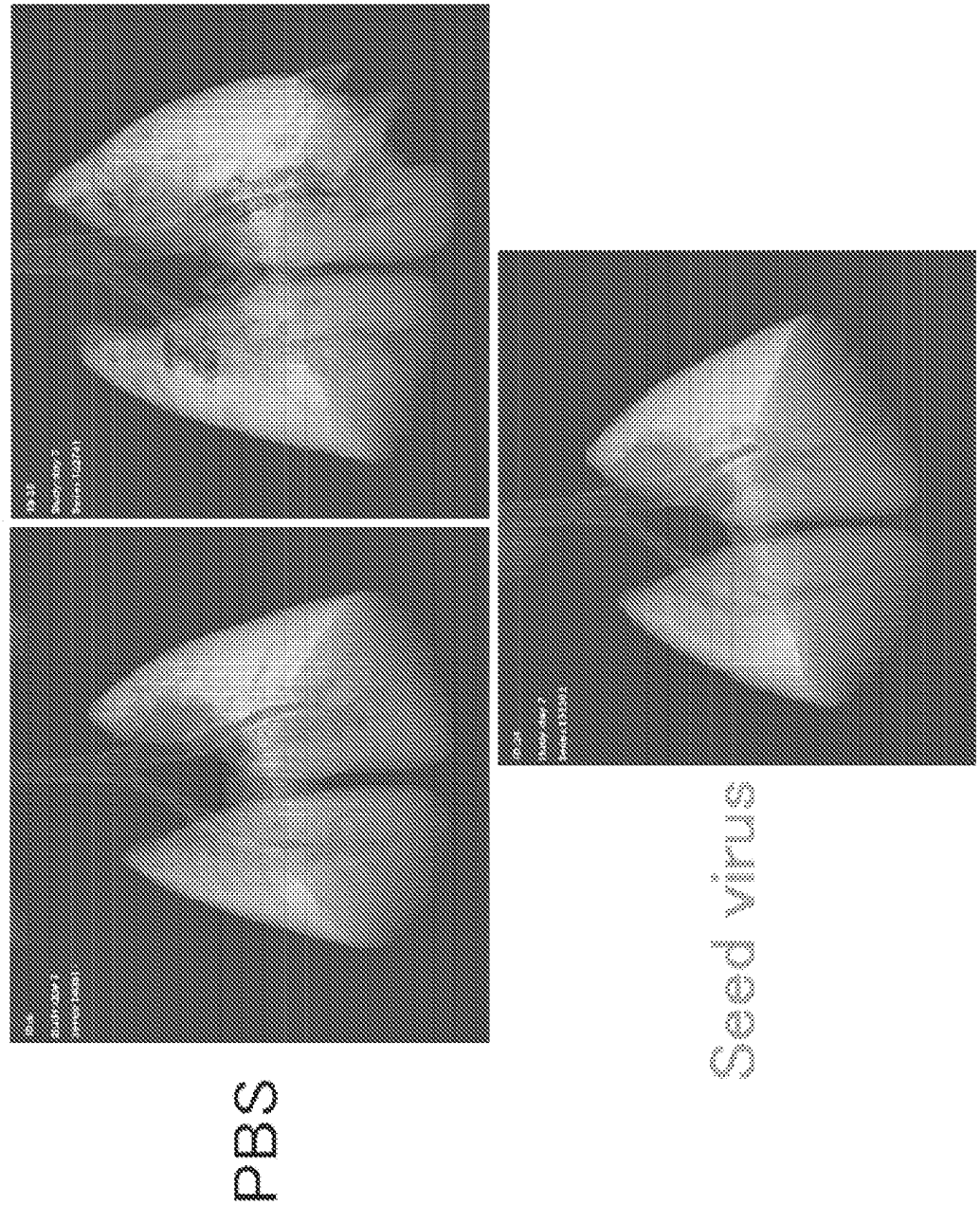


Fig. 8

Immunized with	Neutralization titer against:				
	Wild-type	Beta	Gamma	Delta	BA.1
PBS	<:80	<:80	<:80	<:80	<:10
Vaccine virus	:320	:80	:160	:80	:20
Wild-type virus	:1280	:320	:640	:160	:20

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Fig. 9

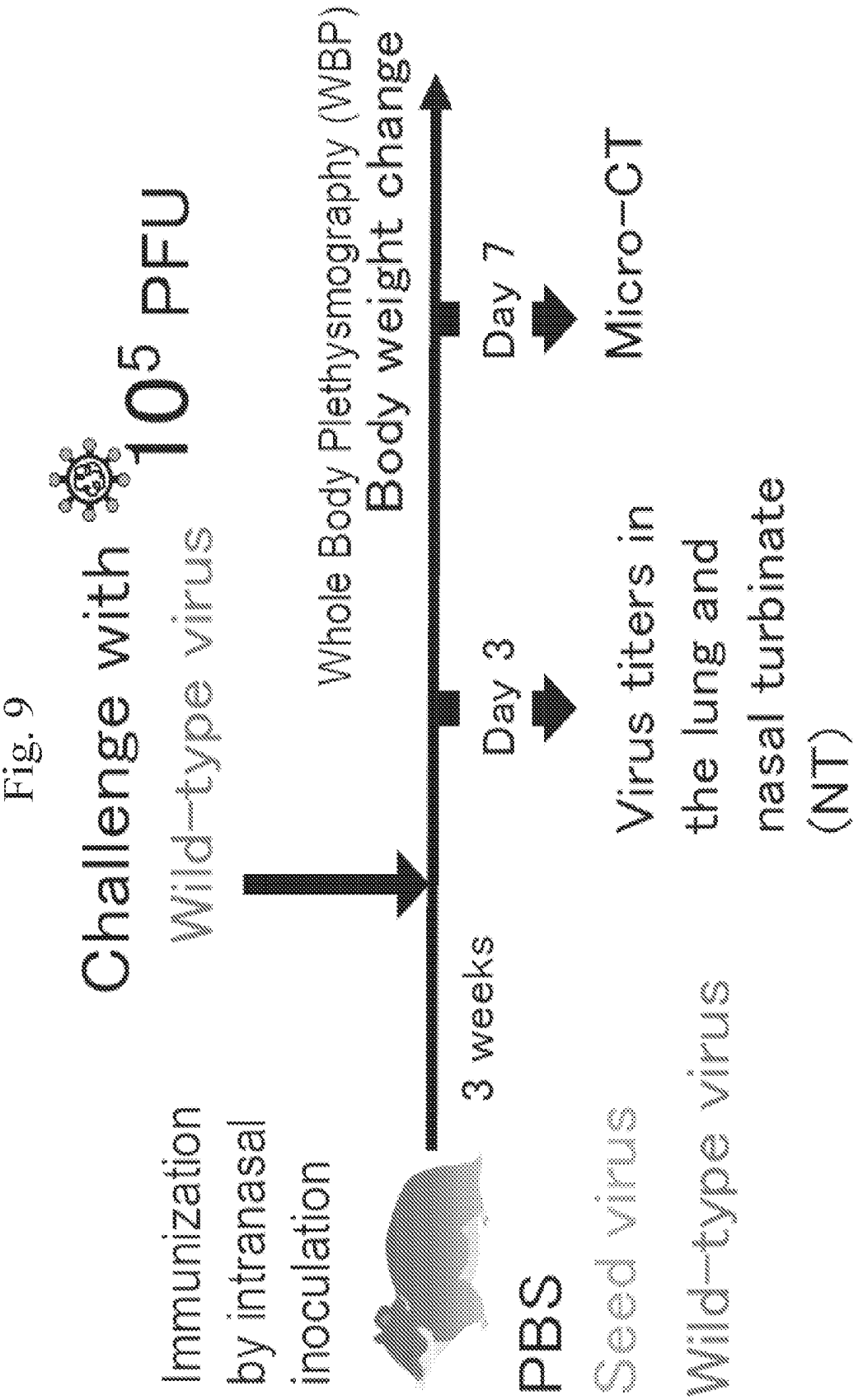

Challenge with 10^5 PFU
Wild-type virus
Whole Body Plethysmography (WBP)
Body weight change
Day 7
Micro-CT
Day 3
Virus titers in
the lung and
nasal turbinate
(NT)
3 weeks
Immunization
by intranasal
inoculation
PBS
Seed virus
Wild-type virus

Fig. 10

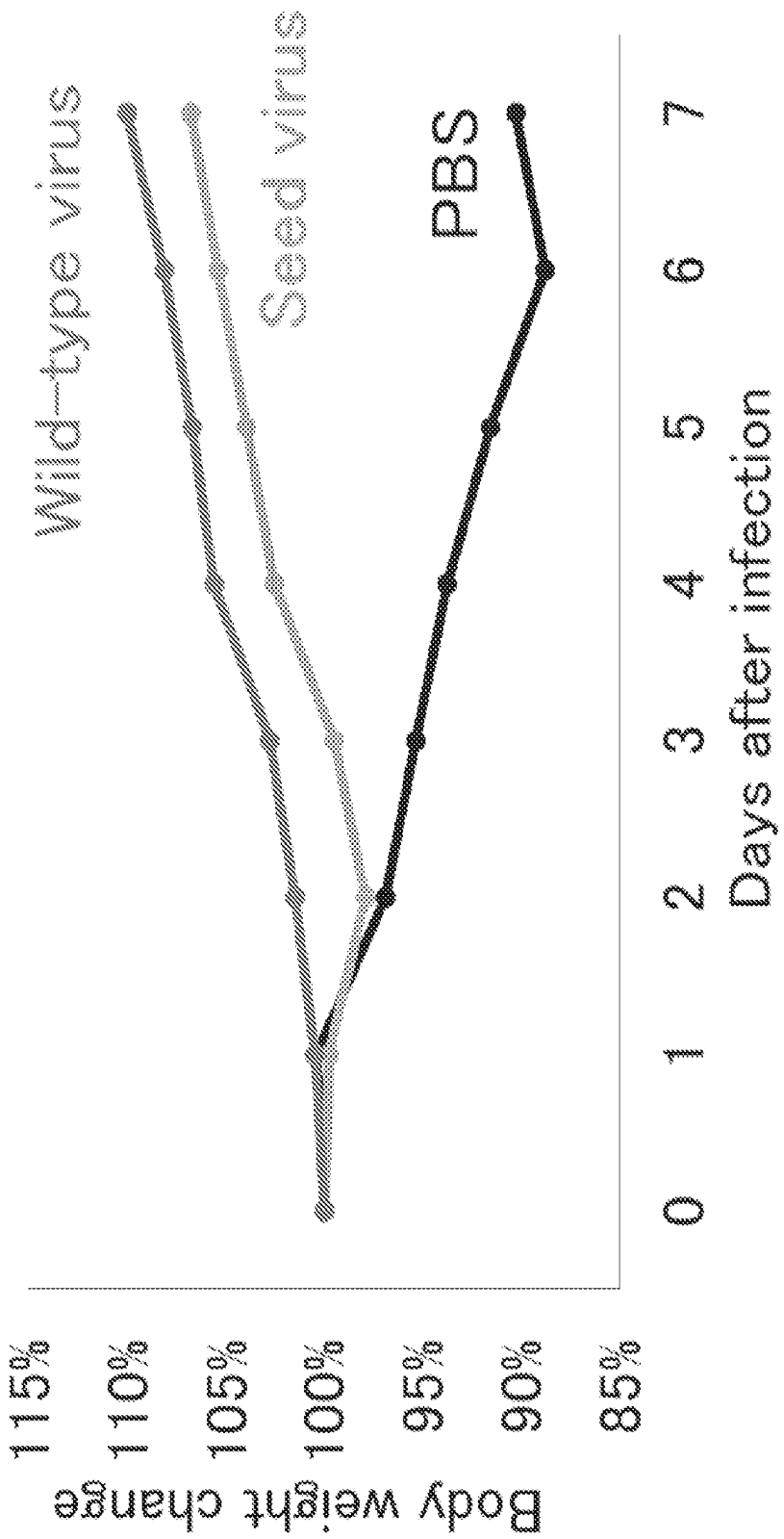
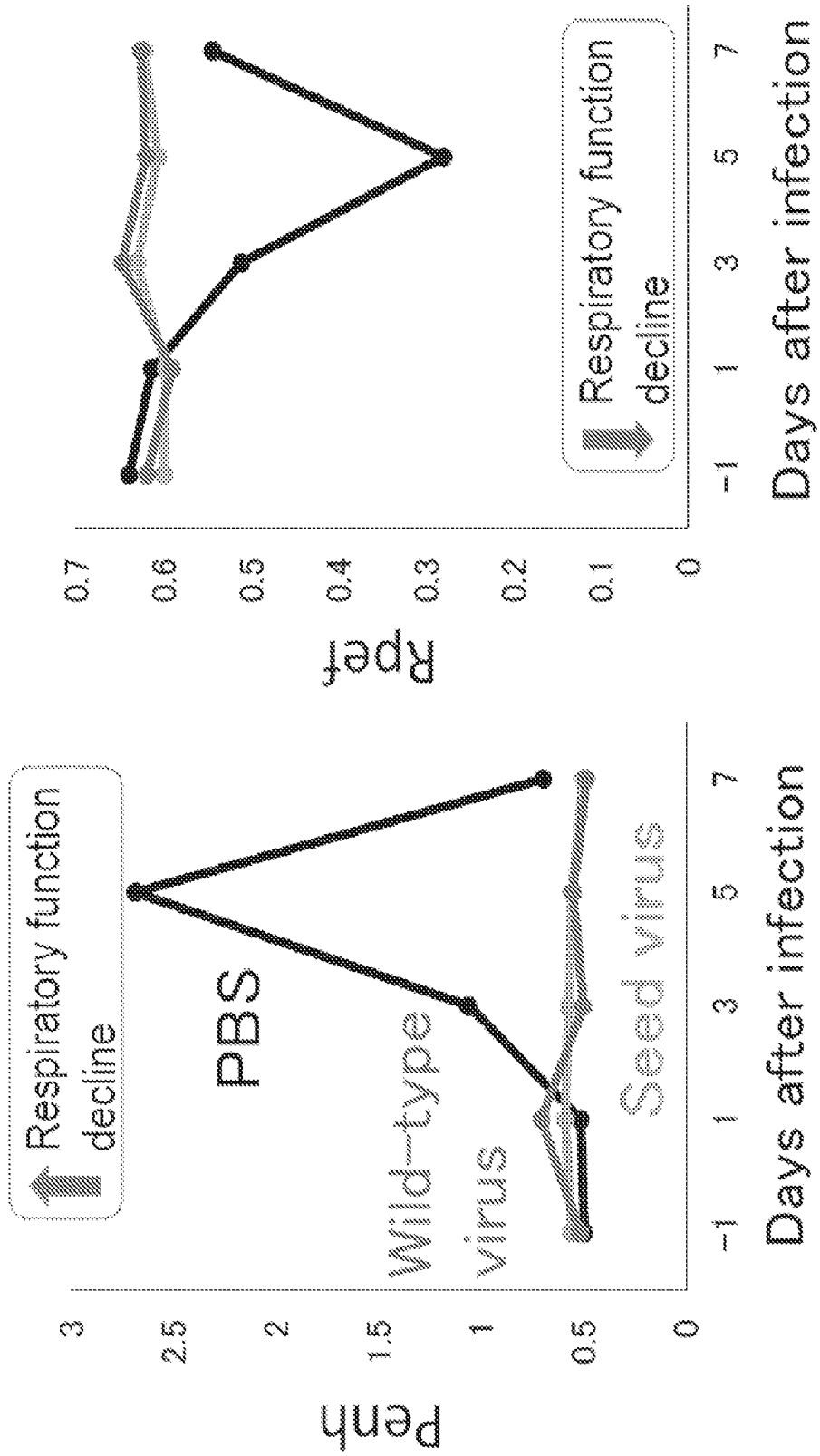

Wild-type virus
Seed virus
PBS
Body weight change
115%
110%
105%
100%
95%
90%
85%
0
1
2
3
4
5
6
7
Days after infection

Fig. 11



Penh: Index that combines the box pressure signals from both inspiration (PIF) and expiration (PEF), with the timing comparison of early and late expiration (PAU). Penh = PAU*(PEF/PIF)

Rpef: Ratio of time required to reach the maximum expiratory flow (PEF) relative to time of expiration (Te).

Fig. 12

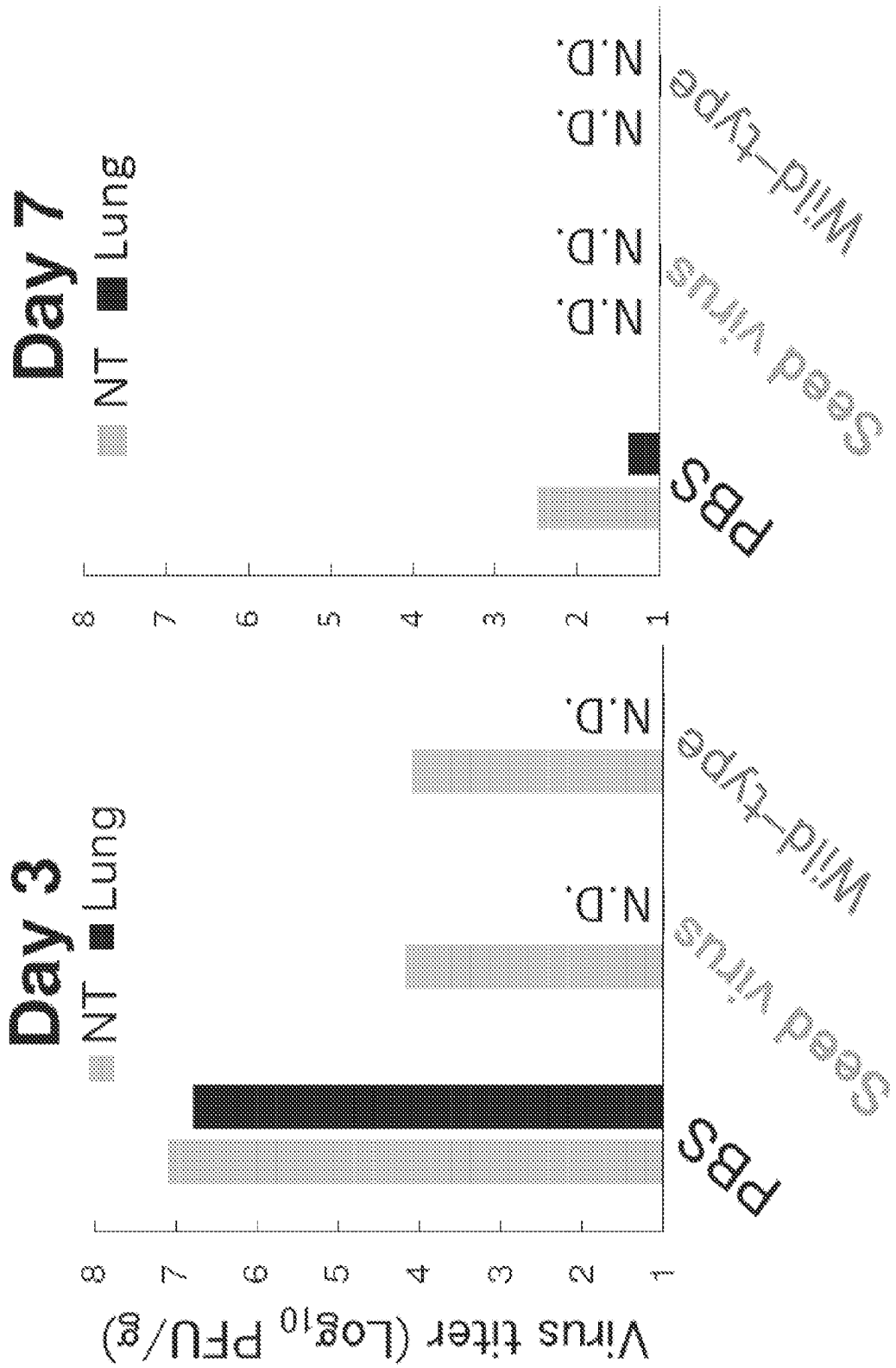

Day 3
NT
Lung
Virus titer (Log10 PFU/g)
8
7
6
5
4
3
2
1
PBS
Seed virus
Wild-type
N.D.
N.D.
Day 7
NT
Lung
PBS
Seed virus
Wild-type
N.D.
N.D.
N.D.
N.D.

Fig. 13

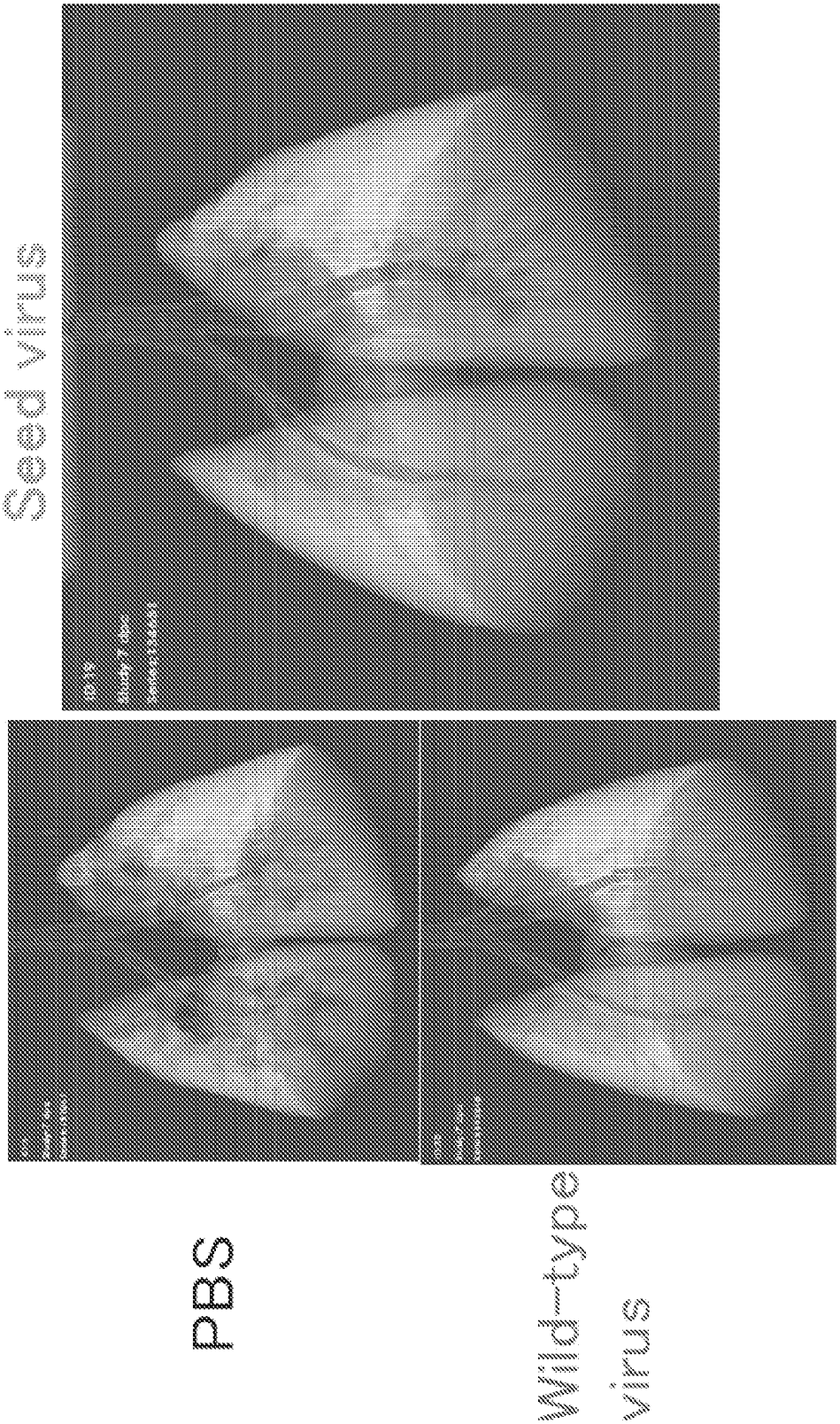
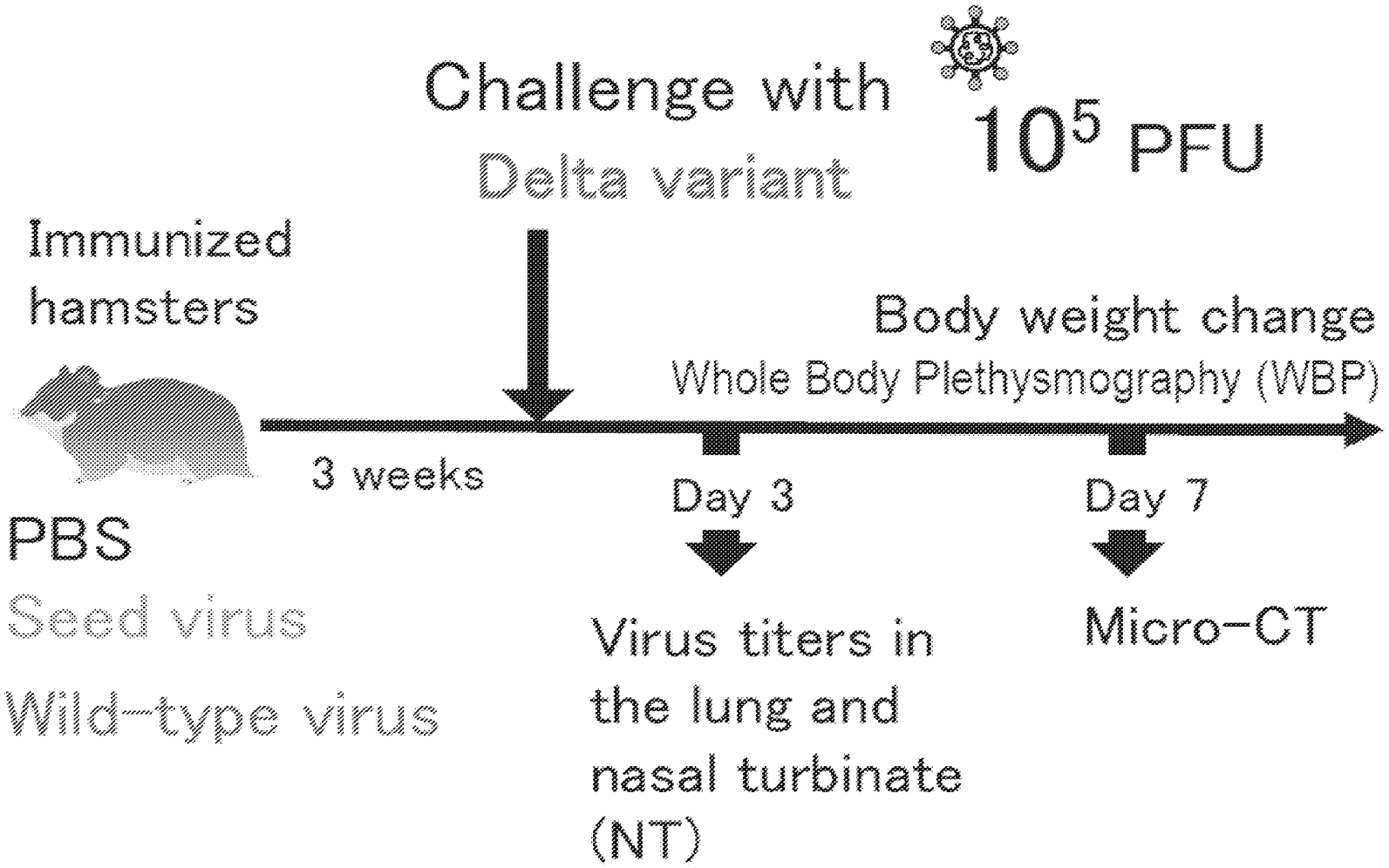


Fig. 14



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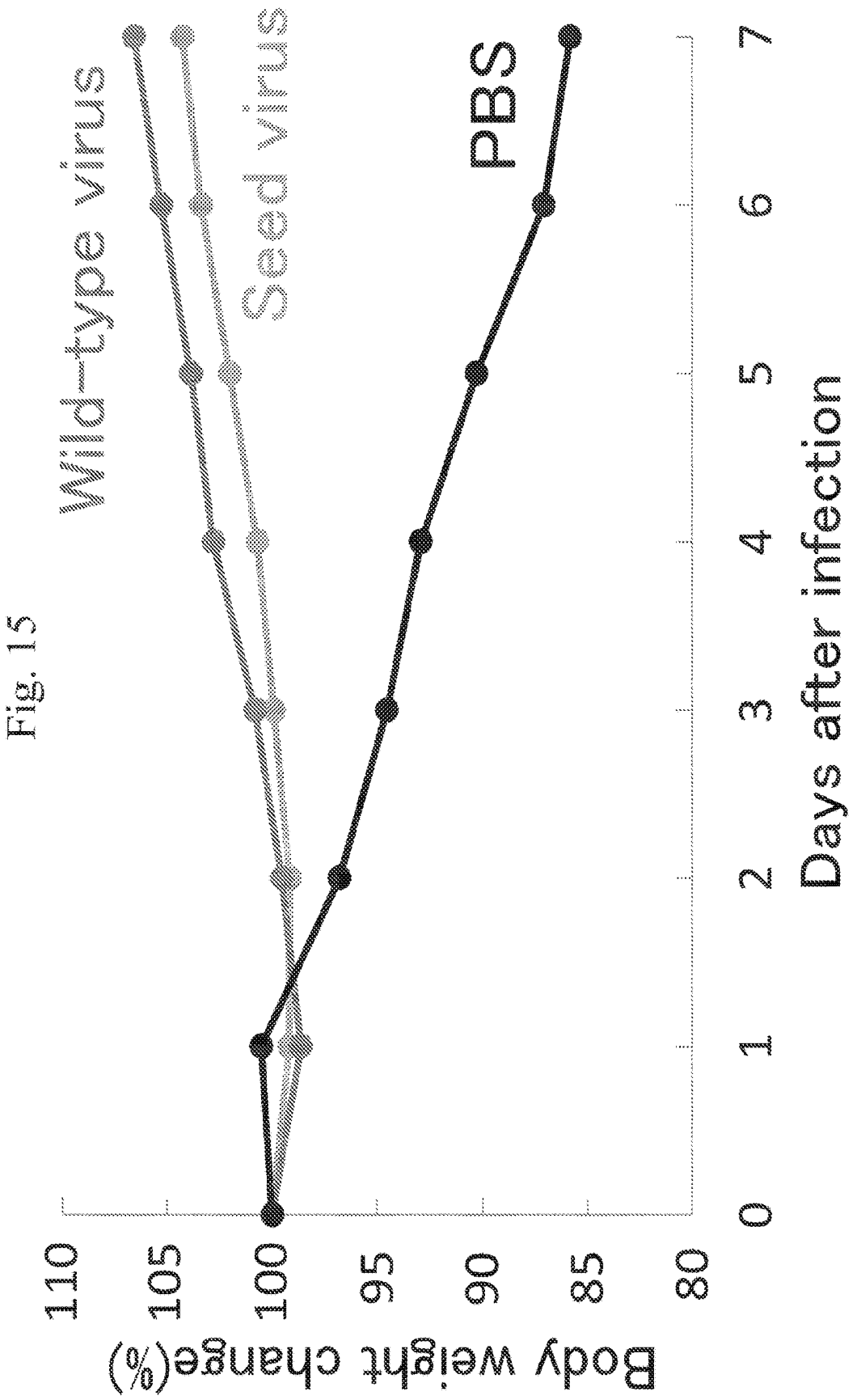
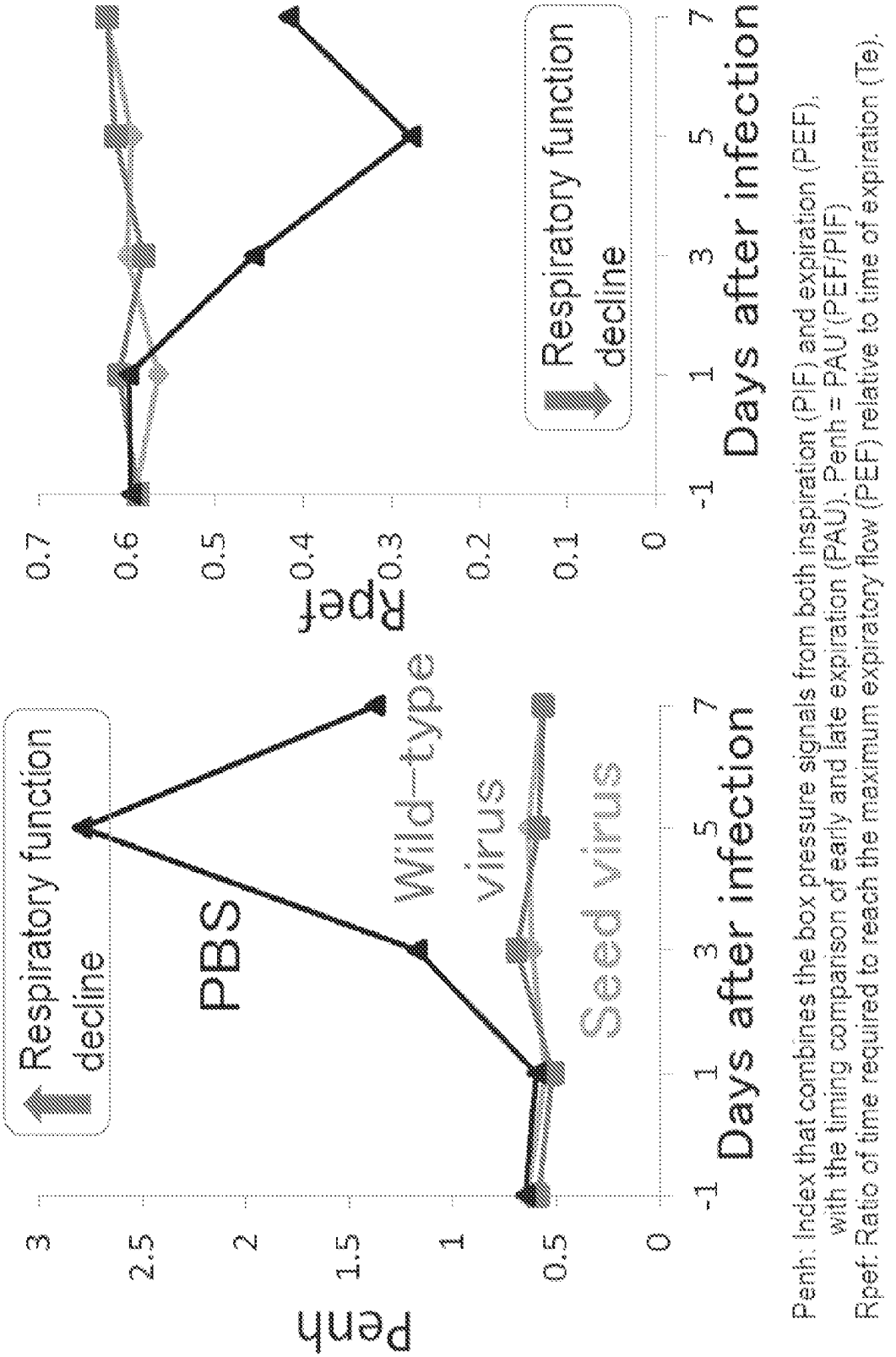

Wild-type virus
Seed virus
PBS
Body weight change(%)
110
105
100
95
90
85
80
0
1
2
3
4
5
6
7
Days after infection

Fig. 15

Fig. 16



Penh: Index that combines the box pressure signals from both inspiration (PIF) and expiration (PEF), with the timing comparison of early and late expiration (PAU). Penh = PAU*(PEF/PIF)
Rpef: Ratio of time required to reach the maximum expiratory flow (PEF) relative to time of expiration (Te).

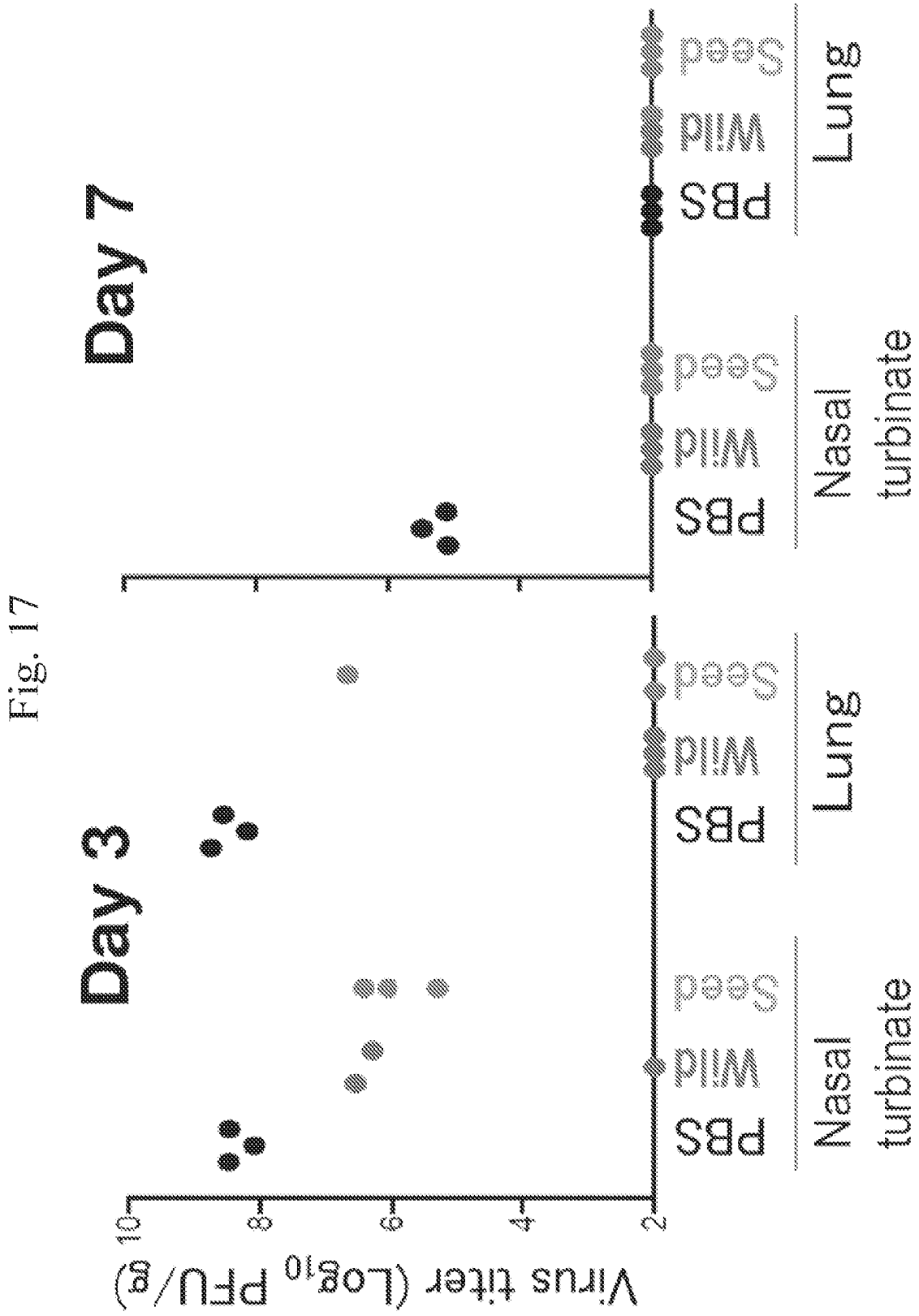

Day 3
Day 7
Virus titer (Log10 PFU/g)
10
8
6
4
2
PBS
Wild
Seed
Nasal turbinate
Lung

Fig. 17

Fig. 18

Fig. 19

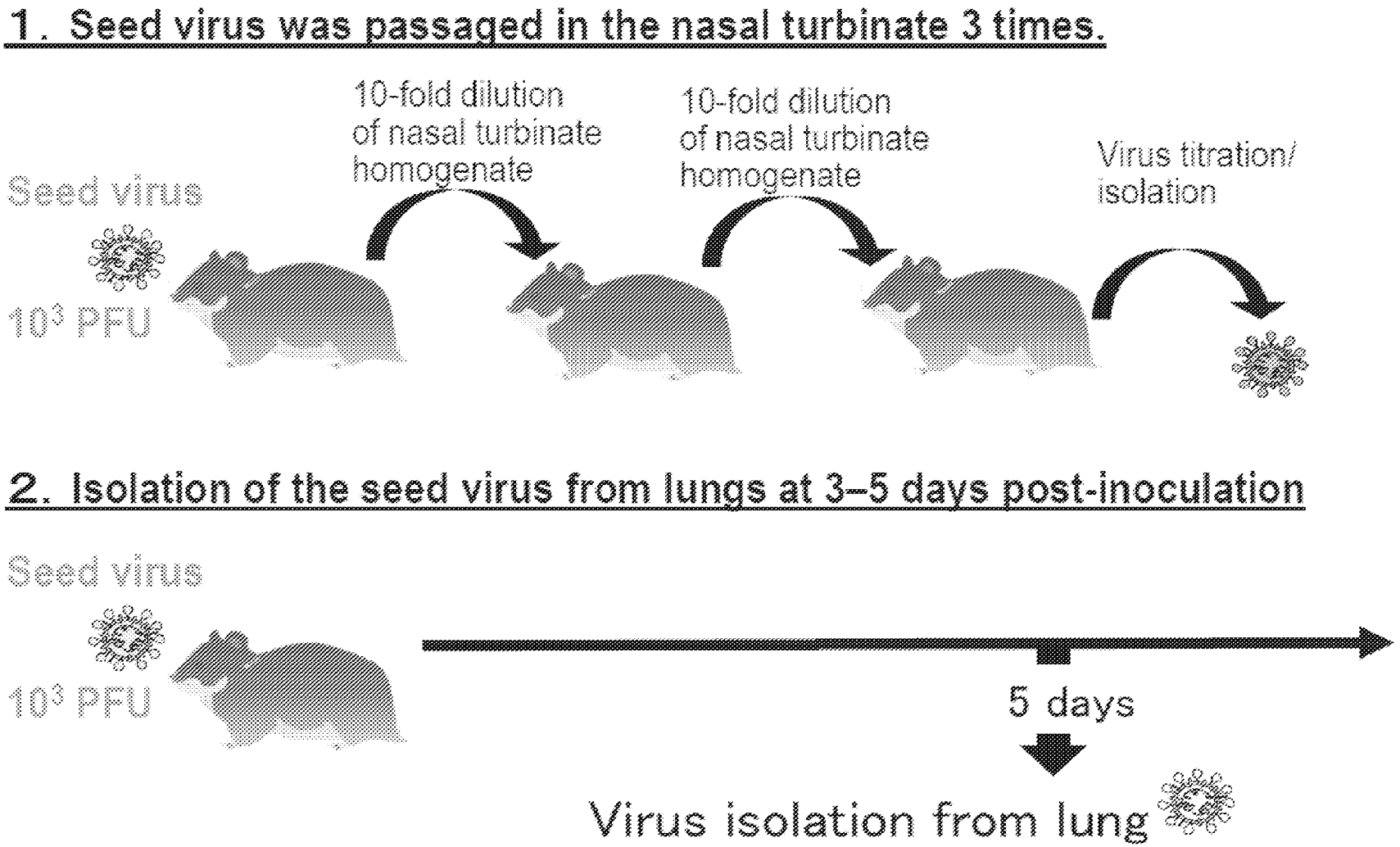


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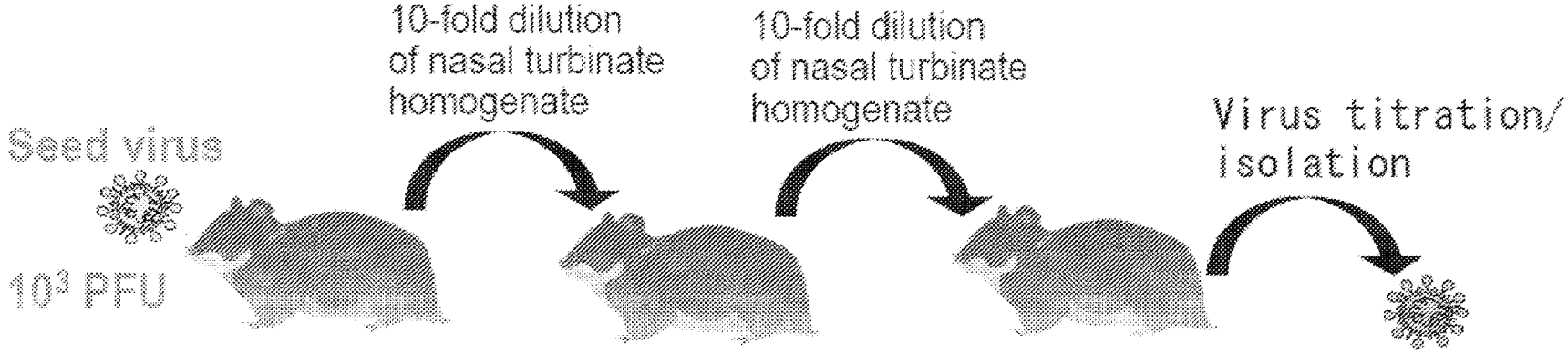
Fig. 20

1. Seed virus was passaged in the nasal turbinate for 3 times.



	1st pass	2nd pass	3rd pass
Line A	1.0×10^7	5.6×10^6	1.1×10^7
Line B	1.8×10^7	1.3×10^7	2.1×10^7
Line C	3.2×10^7	1.5×10^7	7.0×10^6

➡Virus titers were not affected over 3 passages in hamster nasal turbinate.

Properties of passaged viruses were examined.

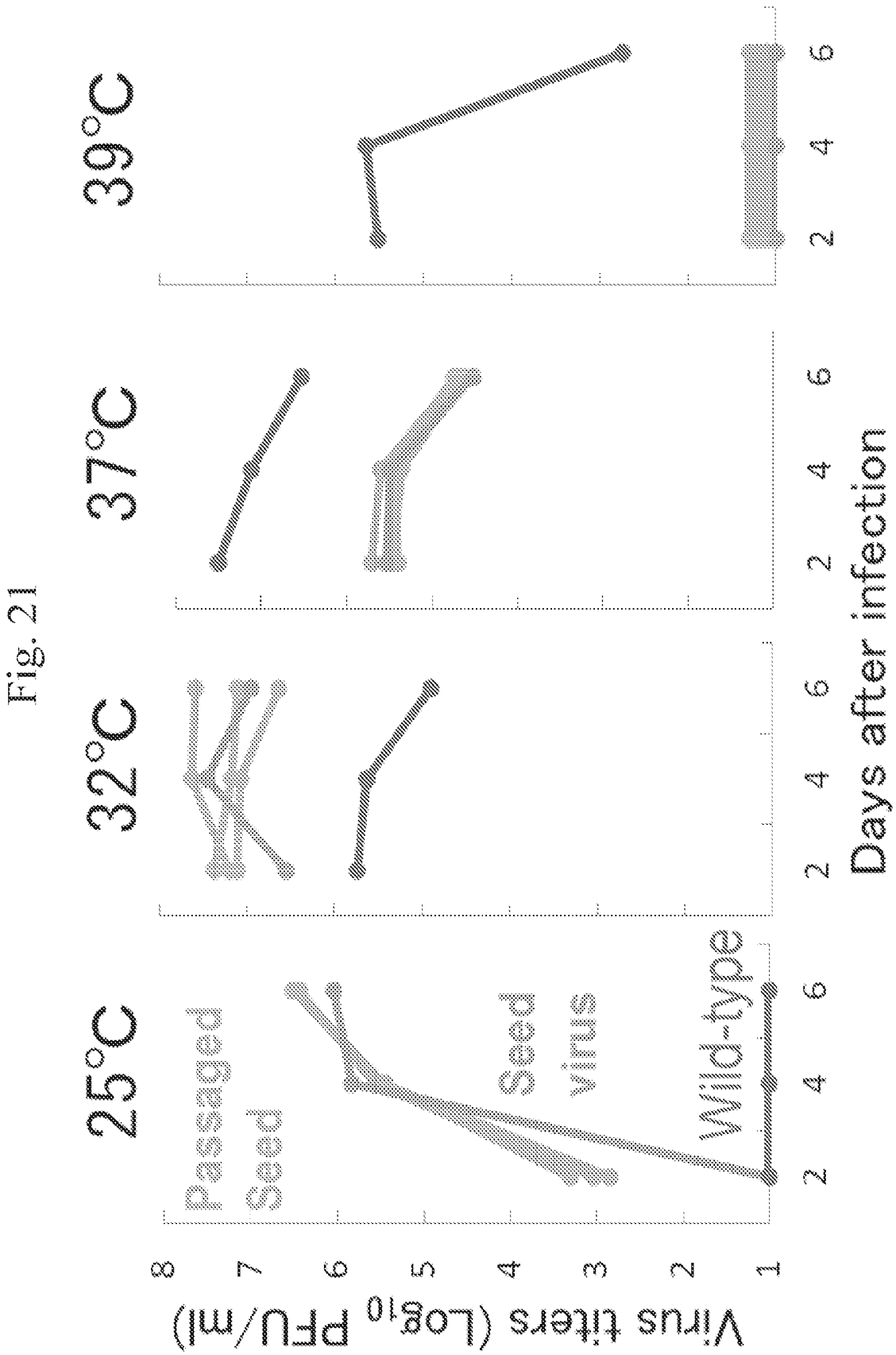

25°C
32°C
37°C
39°C
Passaged Seed
Seed virus
Wild-type
Virus titers (Log10 PFU/ml)
Days after infection

Fig. 21

Fig. 22

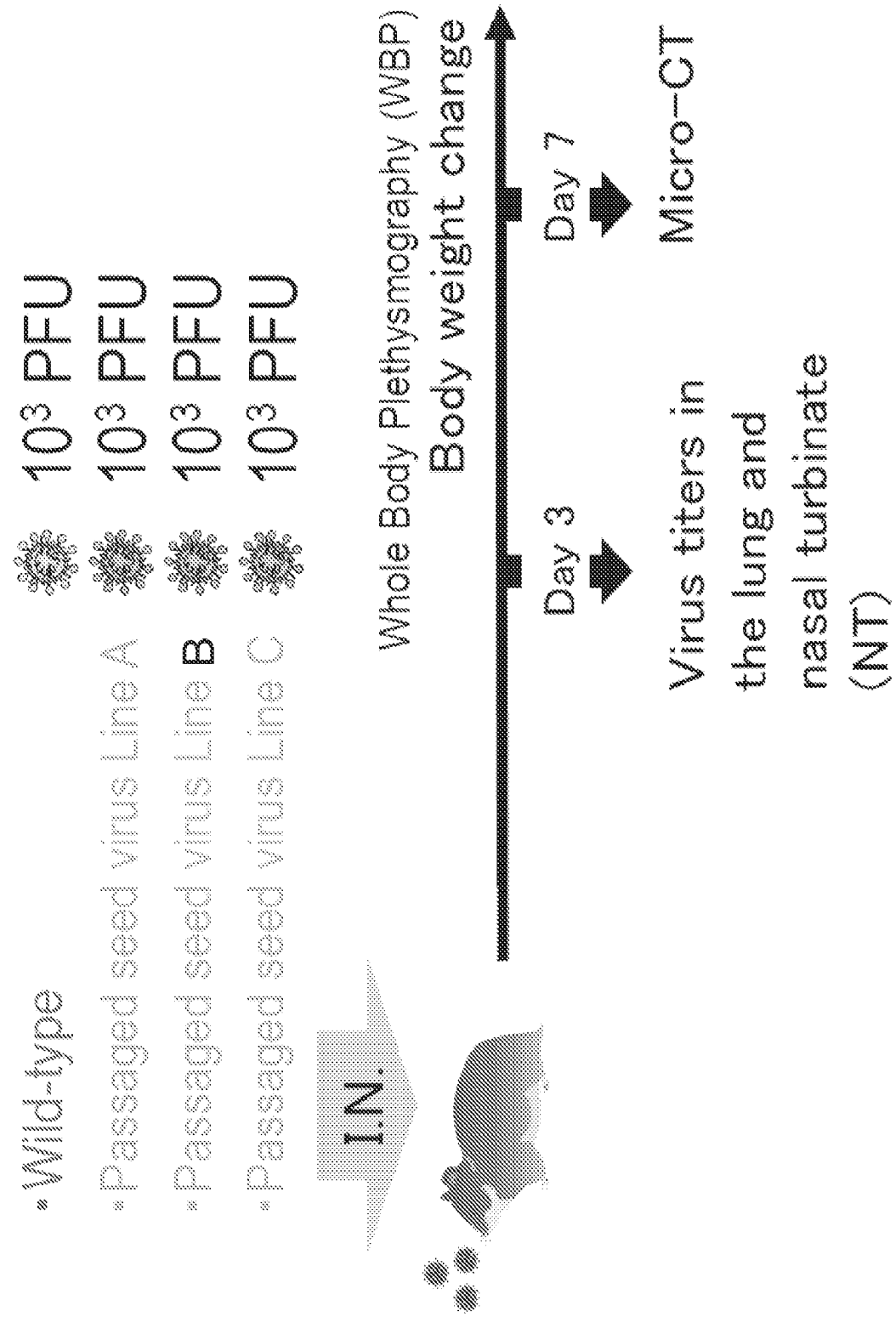

Wild-type
Passaged seed virus Line A
Passaged seed virus Line B
Passaged seed virus Line C
10³ PFU
10³ PFU
10³ PFU
10³ PFU
I.N.
Whole Body Plethysmography (WBP)
Body weight change
Day 3
Day 7
Micro-CT
Virus titers in the lung and nasal turbinate (NT)

Fig. 23

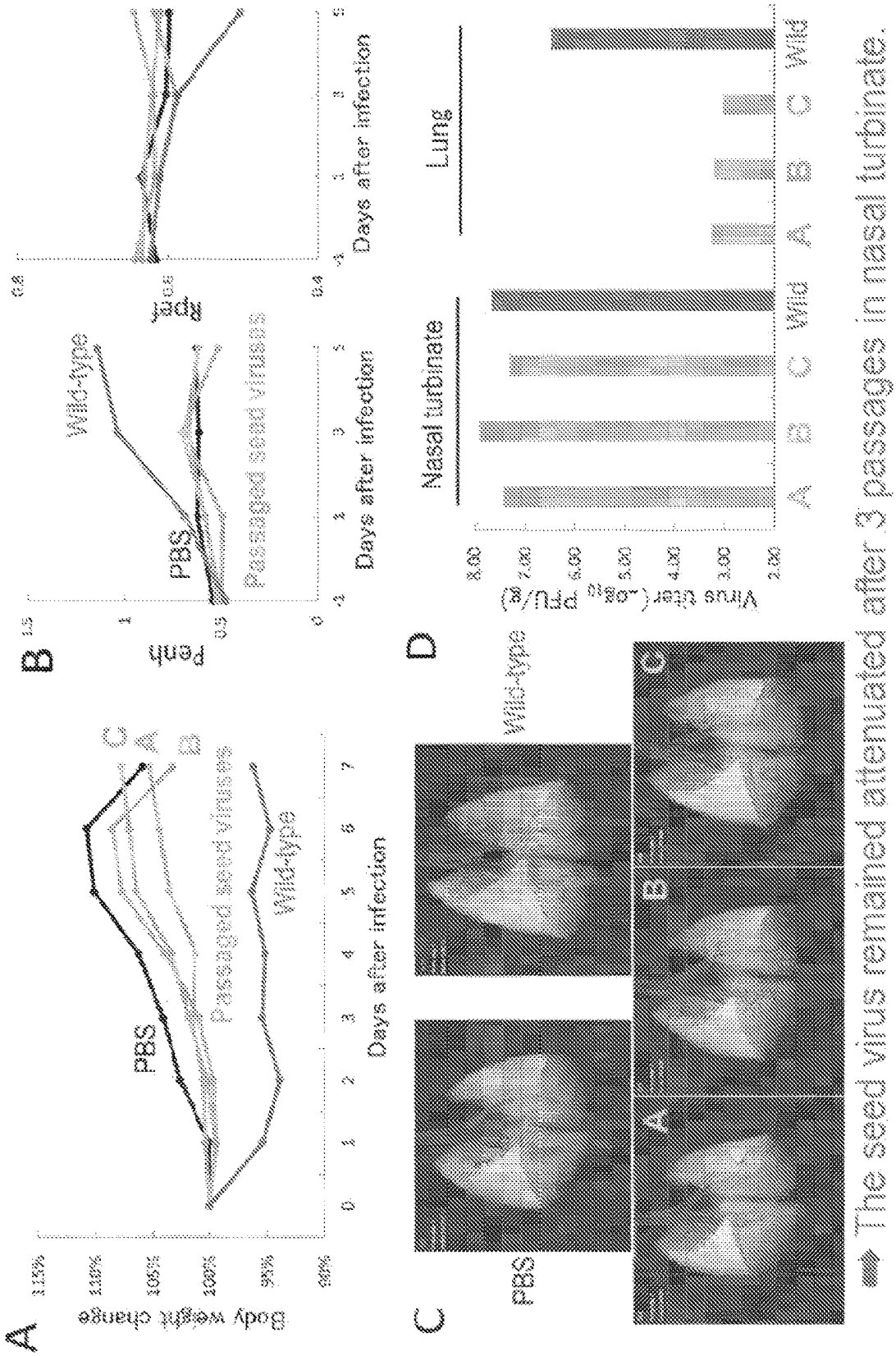
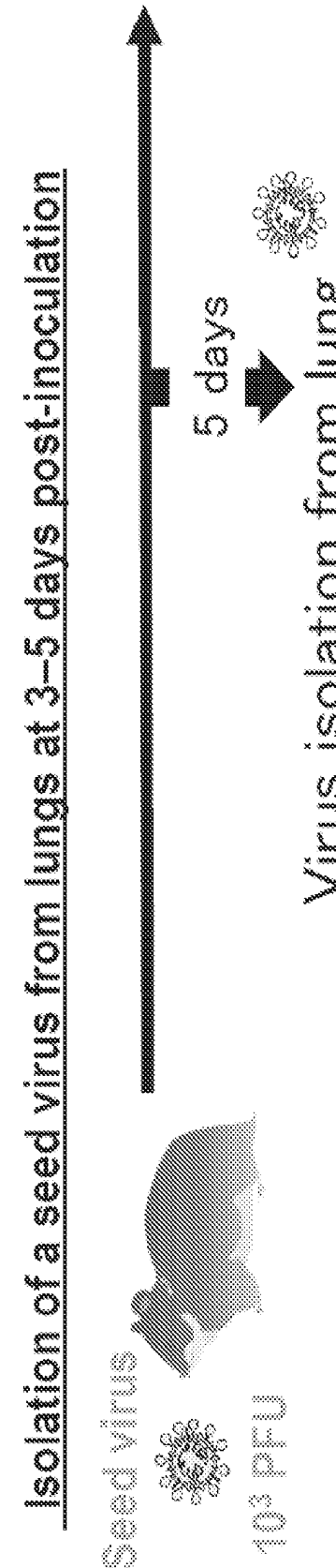

A
Body weight change
PBS
Passaged seed viruses
Wild-type
Days after infection
B
Penh
Rpef
PBS
Wild-type
Passaged seed viruses
Days after infection
C
PBS
Wild-type
A
B
C
D
Nasal turbinate
Lung
Virus titer (-log10 PFU/g)
A B C Wild A B C Wild
The seed virus remained attenuated after 3 passages in nasal turbinate.

Fig. 24



Hamster	Day 5 Titer	Isolation	
No. 1	2.5×10^2	×	
No. 2	5.8×10^2	×	
No. 3	1.4×10^2	○	(3)
No. 4	2.3×10^1	×	
No. 5	8.2×10^1	○	(5)
No. 6	< 10	×	

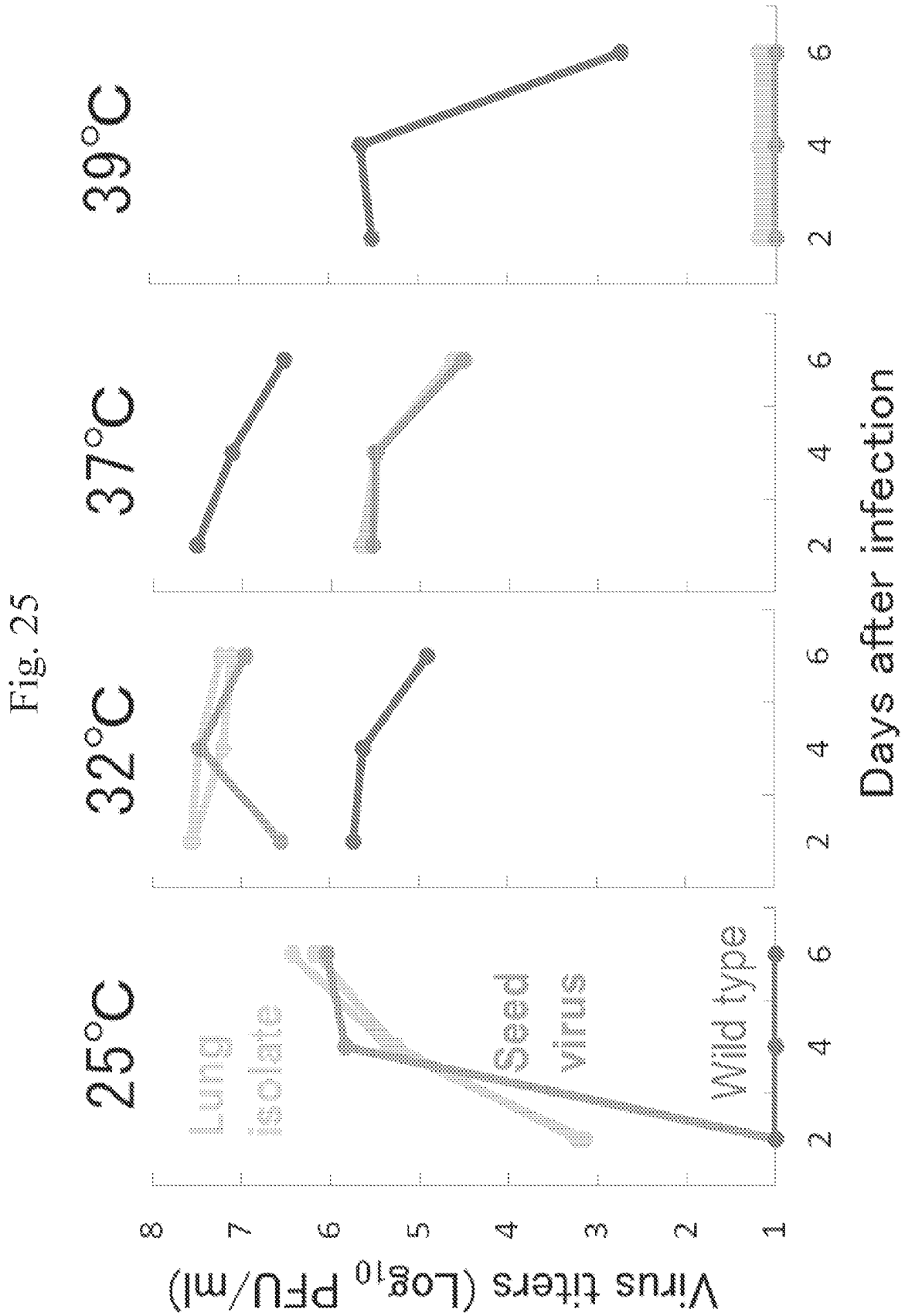

25°C
32°C
37°C
39°C
Lung isolate
Seed virus
Wild type
Virus titers (Log_{10} PFU/ml)
Days after infection

Fig. 25

Fig. 26

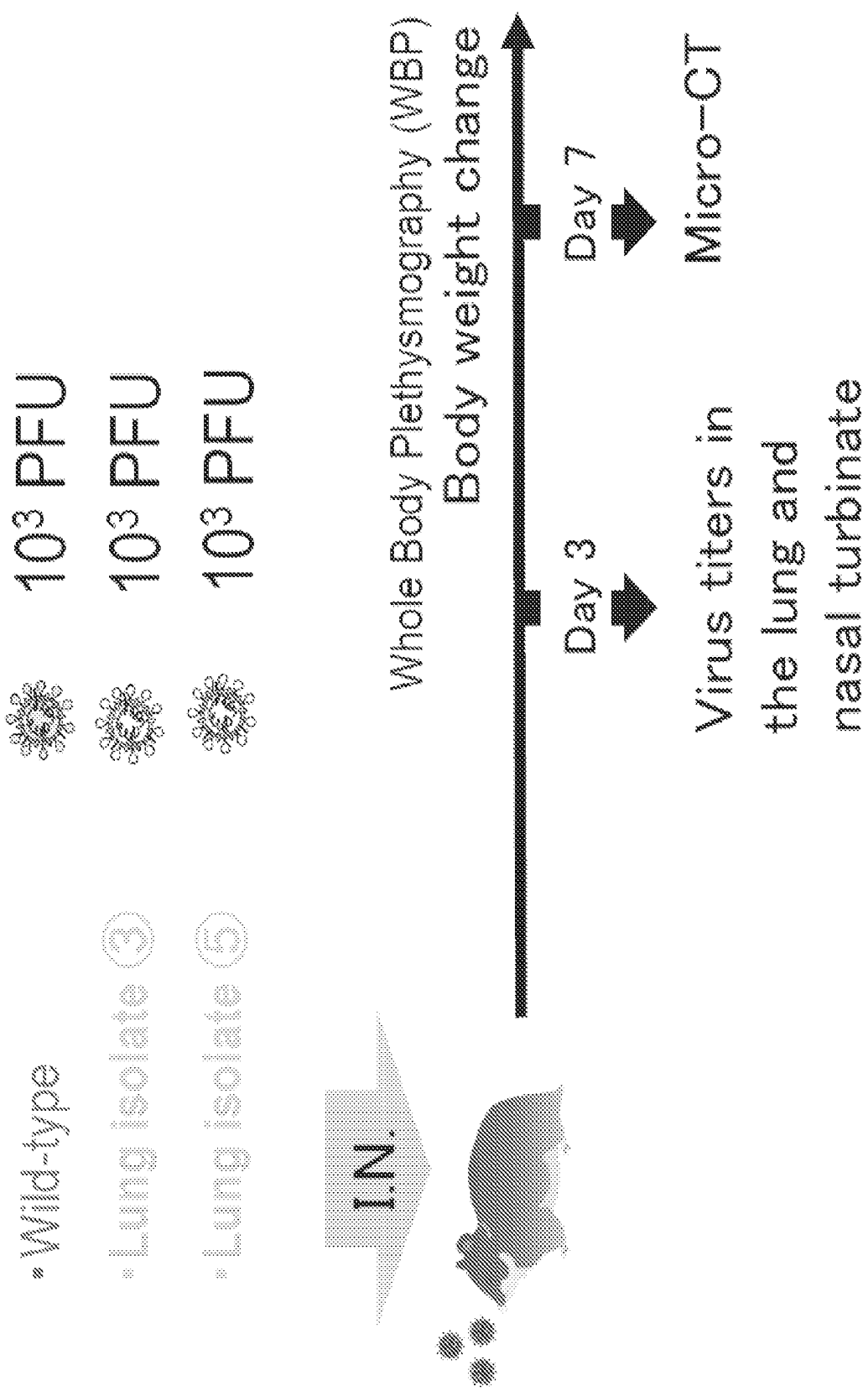

Wild-type
Lung isolate (3)
Lung isolate (5)
10³ PFU
10³ PFU
10³ PFU
I.N.
Whole Body Plethysmography (WBP)
Body weight change
Day 3
Day 7
Virus titers in
the lung and
nasal turbinate
Micro-CT

Fig. 27

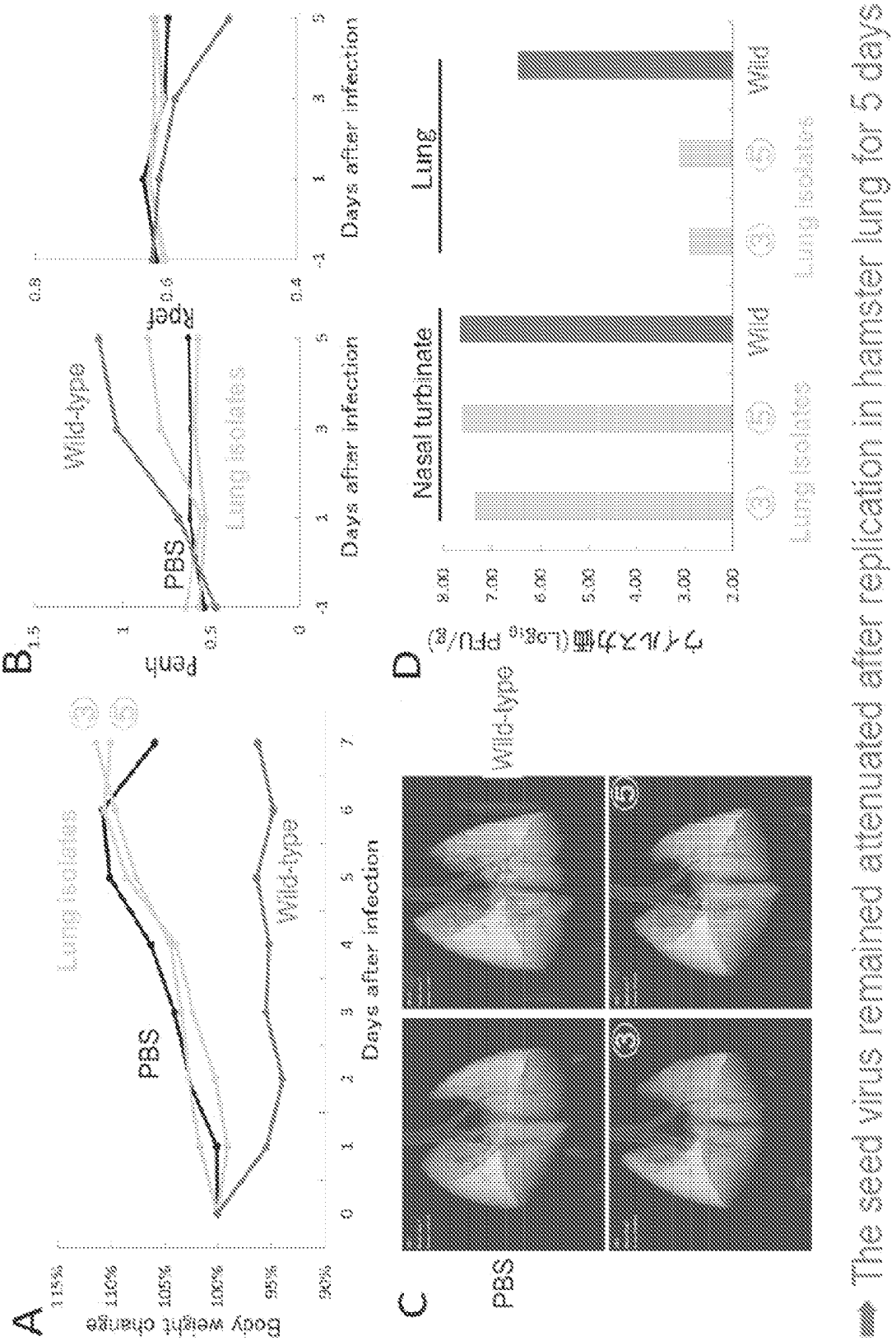

A
Body weight change
Days after infection
PBS
Lung isolates
Wild-type
B
Fenh
Rpet
Days after infection
PBS
Wild-type
Lung isolates
C
PBS
Wild-type
D
Nasal turbinate
Lung
Wild
Lung isolates
The seed virus remained attenuated after replication in hamster lung for 5 days

Fig. 28A

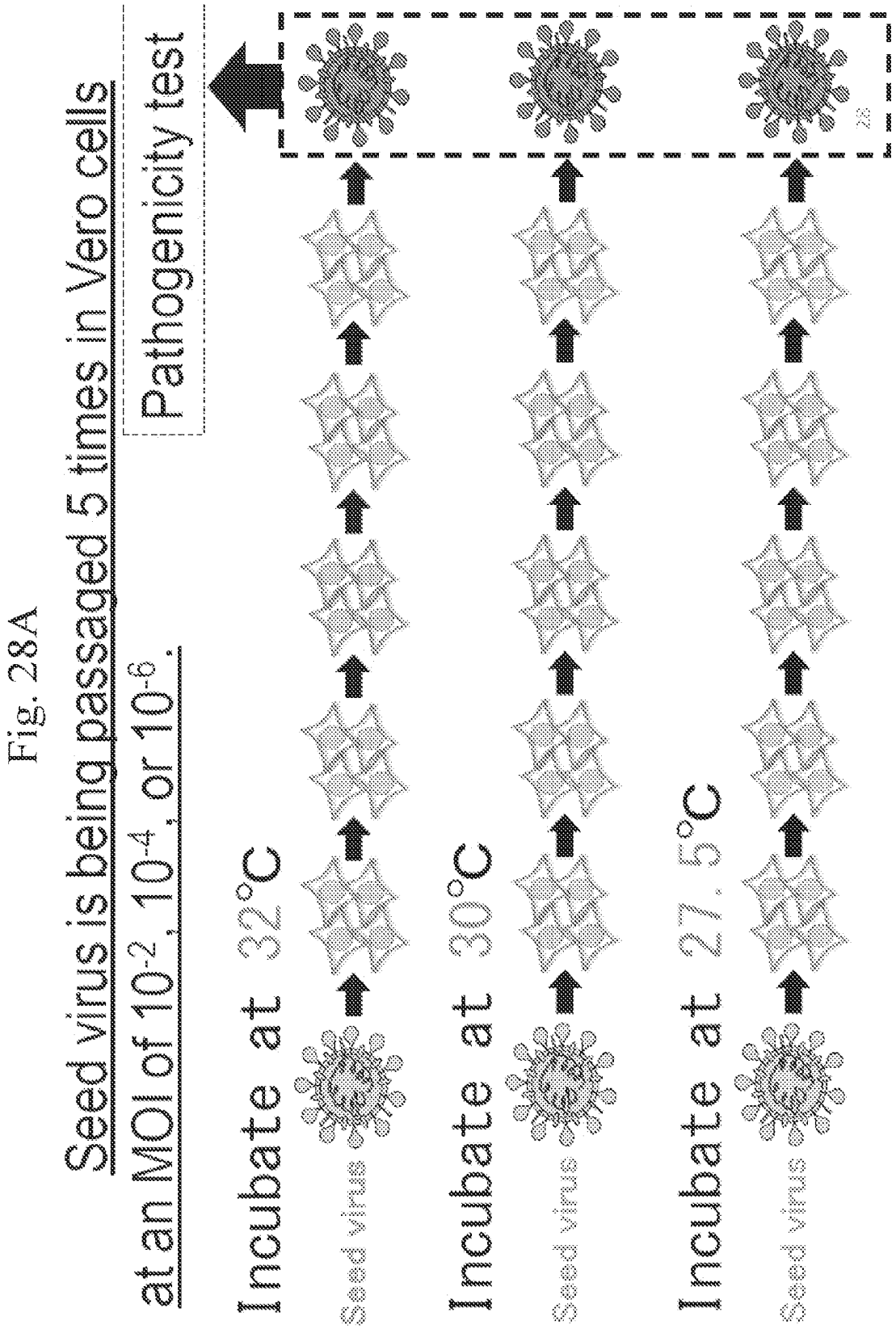

Seed virus is being passaged 5 times in Vero cells
at an MOI of 10^{-2}, 10^{-4}, or 10^{-6}.
Pathogenicity test
Incubate at 32°C
Incubate at 30°C
Incubate at 27.5°C
Seed virus
Seed virus
Seed virus
28

Fig. 28B

Temperature	MOI	Passage 1	Passage 2	Passage 3	Passage 4	Passage 5
32 °C	10^{-2}	6.9×10^{7}	7.2×10^{7}	4.8×10^{7}	1.0×10^{8}	7.2×10^{7}
	10^{-3}	9.2×10^{7}	9.1×10^{7}	1.7×10^{7}	1.3×10^{8}	6.8×10^{7}
	10^{-4}	9.8×10^{7}	1.1×10^{8}	2.5×10^{7}	1.4×10^{8}	7.0×10^{7}
	10^{-5}	7.6×10^{7}	1.1×10^{8}	1.4×10^{7}	1.3×10^{8}	5.7×10^{7}
	10^{-6}	5.6×10^{6}	2.6×10^{7}	7.0×10^{6}	1.1×10^{8}	1.2×10^{7}
30 °C	10^{-2}	5.0×10^{7}	3.5×10^{7}	9.0×10^{7}	9.1×10^{7}	5.7×10^{7}
	10^{-3}	4.2×10^{7}	4.9×10^{7}	8.3×10^{7}	1.0×10^{8}	7.5×10^{7}
	10^{-4}	9.5×10^{7}	6.0×10^{7}	1.0×10^{8}	8.7×10^{7}	6.2×10^{7}
	10^{-5}	1.0×10^{8}	5.4×10^{7}	5.3×10^{7}	4.6×10^{7}	2.2×10^{7}
27.5 °C	10^{-2}	2.1×10^{7}	1.6×10^{7}	2.3×10^{7}	4.2×10^{7}	1.8×10^{7}
	10^{-3}	1.8×10^{7}	1.3×10^{7}	1.5×10^{7}	3.0×10^{7}	1.4×10^{7}
	10^{-4}	1.2×10^{7}	4.4×10^{6}	6.9×10^{6}	5.2×10^{6}	1.8×10^{6}
	10^{-5}	1.5×10^{6}	5.0×10^{5}	6.4×10^{5}	5.6×10^{5}	4.2×10^{4}

(pfu/ml)

Fig. 28C

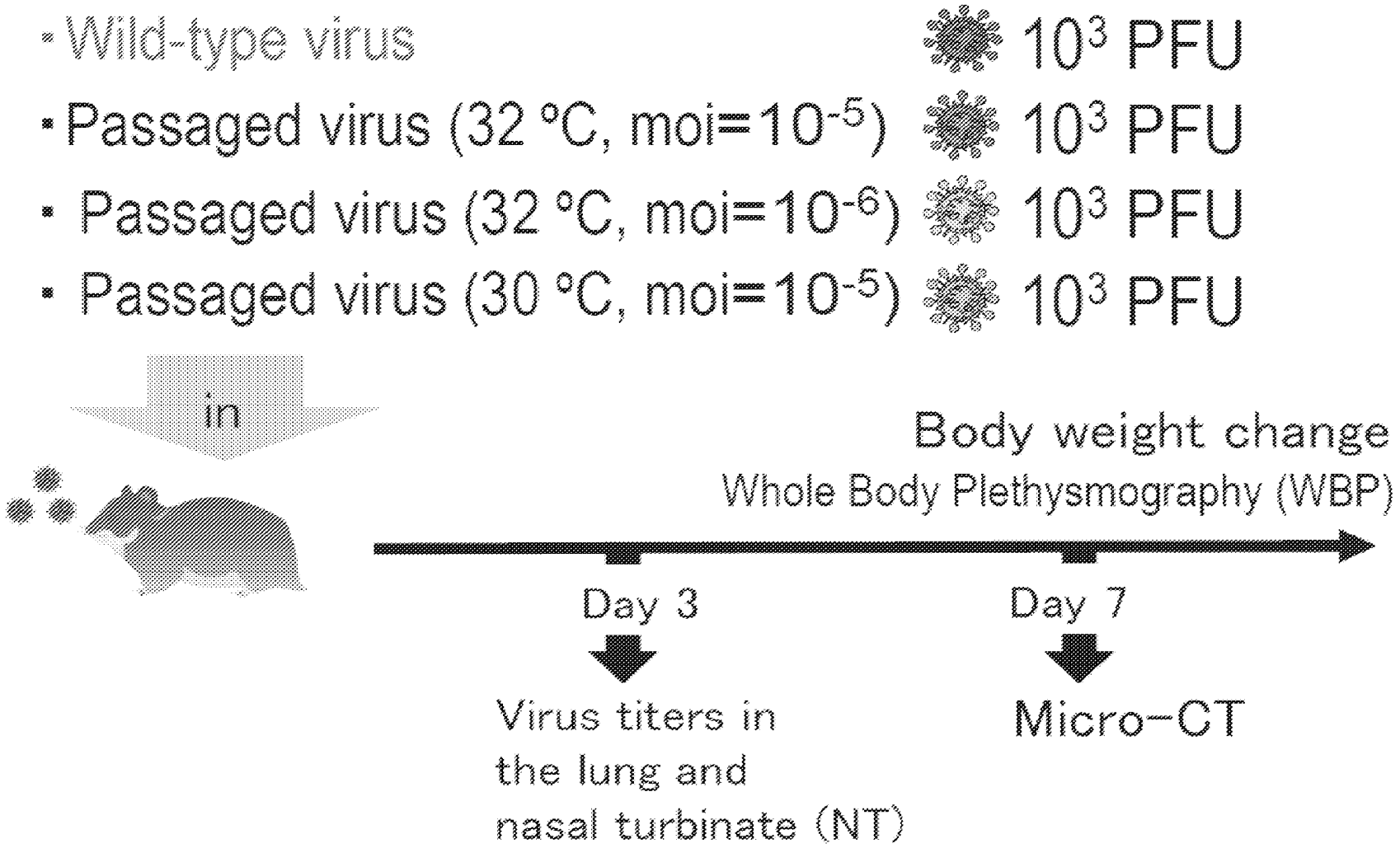


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Fig. 28D

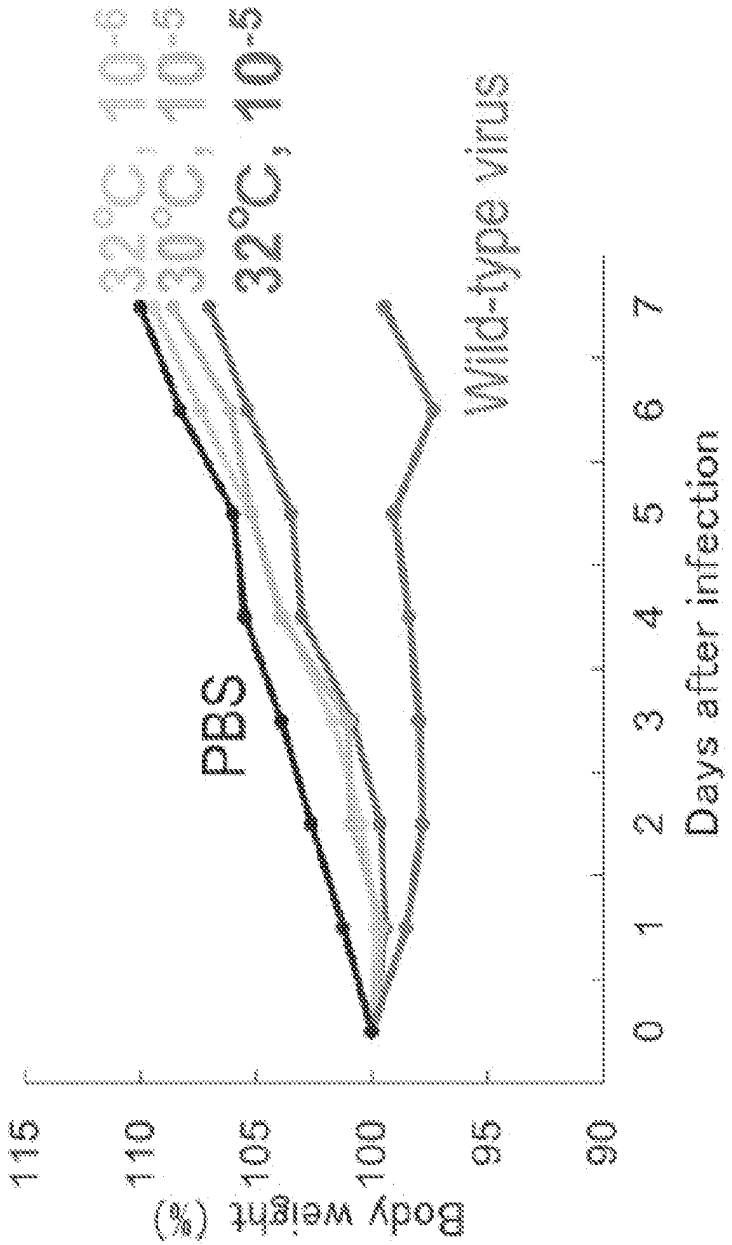
32°C, 10-6
30°C, 10-5
32°C, 10-5
PBS
Wild-type virus
Body weight (%)
115
110
105
100
95
90
0
1
2
3
4
5
6
7
Days after infection

Fig. 28D continued

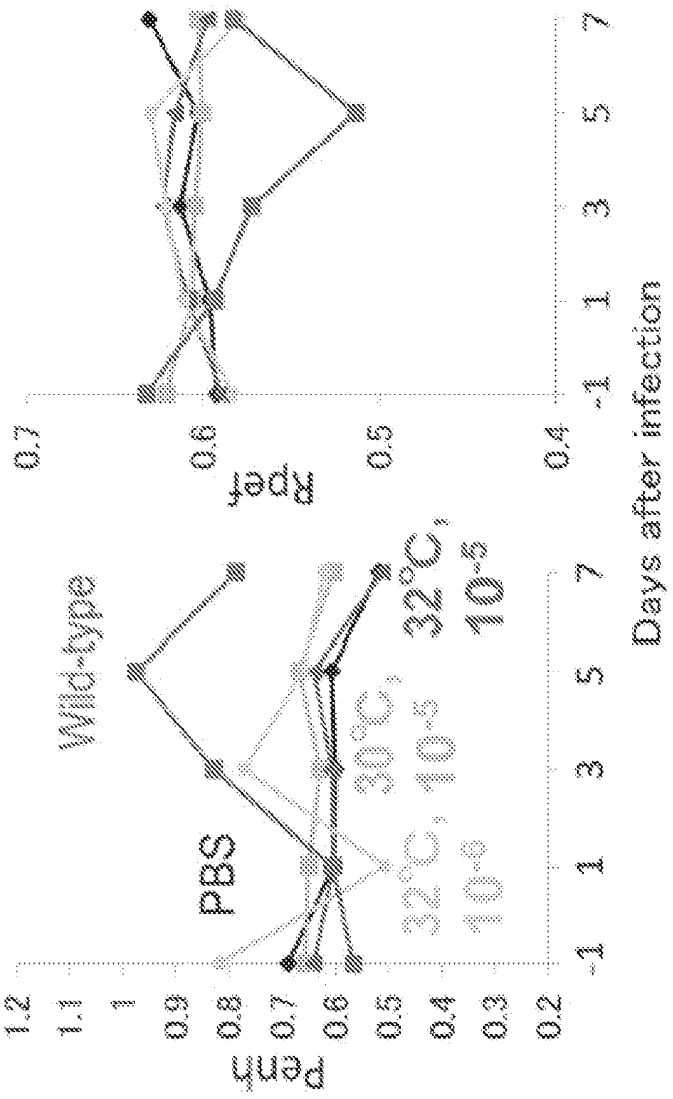

Fig. 28D continued

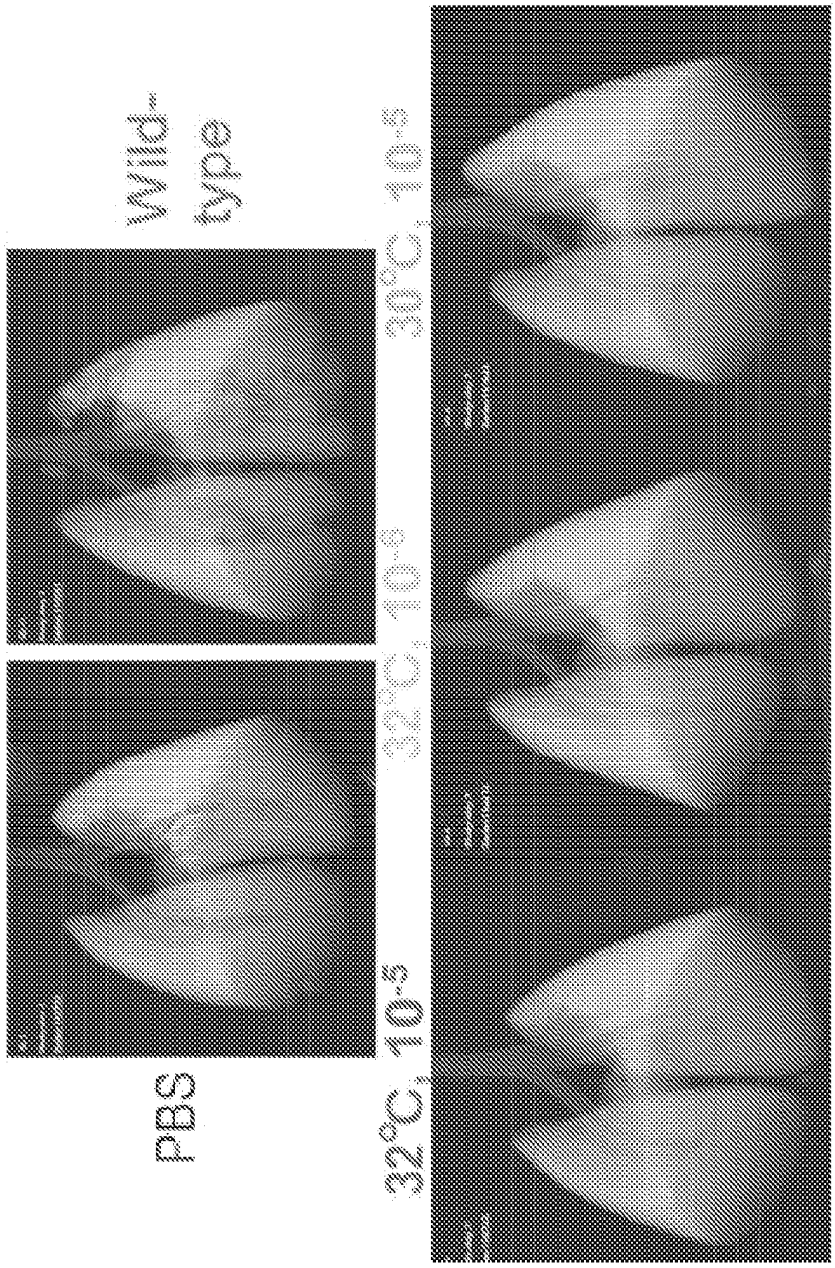

Fig. 28D continued

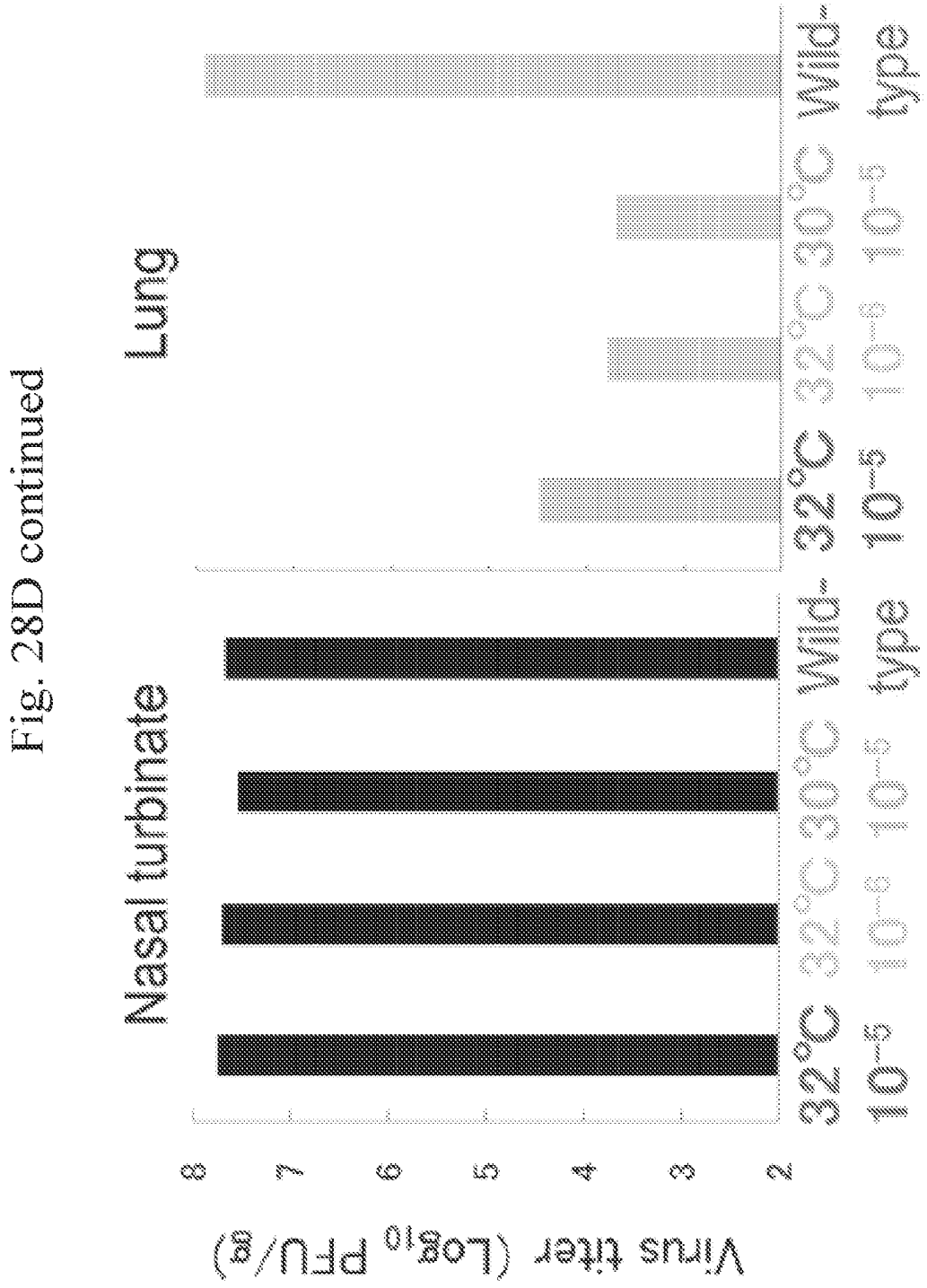

Fig. 29

- ORF1ab-Y1090N contributed to the temperature-sensitive phenotype.
- SV1004F, ORF1ab-M85del, and ORF1ab-L3829F might be important for cold-adaptation.

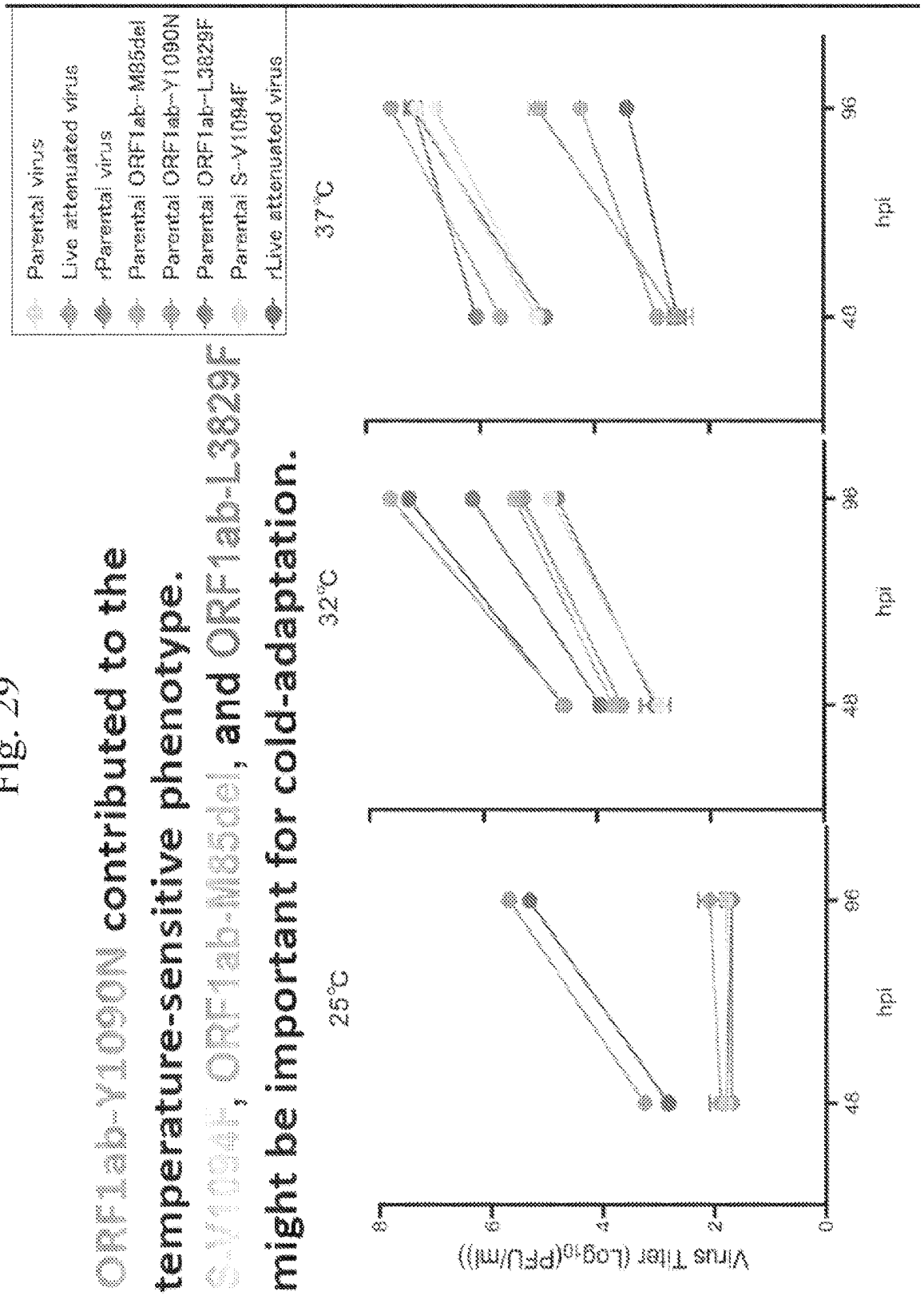

Fig. 30

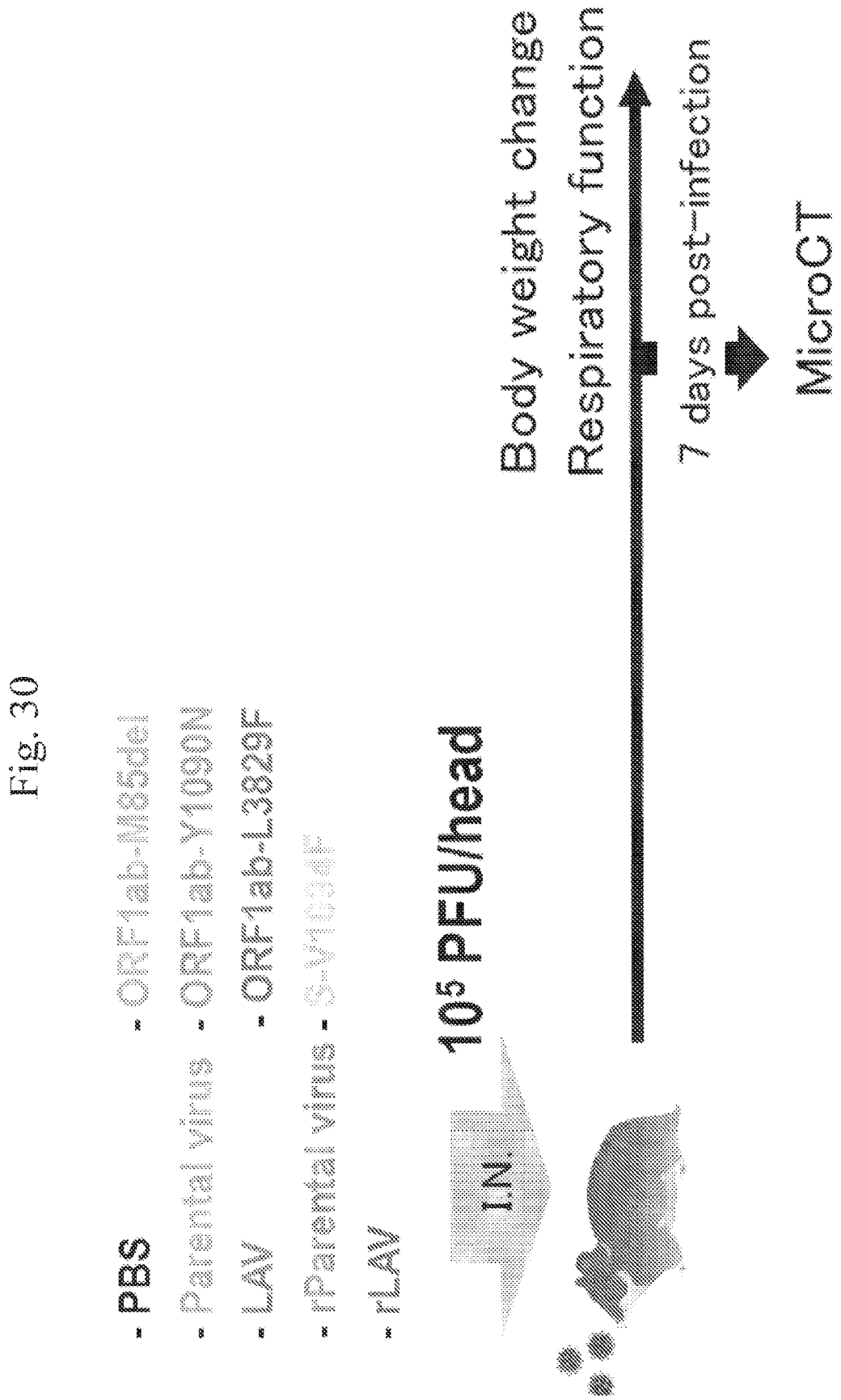
- PBS
- Parental virus
- LAV
- rParental virus
- rLAV
- ORF1ab-M86del
- ORF1ab-Y1090N
- ORF1ab-L3829F
- S-V1034F
I.N.
10⁵ PFU/head
Body weight change
Respiratory function
7 days post-infection
MicroCT

Fig. 31

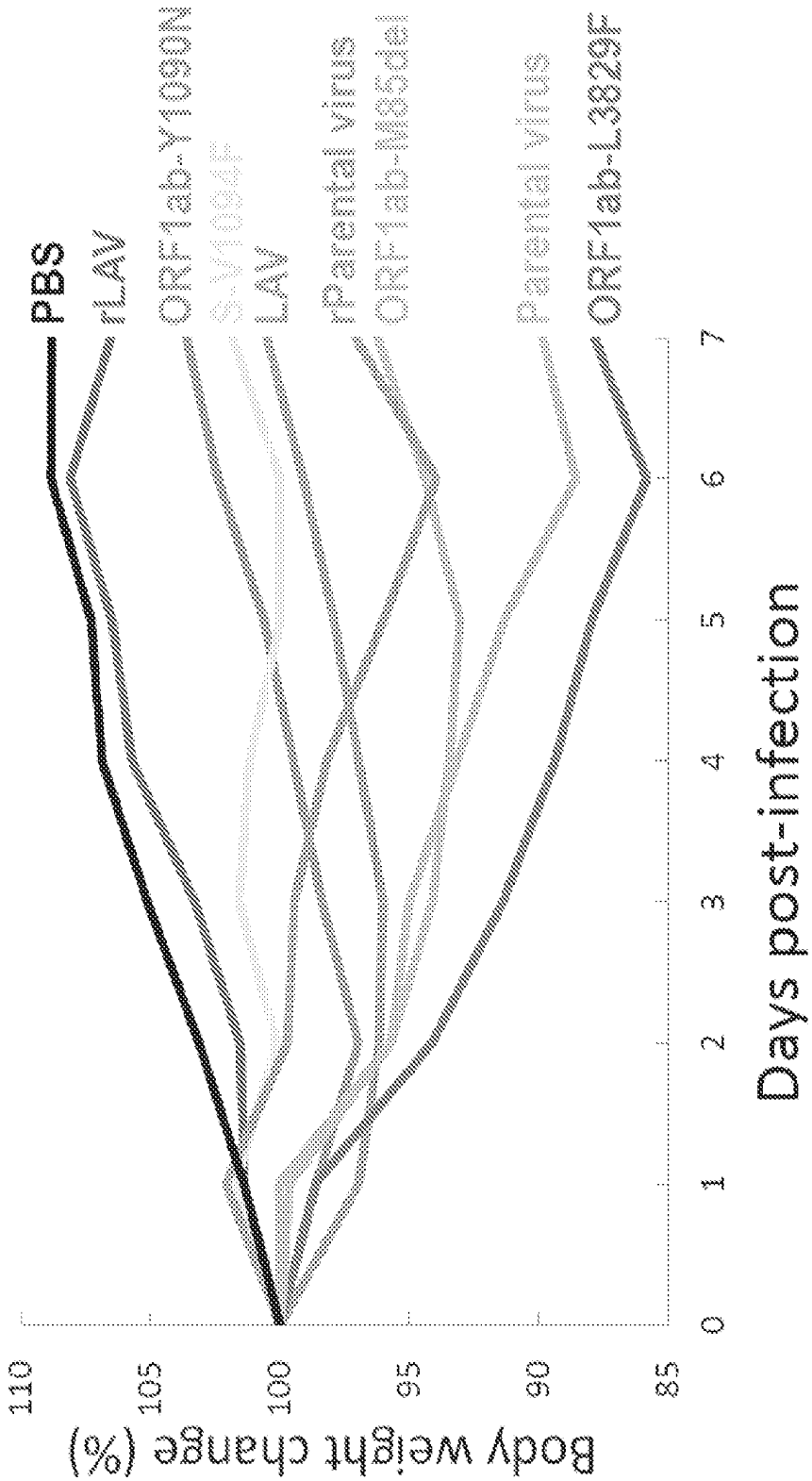
PBS
rLAV
ORF1ab-Y1090N
SV100F
LAV
rParental virus
ORF1ab-M85del
Parental virus
ORF1ab-L382QF
Body weight change (%)
110
105
100
95
90
85
0
1
2
3
4
5
6
7
Days post-infection

Fig. 32

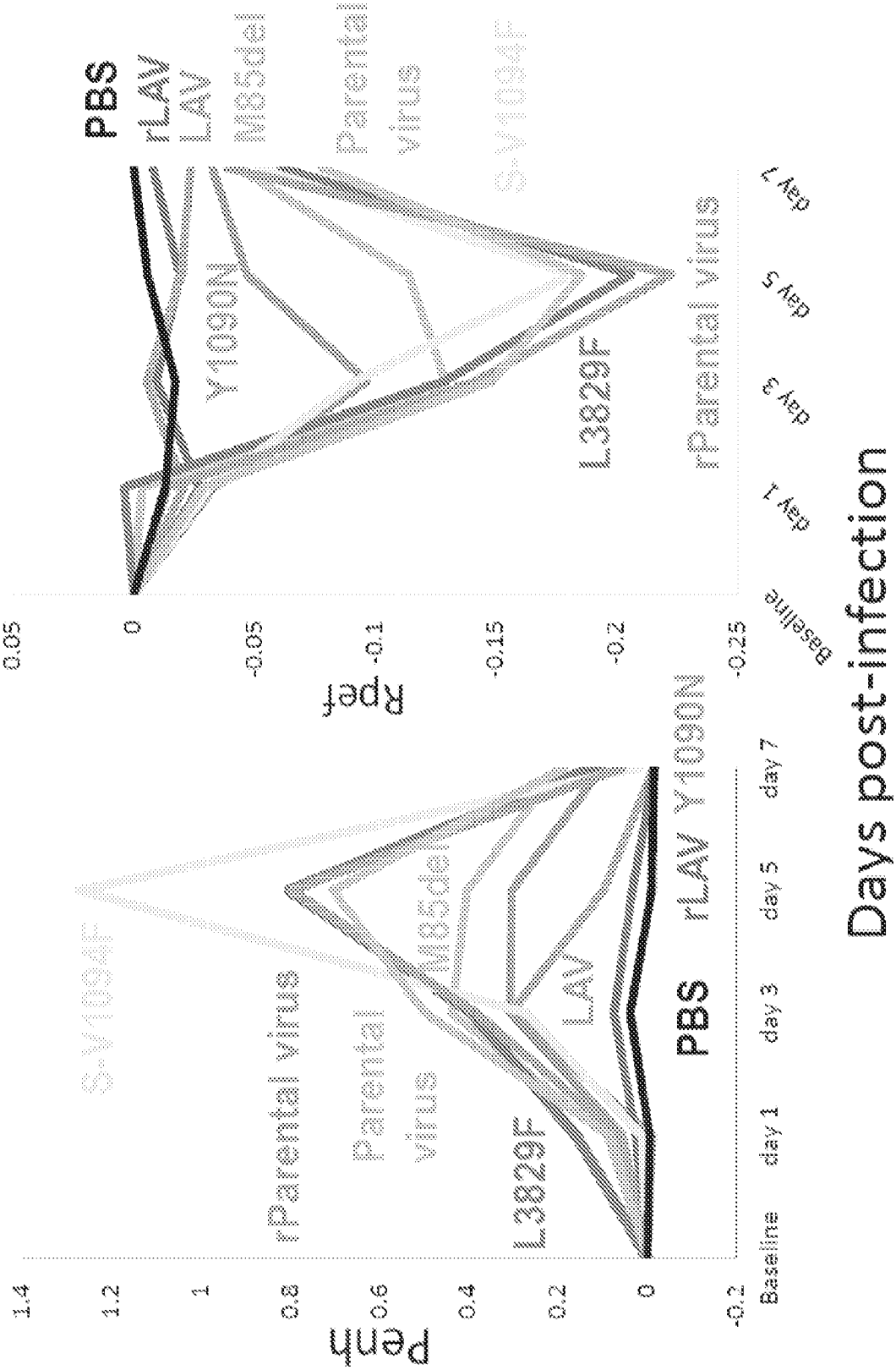
PBS
rLAV
LAV
M85del
Parental virus
S-V1094F
Y1090N
L3829F
rParental virus
Penh
Rpef
Baseline
day 1
day 3
day 5
day 7
Days post-infection

Fig. 33

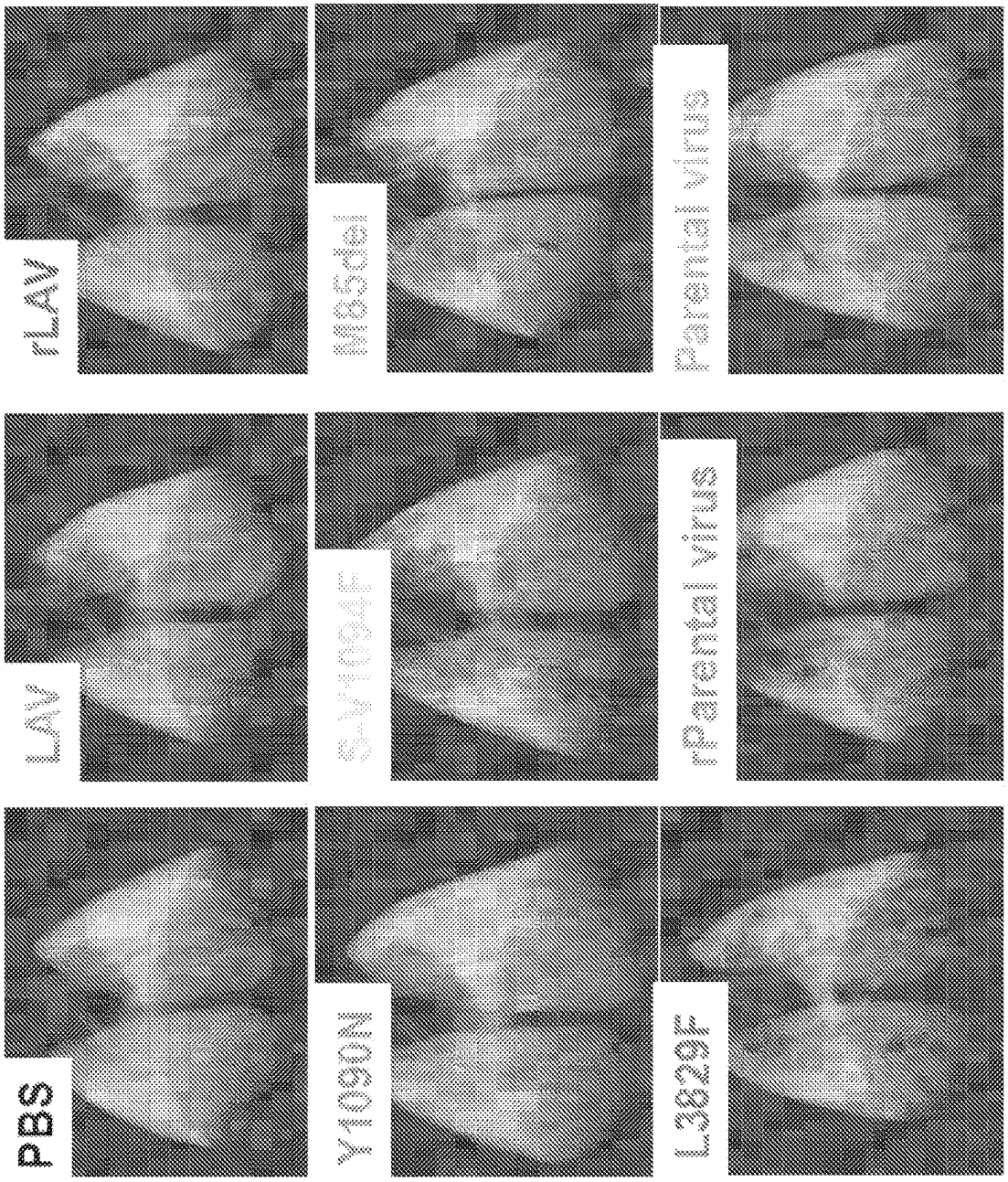
rLAV
M85del
Parental virus
LAV
S-Y1094F
rParental virus
PBS
Y1090N
L3629F

Fig. 34

SEQ ID NO:1

>Parental virus

ATTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCTGTTCTCTAAACGAACTTT
AAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATTAATAACTAATTACTGTCGTTG
ACAGGACACGAGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCAGCCGATCATCAGCACA
TCTAGGTTTTGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAACACACGT
CCAACTTAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTACGTGGCTTTGGAGACTCCGTGGAGGAGGTCTTA
TCAGAGGCACGTCAACATCTTAAAGATGGCACTTGTGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAACTTG
AACAGCCCTATGTGTTCATCAAACGTTCGGATGCTCGAACTGCACCTCATGGTCATGTTATGGTTGAGCTGGTAGC
AGAACTCGAAGGCATTCAGTACGGTCGTAGTGGTGAGACACTTGGTGTCCTTGTCCCTCATGTGGGCGAAATACC
AGTGGCTTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAAGGAGCTGGTGGCCATAGTTACGGCGCCGATCT
AAAGTCATTTGACTTAGGCGACGAGCTTGGCACTGATCCTTATGAAGATTTTCAAGAAAACTGGAACACTAAACAT
AGCAGTGGTGTTACCCGTGAACTCATGCGTGAGCTTAACGGAGGGGCATACACTCGCTATGTCGATAACAACTTCT
GTGGCCCTGATGGCTACCCTCTTGAGTGCATTAAAGACCTTCTAGCACGTGCTGGTAAAGCTTCATGCACTTTGTCC
GAACAACTGGACTTTATTGACACTAAGAGGGGTGTATACTGCTGCCGTGAACATGAGCATGAAATTGCTTGGTAC
ACGGAACGTTCTGAAAAGAGCTATGAATTGCAGACACCTTTTGAAATTAAATTGGCAAAGAAATTTGACACCTTCA
ATGGGGAATGTCCAAATTTTGTATTTCCCTTAAATTCCATAATCAAGACTATTCAACCAAGGGTTGAAAAGAAAAA
GCTTGATGGCTTTATGGGTAGAATTCGATCTGTCTATCCAGTTGCGTCACCAAATGAATGCAACCAAATGTGCCTTT
CAACTCTCATGAAGTGTGATCATTGTGGTGAAACTTCATGGCAGACGGGCGATTTTGTTAAAGCCACTTGCGAATT
TTGTGGCACTGAGAATTTGACTAAAGAAGGTGCCACTACTTGTGGTTACTTACCCCAAAATGCTGTTGTTAAAATTT
ATTGTCCAGCATGTCACAATTCAGAAGTAGGACCTGAGCATAGTCTTGCCGAATACCATAATGAATCTGGCTTGAA
AACCATTCTTCGTAAGGGTGGTCGCACTATTGCCTTTGGAGGCTGTGTGTTCTCTTATGTTGGTTGCCATAACAAGT
GTGCCTATTGGGTTCCACGTGCTAGCGCTAACATAGGTTGTAACCATACAGGTGTTGTTGGAGAAGGTTCCGAAG
GTCTTAATGACAACCTTCTTGAAATACTCCAAAAAGAGAAAGTCAACATCAATATTGTTGGTGACTTTAAACTTAAT
GAAGAGATCGCCATTATTTTGGCATCTTTTTCTGCTTCCACAAGTGCTTTTGTGGAAACTGTGAAAGGTTTGGATTA
TAAAGCATTCAAACAAATTGTTGAATCCTGTGGTAATTTTAAAGTTACAAAAGGAAAAGCTAAAAAAGGTGCCTGG
AATATTGGTGAACAGAAATCAATACTGAGTCCTCTTTATGCATTTGCATCAGAGGCTGCTCGTGTTGTACGATCAAT
TTTCTCCCGCACTCTTGAAACTGCTCAAAATTCTGTGCGTGTTTTACAGAAGGCCGCTATAACAATACTAGATGGAA
TTTCACAGTATTCACTGAGACTCATTGATGCTATGATGTTCACATCTGATTTGGCTACTAACAATCTAGTTGTAATG
GCCTACATTACAGGTGGTGTTGTTCAGTTGACTTCGCAGTGGCTAACTAACATCTTTGGCACTGTTTATGAAAAACT
CAAACCCGTCCTTGATTGGCTTGAAGAGAAGTTTAAGGAAGGTGTAGAGTTTCTTAGAGACGGTTGGGAAATTGT
TAAATTTATCTCAACCTGTGCTTGTGAAATTGTCGGTGGACAAATTGTCACCTGTGCAAAGGAAATTAAGGAGAGT
GTTCAGACATTCTTTAAGCTTGTAAATAAATTTTTGGCTTTGTGTGCTGACTCTATCATTATTGGTGGAGCTAAACTT
AAAGCCTTGAATTTAGGTGAAACATTTGTCACGCACTCAAAGGGATTGTACAGAAAGTGTGTTAAATCCAGAGAA
GAAACTGGCCTACTCATGCCTCTAAAAGCCCCAAAAGAAATTATCTTCTTAGAGGGAGAAACACTTCCCACAGAAG
TGTTAACAGAGGAAGTTGTCTTGAAAACTGGTGATTTACAACCATTAGAACAACCTACTAGTGAAGCTGTTGAAGC
TCCATTGGTTGGTACACCAGTTTGTATTAACGGGCTTATGTTGCTCGAAATCAAAGACACAGAAAAGTACTGTGCC
CTTGCACCTAATATGATGGTAACAAACAATACCTTCACACTCAAAGGCGGTGCACCAACAAAGGTTACTTTTGGTG
ATGACACTGTGATAGAAGTGCAAGGTTACAAGAGTGTGAATATCACTTTTGAACTTGATGAAAGGATTGATAAAG
TACTTAATGAGAAGTGCTCTGCCTATACAGTTGAACTCGGTACAGAAGTAAATGAGTTCGCCTGTGTTGTGGCAGA
TGCTGTCATAAAAACTTTGCAACCAGTATCTGAATTACTTACACCACTGGGCATTGATTTAGATGAGTGGAGTATG
GCTACATACTACTTATTTGATGAGTCTGGTGAGTTTAAATTGGCTTCACATATGTATTGTTCTTTTTACCCTCCAGAT
GAGGATGAAGAAGAAGGTGATTGTGAAGAAGAAGAGTTTGAGCCATCAACTCAATATGAGTATGGTACTGAAGA
TGATTACCAAGGTAAACCTTTGGAATTTGGTGCCACTTCTGCTGCTCTTCAACCTGAAGAAGAGCAAGAAGAAGAT
TGGTTAGATGATGATAGTCAACAAACTGTTGGTCAACAAGACGGCAGTGAGGACAATCAGACAACTACTATTCAA
ACAATTGTTGAGGTTCAACCTCAATTAGAGATGGAACTTACACCAGTTGTTCAGACTATTGAAGTGAATAGTTTTA
GTGGTTATTTAAAACTTACTGACAATGTATACATTAAAAATGCAGACATTGTGGAAGAAGCTAAAAAGGTAAAACC
AACAGTGGTTGTTAATGCAGCCAATGTTTACCTTAAACATGGAGGAGGTGTTGCAGGAGCCTTAAATAAGGCTAC

FIG. 34

TAACAATGCCATGCAAGTTGAATCTGATGATTACATAGCTACTAATGGACCACTTAAAGTGGGTGGTAGTTGTGTT
TTAAGCGGACACAATCTTGCTAAACACTGTCTTCATGTTGTCGGCCCAAATGTTAACAAAGGTGAAGACATTCAAC
TTCTTAAGAGTGCTTATGAAAATTTTAATCAGCACGAAGTTCTACTTGCACCATTATTATCAGCTGGTATTTTTGGTG
CTGACCCTATACATTCTTTAAGAGTTTGTGTAGATACTGTTCGCACAAATGTCTACTTAGCTGTCTTTGATAAAAATC
TCTATGACAAACTTGTTTCAAGCTTTTTGGAAATGAAGAGTGAAAAGCAAGTTGAACAAAAGATCGCTGAGATTCC
TAAAGAGGAAGTTAAGCCATTTATAACTGAAAGTAAACCTTCAGTTGAACAGAGAAAACAAGATGATAAGAAAAT
CAAAGCTTGTGTTGAAGAAGTTACAACAACTCTGGAAGAAACTAAGTTCCTCACAGAAAACTTGTTACTTTATATTG
ACATTAATGGCAATCTTCATCCAGATTCTGCCACTCTTGTTAGTGACATTGACATCACTTTCTTAAAGAAAGATGCTC
CATATATAGTGGGTGATGTTGTTCAAGAGGGTGTTTTAACTGCTGTGGTTATACCTACTAAAAAGGCTGGTGGCAC
TACTGAAATGCTAGCGAAAGCTTTGAGAAAAGTGCCAACAGACAATTATATAACCACTTACCCGGGTCAGGGTTTA
AATGGTTACACTGTAGAGGAGGCAAAGACAGTGCTTAAAAAGTGTAAAAGTGCCTTTTACATTCTACCATCTATTA
TCCCTAATGAGAAGCAAGAAATTCTTGGAACTGTTTCTTGGAATTTGCGAGAAATGCTTGCACATGCAGAAGAAAC
ACGCAAATTAATGCCTGTCTGTGTGGAAACTAAAGCCATAGTTTCAACTATACAGCGTAAATATAAGGGTATTAAA
ATACAAGAGGGTGTGGTTGATTATGGTGCTAGATTTTACTTTTACACCAGTAAAACAACTGTAGCGTCACTTATCA
ACACACTTAACGATCTAAATGAAACTCTTGTTACAATGCCACTTGGCTATGTAACACATGGCTTAAATTTGGAAGAA
GCTGCTCGGTATATGAGATCTCTCAAAGTGCCAGCTACAGTTTCTGTTTCTTCACCTGATGCTGTTACAGCGTATAA
TGGTTATCTTACTTCTTCTTCTAAAACACCTGAAGAACATTTTATTGAAACCATCTCACTTGCTGGTTCCTATAAAGA
TTGGTCCTATTCTGGACAATCTACACAACTAGGTATAGAATTTCTTAAGAGAGGTGATAAAAGTGTATATTACACTA
GTAATCCTACCACATTCCACCTAGATGGTGAAGTTATCACCTTTGACAATCTTAAGACACTTCTTTCTTTGAGAGAA
GTGAGGACTATTAAGGTGTTTACAACAGTAGACAACATTAACCTCCACACGCAAGTTGTGGACATGTCAATGACAT
ATGGACAACAGTTTGGTCCAACTTATTTGGATGGAGCTGATGTTACTAAAATAAAACCTCATAATTCACATGAAGG
TAAAACATTTTATGTTTTACCTAATGATGACACTCTACGTGTTGAGGCTTTTGAGTACTACCACACAACTGATCCTA
GTTTTCTGGGTAGGTACATGTCAGCATTAAATCACACTAAAAAGTGGAAATACCCACAAGTTAATGGTTTAACTTCT
ATTAAATGGGCAGATAACAACTGTTATCTTGCCACTGCATTGTTAACACTCCAACAAATAGAGTTGAAGTTTAATCC
ACCTGCTCTACAAGATGCTTATTACAGAGCAAGGGCTGGTGAAGCTGCTAACTTTTGTGCACTTATCTTAGCCTACT
GTAATAAGACAGTAGGTGAGTTAGGTGATGTTAGAGAAACAATGAGTTACTTGTTTCAACATGCCAATTTAGATTC
TTGCAAAAGAGTCTTGAACGTGGTGTGTAAAACTTGTGGACAACAGCAGACAACCCTTAAGGGTGTAGAAGCTGT
TATGTACATGGGCACACTTTCTTATGAACAATTTAAGAAAGGTGTTCAGATACCTTGTACGTGTGGTAAACAAGCT
ACAAAATATCTAGTACAACAGGAGTCACCTTTTGTTATGATGTCAGCACCACCTGCTCAGTATGAACTTAAGCATG
GTACATTTACTTGTGCTAGTGAGTACACTGGTAATTACCAGTGTGGTCACTATAAACATATAACTTCTAAAGAAACT
TTGTATTGCATAGACGGTGCTTTACTTACAAAGTCCTCAGAATACAAAGGTCCTATTACGGATGTTTTCTACAAAGA
AAACAGTTACACAACAACCATAAAACCAGTTACTTATAAATTGGATGGTGTTGTTTGTACAGAAATTGACCCTAAG
TTGGACAATTATTATAAGAAAGACAATTCTTATTTCACAGAGCAACCAATTGATCTTGTACCAAACCAACCATATCC
AAACGCAAGCTTCGATAATTTTAAGTTTGTATGTGATAATATCAAATTTGCTGATGATTTAAACCAGTTAACTGGTT
ATAAGAAACCTGCTTCAAGAGAGCTTAAAGTTACATTTTTCCCTGACTTAAATGGTGATGTGGTGGCTATTGATTAT
AAACACTACACACCCTCTTTTAAGAAAGGAGCTAAATTGTTACATAAACCTATTGTTTGGCATGTTAACAATGCAAC
TAATAAAGCCACGTATAAACCAAATACCTGGTGTATACGTTGTCTTTGGAGCACAAAACCAGTTGAAACATCAAAT
TCGTTTGATGTACTGAAGTCAGAGGACGCGCAGGGAATGGATAATCTTGCCTGCGAAGATCTAAAACCAGTCTCT
GAAGAAGTAGTGGAAAATCCTACCATACAGAAAGACGTTCTTGAGTGTAATGTGAAAACTACCGAAGTTGTAGGA
GACATTATACTTAAACCAGCAAATAATAGTTTAAAAATTACAGAAGAGGTTGGCCACACAGATCTAATGGCTGCTT
ATGTAGACAATTCTAGTCTTACTATTAAGAAACCTAATGAATTATCTAGAGTATTAGGTTTGAAAACCCTTGCTACT
CATGGTTTAGCTGCTGTTAATAGTGTCCCTTGGGATACTATAGCTAATTATGCTAAGCCTTTTCTTAACAAAGTTGTT
AGTACAACTACTAACATAGTTACACGGTGTTTAAACCGTGTTTGTACTAATTATATGCCTTATTTCTTTACTTTATTG
CTACAATTGTGTACTTTTACTAGAAGTACAAATTCTAGAATTAAAGCATCTATGCCGACTACTATAGCAAAGAATAC
TGTTAAGAGTGTCGGTAAATTTTGTCTAGAGGCTTCATTTAATTATTTGAAGTCACCTAATTTTTCTAAACTGATAAA
TATTATAATTTGGTTTTTACTATTAAGTGTTTGCCTAGGTTCTTTAATCTACTCAACCGCTGCTTTAGGTGTTTTAATG
TCTAATTTAGGCATGCCTTCTTACTGTACTGGTTACAGAGAAGGCTATTTGAACTCTACTAATGTCACTATTGCAAC
CTACTGTACTGGTTCTATACCTTGTAGTGTTTGTCTTAGTGGTTTAGATTCTTTAGACACCTATCCTTCTTTAGAAACT

FIG. 34

ATACAAATTACCATTTCATCTTTTAAATGGGATTTAACTGCTTTTGGCTTAGTTGCAGAGTGGTTTTTTGGCATATATT
CTTTTCACTAGGTTTTTCTATGTACTTGGATTGGCTGCAATCATGCAATTGTTTTTCAGCTATTTTGCAGTACATTTTA
TTAGTAATTCTTGGCTTATGTGGTTAATAATTAATCTTGTACAAATGGCCCCGATTTCAGCTATGGTTAGAATGTAC
ATCTTCTTTGCATCATTTTATTATGTATGGAAAAGTTATGTGCATGTTGTAGACGGTTGTAATTCATCAACTTGTATG
ATGTGTTACAAACGTAATAGAGCAACAAGAGTCGAATGTACAACTATTGTTAATGGTGTTAGAAGGTCCTTTTATG
TCTATGCTAATGGAGGTAAAGGCTTTTGCAAACTACACAATTGGAATTGTGTTAATTGTGATACATTCTGTGCTGGT
AGTACATTTATTAGTGATGAAGTTGCGAGAGACTTGTCACTACAGTTTAAAAGACCAATAAATCCTACTGACCAGT
CTTCTTACATCGTTGATAGTGTTACAGTGAAGAATGGTTCCATCCATCTTTACTTTGATAAAGCTGGTCAAAAGACT
TATGAAAGACATTCTCTCTCTCATTTTGTTAACTTAGACAACCTGAGAGCTAATAACACTAAAGGTTCATTGCCTATT
AATGTTATAGTTTTTGATGGTAAATCAAAATGTGAAGAATCATCTGCAAAATCAGCGTCTGTTTACTACAGTCAGCT
TATGTGTCAACCTATACTGTTACTAGATCAGGCATTAGTGTCTGATGTTGGTGATAGTGCGGAAGTTGCAGTTAAA
ATGTTTGATGCTTACGTTAATACGTTTTCATCAACTTTTAACGTACCAATGGAAAAACTCAAAACACTAGTTGCAAC
TGCAGAAGCTGAACTTGCAAAGAATGTGTCCTTAGACAATGTCTTATCTACTTTTATTTCAGCAGCTCGGCAAGGG
TTTGTTGATTCAGATGTAGAAACTAAAGATGTTGTTGAATGTCTTAAATTGTCACATCAATCTGACATAGAAGTTAC
TGGCGATAGTTGTAATAACTATATGCTCACCTATAACAAAGTTGAAAACATGACACCCCGTGACCTTGGTGCTTGT
ATTGACTGTAGTGCGCGTCATATTAATGCGCAGGTAGCAAAAAGTCACAACATTGCTTTGATATGGAACGTTAAAG
ATTTCATGTCATTGTCTGAACAACTACGAAAACAAATACGTAGTGCTGCTAAAAAGAATAACTTACCTTTTAAGTTG
ACATGTGCAACTACTAGACAAGTTGTTAATGTTGTAACAACAAAGATAGCACTTAAGGGTGGTAAAATTGTTAATA
ATTGGTTGAAGCAGTTAATTAAAGTTACACTTGTGTTCCTTTTTGTTGCTGCTATTTTCTATTTAATAACACCTGTTCA
TGTCATGTCTAAACATACTGACTTTTCAAGTGAAATCATAGGATACAAGGCTATTGATGGTGGTGTCACTCGTGAC
ATAGCATCTACAGATACTTGTTTTGCTAACAAACATGCTGATTTTGACACATGGTTTAGCCAGCGTGGTGGTAGTTA
TACTAATGACAAAGCTTGCCCATTGATTGCTGCAGTCATAACAAGAGAAGTGGGTTTTGTCGTGCCTGGTTTGCCT
GGCACGATATTACGCACAACTAATGGTGACTTTTTGCATTTCTTACCTAGAGTTTTTAGTGCAGTTGGTAACATCTG
TTACACACCATCAAAACTTATAGAGTACACTGACTTTGCAACATCAGCTTGTGTTTTGGCTGCTGAATGTACAATTT
TTAAAGATGCTTCTGGTAAGCCAGTACCATATTGTTATGATACCAATGTACTAGAAGGTTCTGTTGCTTATGAAAGT
TTACGCCCTGACACACGTTATGTGCTCATGGATGGCTCTATTATTCAATTTCCTAACACCTACCTTGAAGGTTCTGTT
AGAGTGGTAACAACTTTTGATTCTGAGTACTGTAGGCACGGCACTTGTGAAAGATCAGAAGCTGGTGTTTGTGTAT
CTACTAGTGGTAGATGGGTACTTAATAATGATTATTACAGATCTTTACCAGGAGTTTTCTGTGGTGTAGATGCTGTA
AATTTACTTACTAATATGTTTACACCACTAATTCAACCTATTGGTGCTTTGGACATATCAGCATCTATAGTAGCTGGT
GGTATTGTAGCTATCGTAGTAACATGCCTTGCCTACTATTTTATGAGGTTTAGAAGAGCTTTTGGTGAATACAGTCA
TGTAGTTGCCTTTAATACTTTACTATTCCTTATGTCATTCACTGTACTCTGTTTAACACCAGTTTACTCATTCTTACCT
GGTGTTTATTCTGTTATTTACTTGTACTTGACATTTTATCTTACTAATGATGTTTCTTTTTTAGCACATATTCAGTGGA
TGGTTATGTTCACACCTTTAGTACCTTTCTGGATAACAATTGCTTATATCATTTGTATTTCCACAAAGCATTTCTATTG
GTTCTTTAGTAATTACCTAAAGAGACGTGTAGTCTTTAATGGTGTTTCCTTTAGTACTTTTGAAGAAGCTGCGCTGT
GCACCTTTTTGTTAAATAAAGAAATGTATCTAAAGTTGCGTAGTGATGTGCTATTACCTCTTACGCAATATAATAGA
TACTTAGCTCTTTATAATAAGTACAAGTATTTTAGTGGAGCAATGGATACAACTAGCTACAGAGAAGCTGCTTGTT
GTCATCTCGCAAAGGCTCTCAATGACTTCAGTAACTCAGGTTCTGATGTTCTTTACCAACCACCACAAACCTCTATC
ACCTCAGCTGTTTTGCAGAGTGGTTTTAGAAAAATGGCATTCCCATCTGGTAAAGTTGAGGGTTGTATGGTACAAG
TAACTTGTGGTACAACTACACTTAACGGTCTTTGGCTTGATGACGTAGTTTACTGTCCAAGACATGTGATCTGCACC
TCTGAAGACATGCTTAACCCTAATTATGAAGATTTACTCATTCGTAAGTCTAATCATAATTTCTTGGTACAGGCTGG
TAATGTTCAACTCAGGGTTATTGGACATTCTATGCAAAATTGTGTACTTAAGCTTAAGGTTGATACAGCCAATCCTA
AGACACCTAAGTATAAGTTTGTTCGCATTCAATCAGGACAGACTTTTTCAGTGTTAGCTTGTTACAATGGTTCACCA
TCTGGTGTTTACCAATGTGCTATGAGGCCCAATTTCACTATTAAGGGTTCATTCCTTAATGGTTCATGTGGTAGTGT
TGGTTTTAACATAGATTATGACTGTGTCTCTTTTTGTTACATGCACCATATGGAATTACCAACTGGAGTTCATGCTG
GCACAGACTTAGAAGGTAACTTTTATGGACCTTTTGTTGACAGGCAAACAGCACAAGCAGCTGGTACGGACACAA
CTATTACAGTTAATGTTTTAGCTTGGTTGTACGCTGCTGTTATAAATGGAGACAGGTGGTTTCTCAATCGATTTACC
ACAACTCTTAATGACTTTAACCTTGTGGCTATGAAGTACAATTATGAACCTCTAACACAAGACCATGTTGACATACT
AGGACCTCTTTCTGCTCAAACTGGAATTGCCGTTTTAGATATGTGTGCTTCATTAAAAGAATTACTGCAAAATGGTA

FIG. 34

TGAATGGACGTACCATATTGGGTAGTGCTTTATTAGAAGATGAATTTACACCTTTTGATGTTGTTAGACAATGCTCA
GGTGTTACTTTCCAAAGTGCAGTGAAAAGAACAATCAAGGGTACACACCACTGGTTGTTACTCACAATTTTGACTT
CACTTTTAGTTTTAGTCCAGAGTACTCAATGGTCTTTGTTCTTTTTTTTTGTATGAAAATGCCTTTTTACCTTTTGCTAT
GGGTATTATTGCTATGTCTGCTTTTGCAATGATGTTTGTCAAACATAAGCATGCATTTCTCTGTTTGTTTTTGTTACC
TTCTCTTGCCACTGTAGCTTATTTTAATATGGTCTATATGCCTGCTAGTTGGGTGATGCGTATTATGACATGGTTGG
ATATGGTTGATACTAGTTTGTCTGGTTTTAAGCTAAAAGACTGTGTTATGTATGCATCAGCTGTAGTGTTACTAATC
CTTATGACAGCAAGAACTGTGTATGATGATGGTGCTAGGAGAGTGTGGACACTTATGAATGTCTTGACACTCGTTT
ATAAAGTTTATTATGGTAATGCTTTAGATCAAGCCATTTCCATGTGGGCTCTTATAATCTCTGTTACTTCTAACTACT
CAGGTGTAGTTACAACTGTCATGTTTTTGGCCAGAGGTATTGTTTTTATGTGTGTTGAGTATTGCCCTATTTTCTTCA
TAACTGGTAATACACTTCAGTGTATAATGCTAGTTTATTGTTTCTTAGGCTATTTTTGTACTTGTTACTTTGGCCTCTT
TTGTTTACTCAACCGCTACTTTAGACTGACTCTTGGTGTTTATGATTACTTAGTTTCTACACAGGAGTTTAGATATAT
GAATTCACAGGGACTACTCCCACCCAAGAATAGCATAGATGCCTTCAAACTCAACATTAAATTGTTGGGTGTTGGT
GGCAAACCTTGTATCAAAGTAGCCACTGTACAGTCTAAAATGTCAGATGTAAAGTGCACATCAGTAGTCTTACTCT
CAGTTTTGCAACAACTCAGAGTAGAATCATCATCTAAATTGTGGGCTCAATGTGTCCAGTTACACAATGACATTCTC
TTAGCTAAAGATACTACTGAAGCCTTTGAAAAAATGGTTTCACTACTTTCTGTTTTGCTTTCCATGCAGGGTGCTGT
AGACATAAACAAGCTTTGTGAAGAAATGCTGGACAACAGGGCAACCTTACAAGCTATAGCCTCAGAGTTTAGTTCC
CTTCCATCATATGCAGCTTTTGCTACTGCTCAAGAAGCTTATGAGCAGGCTGTTGCTAATGGTGATTCTGAAGTTGT
TCTTAAAAAGTTGAAGAAGTCTTTGAATGTGGCTAAATCTGAATTTGACCGTGATGCAGCCATGCAACGTAAGTTG
GAAAAGATGGCTGATCAAGCTATGACCCAAATGTATAAACAGGCTAGATCTGAGGACAAGAGGGCAAAAGTTAC
TAGTGCTATGCAGACAATGCTTTTCACTATGCTTAGAAAGTTGGATAATGATGCACTCAACAACATTATCAACAATG
CAAGAGATGGTTGTGTTCCCTTGAACATAATACCTCTTACAACAGCAGCCAAACTAATGGTTGTCATACCAGACTAT
AACACATATAAAAATACGTGTGATGGTACAACATTTACTTATGCATCAGCATTGTGGGAAATCCAACAGGTTGTAG
ATGCAGATAGTAAAATTGTTCAACTTAGTGAAATTAGTATGGACAATTCACCTAATTTAGCATGGCCTCTTATTGTA
ACAGCTTTAAGGGCCAATTCTGCTGTCAAATTACAGAATAATGAGCTTAGTCCTGTTGCACTACGACAGATGTCTT
GTGCTGCCGGTACTACACAAACTGCTTGCACTGATGACAATGCGTTAGCTTACTACAACACAACAAAGGGAGGTA
GGTTTGTACTTGCACTGTTATCCGATTTACAGGATTTGAAATGGGCTAGATTCCCTAAGAGTGATGGAACTGGTAC
TATCTATACAGAACTGGAACCACCTTGTAGGTTTGTTACAGACACACCTAAAGGTCCTAAAGTGAAGTATTTATACT
TTATTAAAGGATTAAACAACCTAAATAGAGGTATGGTACTTGGTAGTTTAGCTGCCACAGTACGTCTACAAGCTGG
TAATGCAACAGAAGTGCCTGCCAATTCAACTGTATTATCTTTCTGTGCTTTTGCTGTAGATGCTGCTAAAGCTTACA
AAGATTATCTAGCTAGTGGGGGACAACCAATCACTAATTGTGTTAAGATGTTGTGTACACACACTGGTACTGGTCA
GGCAATAACAGTTACACCGGAAGCCAATATGGATCAAGAATCCTTTGGTGGTGCATCGTGTTGTCTGTACTGCCGT
TGCCACATAGATCATCCAAATCCTAAAGGATTTTGTGACTTAAAAGGTAAGTATGTACAAATACCTACAACTTGTGC
TAATGACCCTGTGGGTTTTACACTTAAAAACACAGTCTGTACCGTCTGCGGTATGTGGAAAGGTTATGGCTGTAGT
TGTGATCAACTCCGCGAACCCATGCTTCAGTCAGCTGATGCACAATCGTTTTTAAACGGGTTTGCGGTGTAAGTGC
AGCCCGTCTTACACCGTGCGGCACAGGCACTAGTACTGATGTCGTATACAGGGCTTTTGACATCTACAATGATAAA
GTAGCTGGTTTTGCTAAATTCCTAAAAACTAATTGTTGTCGCTTCCAAGAAAAGGACGAAGATGACAATTTAATTG
ATTCTTACTTTGTAGTTAAGAGACACACTTTCTCTAACTACCAACATGAAGAAACAATTTATAATTTACTTAAGGATT
GTCCAGCTGTTGCTAAACATGACTTCTTTAAGTTTAGAATAGACGGTGACATGGTACCACATATATCACGTCAACGT
CTTACTAAATACACAATGGCAGACCTCGTCTATGCTTTAAGGCATTTTGATGAAGGTAATTGTGACACATTAAAAG
AAATACTTGTCACATACAATTGTTGTGATGATGATTATTTCAATAAAAAGGACTGGTATGATTTTGTAGAAAACCCA
GATATATTACGCGTATACGCCAACTTAGGTGAACGTGTACGCCAAGCTTTGTTAAAAACAGTACAATTCTGTGATG
CCATGCGAAATGCTGGTATTGTTGGTGTACTGACATTAGATAATCAAGATCTCAATGGTAACTGGTATGATTTCGG
TGATTTCATACAAACCACGCCAGGTAGTGGAGTTCCTGTTGTAGATTCTTATTATTCATTGTTAATGCCTATATTAAC
CTTGACCAGGGCTTTAACTGCAGAGTCACATGTTGACACTGACTTAACAAAGCCTTACATTAAGTGGGATTTGTTA
AAATATGACTTCACGGAAGAGAGGTTAAAACTCTTTGACCGTTATTTTAAATATTGGGATCAGACATACCACCCAA
ATTGTGTTAACTGTTTGGATGACAGATGCATTCTGCATTGTGCAAACTTTAATGTTTTATTCTCTACAGTGTTCCCAC
TTACAAGTTTTGGACCACTAGTGAGAAAAATATTTGTTGATGGTGTTCCATTTGTAGTTTCAACTGGATACCACTTC
AGAGAGCTAGGTGTTGTACATAATCAGGATGTAAACTTACATAGCTCTAGACTTAGTTTTAAGGAATTACTTGTGT

FIG. 34

ATGCTGCTGACCCTGCTATGCACGCTGCTTCTGGTAATCTATTACTAGATAAACGCACTACGTGCTTTTCAGTAGCT
GCACTTACTAACAATGTTGCTTTTCAAACTGTCAAACCCGGTAATTTTAACAAAGACTTCTATGACTTTGTTGTGTCT
AAGGGTTTCTTTAAGGAAGGAAGTTCTGTTGAATTAAAACACTTCTTCTTTGCTCAGGATGGTAATGCTGCTATCAG
CGATTATGACTACTATCGTTATAATCTACCAACAATGTGTGATATCAGACAACTACTATTTGTAGTTGAAGTTGTTG
ATAAGTACTTTGATTGTTACGATGGTGGCTGTATTAATGCTAACCAAGTCATCGTCAACAACCTAGACAAATCAGCT
GGTTTTCCATTTAATAAATGGGGTAAGGCTAGACTTTATTATGATTCAATGAGTTATGAGGATCAAGATGCACTTTT
CGCATATACAAAACGTAATGTCATCCCTACTATAACTCAAATGAATCTTAAGTATGCCATTAGTGCAAAGAATAGA
GCTCGCACCGTAGCTGGTGTCTCTATCTGTAGTACTATGACCAATAGACAGTTTCATCAAAAATTATTGAAATCAAT
AGCCGCCACTAGAGGAGCTACTGTAGTAATTGGAACAAGCAAATTCTATGGTGGTTGGCACAACATGTTAAAAAC
TGTTTATAGTGATGTAGAAAACCCTCACCTTATGGGTTGGGATTATCCTAAATGTGATAGAGCCATGCCTAACATG
CTTAGAATTATGGCCTCACTTGTTCTTGCTCGCAAACATACAACGTGTTGTAGCTTGTCACACCGTTTCTATAGATTA
GCTAATGAGTGTGCTCAAGTATTGAGTGAAATGGTCATGTGTGGCGGTTCACTATATGTTAAACCAGGTGGAACCT
CATCAGGAGATGCCACAACTGCTTATGCTAATAGTGTTTTTAACATTTGTCAAGCTGTCACGGCCAATGTTAATGCA
CTTTTATCTACTGATGGTAACAAAATTGCCGATAAGTATGTCCGCAATTTACAACACAGACTTTATGAGTGTCTCTA
TAGAAATAGAGATGTTGACACAGACTTTGTGAATGAGTTTTACGCATATTTGCGTAAACATTTCTCAATGATGATAC
TCTCTGACGATGCTGTTGTGTGTTTCAATAGCACTTATGCATCTCAAGGTCTAGTGGCTAGCATAAAGAACTTTAAG
TCAGTTCTTTATTATCAAAACAATGTTTTTATGTCTGAAGCAAAATGTTGGACTGAGACTGACCTTACTAAAGGACC
TCATGAATTTTGCTCTCAACATACAATGCTAGTTAAACAGGGTGATGATTATGTGTACCTTCCTTACCCAGATCCAT
CAAGAATCCTAGGGGCCGGCTGTTTTGTAGATGATATCGTAAAAACAGATGGTACACTTATGATTGAACGGTTCGT
GTCTTTAGCTATAGATGCTTACCCACTTACTAAACATCCTAATCAGGAGTATGCTGATGTCTTTCATTTGTACTTACA
ATACATAAGAAAGCTACATGATGAGTTAACAGGACACATGTTAGACATGTATTCTGTTATGCTTACTAATGATAAC
ACTTCAAGGTATTGGGAACCTGAGTTTTATGAGGCTATGTACACACCGCATACAGTCTTACAGGCTGTTGGGGCTT
GTGTTCTTTGCAATTCACAGACTTCATTAAGATGTGGTGCTTGCATACGTAGACCATTCTTATGTTGTAAATGCTGT
TACGACCATGTCATATCAACATCACATAAATTAGTCTTGTCTGTTAATCCGTATGTTTGCAATGCTCCAGGTTGTGAT
GTCACAGATGTGACTCAACTTTACTTAGGAGGTATGAGCTATTATTGTAAATCACATAAACCACCCATTAGTTTTCC
ATTGTGTGCTAATGGACAAGTTTTTGGTTTATATAAAAATACATGTGTTGGTAGCGATAATGTTACTGACTTTAATG
CAATTGCAACATGTGACTGGACAAATGCTGGTGATTACATTTTAGCTAACACCTGTACTGAAAGACTCAAGCTTTTT
GCAGCAGAAACGCTCAAAGCTACTGAGGAGACATTTAAACTGTCTTATGGTATTGCTACTGTACGTGAAGTGCTGT
CTGACAGAGAATTACATCTTTCATGGGAAGTTGGTAAACCTAGACCACCACTTAACCGAAATTATGTCTTTACTGGT
TATCGTGTAACTAAAAACAGTAAAGTACAAATAGGAGAGTACACCTTTGAAAAAGGTGACTATGGTGATGCTGTT
GTTTACCGAGGTACAACAACTTACAAATTAAATGTTGGTGATTATTTTGTGCTGACATCACATACAGTAATGCCATT
AAGTGCACCTACACTAGTGCCACAAGAGCACTATGTTAGAATTACTGGCTTATACCCAACACTCAATATCTCAGATG
AGTTTTCTAGCAATGTTGCAAATTATCAAAAGGTTGGTATGCAAAAGTATTCTACACTCCAGGGACCACCTGGTAC
TGGTAAGAGTCATTTTGCTATTGGCCTAGCTCTCTACTACCCTTCTGCTCGCATAGTGTATACAGCTTGCTCTCATGC
CGCTGTTGATGCACTATGTGAGAAGGCATTAAAATATTTGCCTATAGATAAATGTAGTAGAATTATACCTGCACGT
GCTCGTGTAGAGTGTTTTGATAAATTCAAAGTGAATTCAACATTAGAACAGTATGTCTTTTGTACTGTAAATGCATT
GCCTGAGACGACAGCAGATATAGTTGTCTTTGATGAAATTTCAATGGCCACAAATTATGATTTGAGTGTTGTCAAT
GCCAGATTACGTGCTAAGCACTATGTGTACATTGGCGACCCTGCTCAATTACCTGCACCACGCACATTGCTAACTAA
GGGCACACTAGAACCAGAATATTTCAATTCAGTGTGTAGACTTATGAAAACTATAGGTCCAGACATGTTCCTCGGA
ACTTGTCGGCGTTGTCCTGCTGAAATTGTTGACACTGTGAGTGCTTTGGTTTATGATAATAAGCTTAAAGCACATAA
AGACAAATCAGCTCAATGCTTTAAAATGTTTTATAAGGGTGTTATCACGCATGATGTTTCATCTGCAATTAACAGGC
CACAAATAGGCGTGGTAAGAGAATTCCTTACACGTAACCCTGCTTGGAGAAAAGCTGTCTTTATTTCACCTTATAAT
TCACAGAATGCTGTAGCCTCAAAGATTTTGGGACTACCAACTCAAACTGTTGATTCATCACAGGGCTCAGAATATG
ACTATGTCATATTCACTCAAACCACTGAAACAGCTCACTCTTGTAATGTAAACAGATTTAATGTTGCTATTACCAGA
GCAAAAGTAGGCATACTTTGCATAATGTCTGATAGAGACCTTTATGACAAGTTGCAATTTACAAGTCTTGAAATTCC
ACGTAGGAATGTGGCAACTTTACAAGCTGAAAATGTAACAGGACTCTTTAAAGATTGTAGTAAGGTAATCACTGG
GTTACATCCTACACAGGCACCTACACACCTCAGTGTTGACACTAAATTCAAAACTGAAGGTTTATGTGTTGACATAC
CTGGCATACCTAAGGACATGACCTATAGAAGACTCATCTCTATGATGGGTTTTAAAATGAATTATCAAGTTAATGG

FIG. 34

TTACCCTAACATGTTTATCACCCGCGAAGAAGCTATAAGACATGTACGTGCATGGATTGGCTTCGATGTCGAGGGG
TGTCATGCTACTAGAGAAGCTGTTGGTACCAATTTACCTTTACAGCTAGGTTTTTCTACAGGTGTTAACCTAGTTGC
TGTACCTACAGGTTATGTTGATACACCTAATAATACAGATTTTTCCAGAGTTAGTGCTAAACCACCGCCTGGAGATC
AATTTAAACACCTCATACCACTTATGTACAAAGGACTTCCTTGGAATGTAGTGCGTATAAAGATTGTACAAATGTTA
AGTGACACACTTAAAAATCTCTCTGACAGAGTCGTATTTGTCTTATGGGCACATGGCTTTGAGTTGACATCTATGAA
GTATTTTGTGAAAATAGGACCTGAGCGCACCTGTTGTCTATGTGATAGACGTGCCACATGCTTTTCCACTGCTTCAG
ACACTTATGCCTGTTGGCATCATTCTATTGGATTTGATTACGTCTATAATCCGTTTATGATTGATGTTCAACAATGGG
GTTTTACAGGTAACCTACAAAGCAACCATGATCTGTATTGTCAAGTCCATGGTAATGCACATGTAGCTAGTTGTGA
TGCAATCATGACTAGGTGTCTAGCTGTCCACGAGTGCTTTGTTAAGCGTGTTGACTGGACTATTGAATATCCTATAA
TTGGTGATGAACTGAAGATTAATGCGGCTTGTAGAAAGGTTCAACACATGGTTGTTAAAGCTGCATTATTAGCAGA
CAAATTCCCAGTTCTTCACGACATTGGTAACCCTAAAGCTATTAAGTGTGTACCTCAAGCTGATGTAGAATGGAAG
TTCTATGATGCACAGCCTTGTAGTGACAAAGCTTATAAAATAGAAGAATTATTCTATTCTTATGCCACACATTCTGA
CAAATTCACAGATGGTGTATGCCTATTTTGGAATTGCAATGTCGATAGATATCCTGCTAATTCCATTGTTTGTAGAT
TTGACACTAGAGTGCTATCTAACCTTAACTTGCCTGGTTGTGATGGTGGCAGTTTGTATGTAAATAAACATGCATTC
CACACACCAGCTTTTGATAAAAGTGCTTTTGTTAATTTAAAACAATTACCATTTTTCTATTACTCTGACAGTCCATGT
GAGTCTCATGGAAAACAAGTAGTGTCAGATATAGATTATGTACCACTAAAGTCTGCTACGTGTATAACACGTTGCA
ATTTAGGTGGTGCTGTCTGTAGACATCATGCTAATGAGTACAGATTGTATCTCGATGCTTATAACATGATGATCTCA
GCTGGCTTTAGCTTGTGGGTTTACAAACAATTTGATACTTATAACCTCTGGAACACTTTTACAAGACTTCAGAGTTT
AGAAAATGTGGCTTTTAATGTTGTAAATAAGGGACACTTTGATGGACAACAGGGTGAAGTACCAGTTTCTATCATT
AATAACACTGTTTACACAAAAGTTGATGGTGTTGATGTAGAATTGTTTGAAAATAAAACAACATTACCTGTTAATGT
AGCATTTGAGCTTTGGGCTAAGCGCAACATTAAACCAGTACCAGAGGTGAAAATACTCAATAATTTGGGTGTGGA
CATTGCTGCTAATACTGTGATCTGGGACTACAAAAGAGATGCTCCAGCACATATATCTACTATTGGTGTTTGTTCTA
TGACTGACATAGCCAAGAAACCAACTGAAACGATTTGTGCACCACTCACTGTCTTTTTTGATGGTAGAGTTGATGG
TCAAGTAGACTTATTTAGAAATGCCCGTAATGGTGTTCTTATTACAGAAGGTAGTGTTAAAGGTTTACAACCATCTG
TAGGTCCCAAACAAGCTAGTCTTAATGGAGTCACATTAATTGGAGAAGCCGTAAAAACACAGTTCAATTATTATAA
GAAAGTTGATGGTGTTGTCCAACAATTACCTGAAACTTACTTTACTCAGAGTAGAAATTTACAAGAATTTAAACCCA
GGAGTCAAATGGAAATTGATTTCTTAGAATTAGCTATGGATGAATTCATTGAACGGTATAAATTAGAAGGCTATGC
CTTCGAACATATCGTTTATGGAGATTTTAGTCATAGTCAGTTAGGTGGTTTACATCTACTGATTGGACTAGCTAAAC
GTTTTAAGGAATCACCTTTTGAATTAGAAGATTTTATTCCTATGGACAGTACAGTTAAAAACTATTTCATAACAGAT
GCGCAAACAGGTTCATCTAAGTGTGTGTGTTCTGTTATTGATTTATTACTTGATGATTTTGTTGAAATAATAAAATC
CCAAGATTTATCTGTAGTTTCTAAGGTTGTCAAAGTGACTATTGACTATACAGAAATTTCATTTATGCTTTGGTGTA
AAGATGGCCATGTAGAAACATTTTACCCAAAATTACAATCTAGTCAAGCGTGGCAACCGGGTGTTGCTATGCCTAA
TCTTTACAAAATGCAAAGAATGCTATTAGAAAAGTGTGACCTTCAAAATTATGGTGATAGTGCAACATTACCTAAA
GGCATAATGATGAATGTCGCAAAATATACTCAACTGTGTCAATATTTAAACACATTAACATTAGCTGTACCCTATAA
TATGAGAGTTATACATTTTGGTGCTGGTTCTGATAAAGGAGTTGCACCAGGTACAGCTGTTTTAAGACAGTGGTTG
CCTACGGGTACGCTGCTTGTCGATTCAGATCTTAATGACTTTGTCTCTGATGCAGATTCAACTTTGATTGGTGATTG
TGCAACTGTACATACAGCTAATAAATGGGATCTCATTATTAGTGATATGTACGACCCTAAGACTAAAAATGTTACA
AAAGAAAATGACTCTAAAGAGGGTTTTTTCACTTACATTTGTGGGTTTATACAACAAAAGCTAGCTCTTGGAGGTT
CCGTGGCTATAAAGATAACAGAACATTCTTGGAATGCTGATCTTTATAAGCTCATGGGACACTTCGCATGGTGGAC
AGCCTTTGTTACTAATGTGAATGCCTCATCATCTGAAGCATTTTTAATTGGATGTAATTATCTTGGCAAACCACGCG
AACAAATAGATGGTTATGTCATGCATGCAAATTACATATTTTGGAGGAATACAAATCCAATTCAGTTGTCTTCCTAT
TCTTTATTTGACATGAGTAAATTTCCCCTTAAATTAAGGGGTACTGCTGTTATGTCTTTAAAAGAAGGTCAAATCAA
TGATATGATTTTATCTCTTCTTAGTAAAGGTAGACTTATAATTAGAGAAAACAACAGAGTTGTTATTTCTAGTGATG
TTCTTGTTAACAACTAAACGAACAATGTTTGTTTTTCTTGTTTTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTA
CAACCAGAACTCAATTACCCCCTGCATACACTAATTCTTTCACACGTGGTGTTTATTACCCTGACAAAGTTTTCAGAT
CCTCAGTTTTACATTCAACTCAGGACTTGTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTC
TGGGACCAATGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTCCACTGAGA
AGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCCAGTCCCTACTTATTGTTAATAAC

FIG. 34

GCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAAC
AACAAAAGTTGGATGGAAAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGC
CTTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTTCAAAAATCTTAGGGAATTTGTGTTTAAGAATATTGATGG
TTATTTTAAAATATATTCTAAGCACACGCCTATTAATTTAGTGCGTGATCTCCCTCAGGGTTTTTCGGCTTTAGAACC
ATTGGTAGATTTGCCAATAGGTATTAACATCACTAGGTTTCAAACTTTACTTGCTTTACATAGAAGTTATTTGACTCC
TGGTGATTCTTCTTCAGGTTGGACAGCTGGTGCTGCAGCTTATTATGTGGGTTATCTTCAACCTAGGACTTTTCTAT
TAAAATATAATGAAAATGGAACCATTACAGATGCTGTAGACTGTGCACTTGACCCTCTCTCAGAAACAAAGTGTAC
GTTGAAATCCTTCACTGTAGAAAAAGGAATCTATCAAACTTCTAACTTTAGAGTCCAACCAACAGAATCTATTGTTA
GATTTCCTAATATTACAAACTTGTGCCCTTTTGGTGAAGTTTTTAACGCCACCAGATTTGCATCTGTTTATGCTTGGA
ACAGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGTCCTATATAATTCCGCATCATTTTCCACTTTTAAGTGTT
ATGGAGTGTCTCCTACTAAATTAAATGATCTCTGCTTTACTAATGTCTATGCAGATTCATTTGTAATTAGAGGTGAT
GAAGTCAGACAAATCGCTCCAGGGCAAACTGGAAAGATTGCTGATTATAATTATAAATTACCAGATGATTTTACAG
GCTGCGTTATAGCTTGGAATTCTAACAATCTTGATTCTAAGGTTGGTGGTAATTATAATTACCTGTATAGATTGTTT
AGGAAGTCTAATCTCAAACCTTTTGAGAGAGATATTTCAACTGAAATCTATCAGGCCGGTAGCACACCTTGTAATG
GTGTTGAAGGTTTTAATTGTTACTTTCCTTTACAATCATATGGTTTCCAACCCACTAATGGTGTTGGTTACCAACCAT
ACAGAGTAGTAGTACTTTCTTTTGAACTTCTACATGCACCAGCAACTGTTTGTGGACCTAAAAAGTCTACTAATTTG
GTTAAAAACAAATGTGTCAATTTCAACTTCAATGGTTTAACAGGCACAGGTGTTCTTACTGAGTCTAACAAAAAGTT
TCTGCCTTTCCAACAATTTGGCAGAGACATTGCTGACACTACTGATGCTGTCCGTGATCCACAGACACTTGAGATTC
TTGACATTACACCATGTTCTTTTGGTGGTGTCAGTGTTATAACACCAGGAACAAATACTTCTAACCAGGTTGCTGTT
CTTTATCAGGGTGTTAACTGCACAGAAGTCCCTGTTGCTATTCATGCAGATCAACTTACTCCTACTTGGCGTGTTTAT
TCTACAGGTTCTAATGTTTTTCAAACACGTGCAGGCTGTTTAATAGGGGCTGAACATGTCAACAACTCATATGAGT
GTGACATACCCATTGGTGCAGGTATATGCGCTAGTTATCAGACTCAGACTAATTCTCCTCGGCGGGCACGTAGTGT
AGCTAGTCAATCCATCATTGCCTACACTATGTCACTTGGTGCAGAAAATTCAGTTGCTTACTCTAATAACTCTATTGC
CATACCCACAAATTTTACTATTAGTGTTACCACAGAAATTCTACCAGTGTCTATGACCAAGACATCAGTAGATTGTA
CAATGTACATTTGTGGTGATTCAACTGAATGCAGCAATCTTTTGTTGCAATATGGCAGTTTTTGTACACAATTAAAC
CGTGCTTTAACTGGAATAGCTGTTGAACAAGACAAAAACACCCAAGAAGTTTTTGCACAAGTCAAACAAATTTACA
AAACACCACCAATTAAAGATTTTGGTGGTTTTAATTTTTCACAAATATTACCAGATCCATCAAAACCAAGCAAGAGG
TCATTTATTGAAGATCTACTTTTCAACAAAGTGACACTTGCAGATGCTGGCTTCATCAAACAATATGGTGATTGCCT
TGGTGATATTGCTGCTAGAGACCTCATTTGTGCACAAAAGTTTAACGGCCTTACTGTTTTGCCACCTTTGCTCACAG
ATGAAATGATTGCTCAATACACTTCTGCACTGTTAGCGGGTACAATCACTTCTGGTTGGACCTTTGGTGCAGGTGCT
GCATTACAAATACCATTTGCTATGCAAATGGCTTATAGGTTTAATGGTATTGGAGTTACACAGAATGTTCTCTATGA
GAACCAAAAATTGATTGCCAACCAATTTAATAGTGCTATTGGCAAAATTCAAGACTCACTTTCTTCCACAGCAAGTG
CACTTGGAAAACTTCAAGATGTGGTCAACCAAAATGCACAAGCTTTAAACACGCTTGTTAAACAACTTAGCTCCAA
TTTTGGTGCAATTTCAAGTGTTTTAAATGATATCCTTTCACGTCTTGACAAAGTTGAGGCTGAAGTGCAAATTGATA
GGTTGATCACAGGCAGACTTCAAAGTTTGCAGACATATGTGACTCAACAATTAATTAGAGCTGCAGAAATCAGAG
CTTCTGCTAATCTTGCTGCTACTAAAATGTCAGAGTGTGTACTTGGACAATCAAAAAGAGTTGATTTTTGTGGAAA
GGGCTATCATCTTATGTCCTTCCCTCAGTCAGCACCTCATGGTGTAGTCTTCTTGCATGTGACTTATGTCCCTGCACA
AGAAAAGAACTTCACAACTGCTCCTGCCATTTGTCATGATGGAAAAGCACACTTTCCTCGTGAAGGTGTCTTTGTTT
CAAATGGCACACACTGGTTTGTAACACAAAGGAATTTTTATGAACCACAAATCATTACTACAGACAACACATTTGT
GTCTGGTAACTGTGATGTTGTAATAGGAATTGTCAACAACACAGTTTATGATCCTTTGCAACCTGAATTAGACTCAT
TCAAGGAGGAGTTAGATAAATATTTTAAGAATCATACATCACCAGATGTTGATTTAGGTGACATCTCTGGCATTAA
TGCTTCAGTTGTAAACATTCAAAAAGAAATTGACCGCCTCAATGAGGTTGCCAAGAATTTAAATGAATCTCTCATCG
ATCTCCAAGAACTTGGAAAGTATGAGCAGTATATAAAATGGCCATGGTACATTTGGCTAGGTTTTATAGCTGGCTT
GATTGCCATAGTAATGGTGACAATTATGCTTTGCTGTATGACCAGTTGCTGTAGTTGTCTCAAGGGCTGTTGTTCTT
GTGGATCCTGCTGCAAATTTGATGAAGACGACTCTGAGCCAGTGCTCAAAGGAGTCAAATTACATTACACATAAAC
GAACTTATGGATTTGTTTATGAGAATCTTCACAATTGGAACTGTAACTTTGAAGCAAGGTGAAATCAAGGATGCTA
CTCCTTCAGATTTTGTTCGCGCTACTGCAACGATACCGATACAAGCCTCACTCCCTTTCGGATGGCTTATTGTTGGC
GTTGCACTTCTTGCTGTTTTTCAGAGCGCTTCCAAAATCATAACCCTCAAAAAGAGATGGCAACTAGCACTCTCCAA

FIG. 34

GGGTGTTCACTTTGTTTGCAACTTGCTGTTGTTGTTTGTAACAGTTTACTCACACCTTTTGCTCGTTGCTGCTGGCCT
TGAAGCCCCTTTTCTCTATCTTTATGCTTTAGTCTACTTCTTGCAGAGTATAAACTTTGTAAGAATAATAATGAGGCT
TTGGCTTTGCTGGAAATGCCGTTCCAAAAACCCATTACTTTATGATGCCAACTATTTTCTTTGCTGGCATACTAATTG
TTACGACTATTGTATACCTTACAATAGTGTAACTTCTTCAATTGTCATTACTTCAGGTGATGGCACAACAAGTCCTAT
TTCTGAACATGACTACCAGATTGGTGGTTATACTGAAAAATGGGAATCTGGAGTAAAAGACTGTGTTGTATTACAC
AGTTACTTCACTTCAGACTATTACCAGCTGTACTCAACTCAATTGAGTACAGACACTGGTGTTGAACATGTTACCTT
CTTCATCTACAATAAAATTGTTGATGAGCCTGAAGAACATGTCCAAATTCACACAATCGACGGTTCATCCGGAGTT
GTTAATCCAGTAATGGAACCAATTTATGATGAACCGACGACGACTACTAGCGTGCCTTTGTAAGCACAAGCTGATG
AGTACGAACTTATGTACTCATTCGTTTCGGAAGAGACAGGTACGTTAATAGTTAATAGCGTACTTCTTTTTCTTGCT
TTCGTGGTATTCTTGCTAGTTACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATATTGTTAAC
GTGAGTCTTGTAAAACCTTCTTTTTACGTTTACTCTCGTGTTAAAAATCTGAATTCTTCTAGAGTTCCTGATCTTCTG
GTCTAAACGAACTAAATATTATATTAGTTTTTCTGTTTGGAACTTTAATTTTAGCCATGGCAGATTCCAACGGTACTA
TTACCGTTGAAGAGCTTAAAAAGCTCCTTGAACAATGGAACCTAGTAATAGGTTTCCTATTCCTTACATGGATTTGT
CTTCTACAATTTGCCTATGCCAACAGGAATAGGTTTTTGTATATAATTAAGTTAATTTTCCTCTGGCTGTTATGGCCA
GTAACTTTAGCTTGTTTTGTGCTTGCTGCTGTTTACAGAATAAATTGGATCACCGGTGGAATTGCTATCGCAATGGC
TTGTCTTGTAGGCTTGATGTGGCTCAGCTACTTCATTGCTTCTTTCAGACTGTTTGCGCGTACGCGTTCCATGTGGTC
ATTCAATCCAGAAACTAACATTCTTCTCAACGTGCCACTCCATGGCACTATTCTGACCAGACCGCTTCTAGAAAGTG
AACTCGTAATCGGAGCTGTGATCCTTCGTGGACATCTTCGTATTGCTGGACACCATCTAGGACGCTGTGACATCAA
GGACCTGCCTAAAGAAATCACTGTTGCTACATCACGAACGCTTTCTTATTACAAATTGGGAGCTTCGCAGCGTGTA
GCAGGTGACTCAGGTTTTGCTGCATACAGTCGCTACAGGATTGGCAACTATAAATTAAACACAGACCATTCCAGTA
GCAGTGACAATATTGCTTTGCTTGTACAGTAAGTGACAACAGATGTTTCATCTCGTTGACTTTCAGGTTACTATAGC
AGAGATATTACTAATTATTATGAGGACTTTTAAAGTTTCCATTTGGAATCTTGATTACATCATAAACCTCATAATTAA
AAATTTATCTAAGTCACTAACTGAGAATAAATATTCTCAATTAGATGAAGAGCAACCAATGGAGATTGATTAAACG
AACATGAAAATTATTCTTTTCTTGGCACTGATAACACTCGCTACTTGTGAGCTTTATCACTACCAAGAGTGTGTTAG
AGGTACAACAGTACTTTTAAAAGAACCTTGCTCTTCTGGAACATACGAGGGCAATTCACCATTTCATCCTCTAGCTG
ATAACAAATTTGCACTGACTTGCTTTAGCACTCAATTTGCTTTTGCTTGTCCTGACGGCGTAAAACACGTCTATCAGT
TACGTGCCAGATCAGTTTCACCTAAACTGTTCATCAGACAAGAGGAAGTTCAAGAACTTTACTCTCCAATTTTTCTT
ATTGTTGCGGCAATAGTGTTTATAACACTTTGCTTCACACTCAAAAGAAAGACAGAATGATTGAACTTTCATTAATT
GACTTCTATTTGTGCTTTTTAGCCTTTCTGCTATTCCTTGTTTTAATTATGCTTATTATCTTTTGGTTCTCACTTGAACT
GCAAGATCATAATGAAACTTGTCACGCCTAAACGAACATGAAATTTCTTGTTTTCTTAGGAATCATCACAACTGTAG
CTGCATTTCACCAAGAATGTAGTTTACAGTCATGTACTCAACATCAACCATATGTAGTTGATGACCCGTGTCCTATT
CACTTCTATTCTAAATGGTATATTAGAGTAGGAGCTAGAAAATCAGCACCTTTAATTGAATTGTGCGTGGATGAGG
CTGGTTCTAAATCACCCATTCAGTACATCGATATCGGTAATTATACAGTTTCCTGTTTACCTTTTACAATTAATTGCC
AGGAACCTAAATTGGGTAGTCTTGTAGTGCGTTGTTCGTTCTATGAAGACTTTTTAGAGTATCATGACGTTCGTGTT
GTTTTAGATTTCATCTAAACGAACAAACTAAAATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCAT
TACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGAGAACGCAGTGGGGCGCGATCAAAACAAC
GTCGGCCCCAAGGTTTACCCAATAATACTGCGTCTTGGTTCACCGCTCTCACTCAACATGGCAAGGAAGACCTTAA
ATTCCCTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATGACCAAATTGGCTACTACCGAAGAGCT
ACCAGACGAATTCGTGGTGGTGACGGTAAAATGAAAGATCTCAGTCCAAGATGGTATTTCTACTACCTAGGAACT
GGGCCAGAAGCTGGACTTCCCTATGGTGCTAACAAAGACGGCATCATATGGGTTGCAACTGAGGGAGCCTTGAAT
ACACCAAAAGATCACATTGGCACCCGCAATCTTGCTAACAATGCTGCAATCGTGCTACAACTTCCTCAAGGAACAA
CATTGCCAAAAGGCTTCTACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCATCACGTAGTC
GCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAAACGAACTTCTCCTGCTAGAATGGCTGGCAATGGCGGTG
ATGCTGCTCTTGCTTTGCTGCTGCTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTGGTAAAGGCCAACAACA
ACAAGGCCAAACTGTCACTAAGAAATCTGCTGCTGAGGCTTCTAAGAAGCCTCGGCAAAAACGTACTGCCACTAA
AGCATACAATGTAACACAAGCTTTCGGCAGACGTGGTCCAGAACAAACCCAAGGAAATTTTGGGGACCAGGAACT
AATCAGACAAGGAACTGATTACAAACATTGGCCGCAAATTGCACAATTTGCCCCCAGCGCTTCAGCGTTCTTCGGA
ATGTCGCGCATTGGCATGGAAGTCACACCTTCGGGAACGTGGTTGACCTACACAGGTGCCATCAAATTGGATGAC

FIG. 34

AAAGATCCAAATTTCAAAGATCAAGTCATTTTGCTGAATAAGCATATTGACGCATACAAAACATTCCCACCAACAG
AGCCTAAAAAAGGACAAAAAGAAGAAGGCTGATGAAACTCAAGCCTTACCGCAGAGACAGAAGAAACAGCAAACT
GTGACTCTTCTTCCTGCTGCAGATTTGGATGATTTCTCCAAACAATTGCAACAATCCATGAGCAGTGCTGACTCAAC
TCAGGCCTAAACTCATGCAGACCACACAAGGCAGATGGGCTATATAAACGTTTTCGCTTTTCCGTTTACGATATATA
GTCTACTCTTGTGCAGAATGAATTCTCGTAACTACATAGCACAAGTAGATGTAGTTAACTTTAATCTCACATAGCAA
TCTTTAATCAGTGTTTAACATTAGGGAGGACTTGAAAGAGCCACCACATTTTCACCGAGGCCACGCGGAGTACGAT
CGAGTGTACAGTGAACAATGCTAGGGAGAGCTGCCTATATGGAAGAGCCCTAATGTGTAAAATTAATTTTAGTAG
TGCTATCCCCATGTGATTTTAATAGCTTCTTAGGAGAATGACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

FIG. 35

SEQ ID NO:2

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   1 attaaaggtt tataccttcc caggtaacaa accaaccaac tttcgatctc ttgtagatct
  61 gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcatgc ttagtgcact
 121 cacgcagtat aattaataac taattactgt cgttgacagg acacgagtaa ctcgtctatc
 181 ttctgcaggc tgcttacggt ttcgtccgtg ttgcagccga tcatcagcac atctaggttt
 241 cgtccgggtg tgaccgaaag gtaagatgga cagccttgtc cctggtttca acgagaaaac
 301 acacgtccaa ctcagtttgc ctgttttaca ggttcgcgac gtgctcgtac gtggctttgg
 361 agactccgtg gaggaggtct tatcagaggc acgtcaacat cttaaagatg gcactttgtgg
 421 cttagtagaa gttgaaaaag gcgttttgcc tcaacttgaa cagccctatg tgttcatcaa
 481 acgttcggat gctcgaactg cacctcatgg tcatgttatg gttgagctgg tagcagaact
 541 cgaaggcatt cagtacggtc gtagtggtga cacacttggt gtccttgtcc ctcatgtggg
 601 cgaaatacca gtggcttacc gcaaggttct tcttcgtaag aacggtaata aaggagctgg
 661 tggccatagt tacgggcgcg atctaaagtc atttgactta ggcgacgagc ttggcactga
 721 tccttatgaa gattttcaag aaaactggaa cactaaacat agcagtggtg ttacccgtga
 781 actcatgcgt gagcttaacg gaggggcata cactcgctat gtcgataaca acttctgtgg
 841 ccctgatggc tacccttttg agtgcattaa agaccttcta gcacgtgctg gtaaagcttc
 901 atgcactttg tccgaacaac tggactttat tgacactaag aggggtgtat actgctgccg
 961 tgaacatgag catgaaattg cttggtacac cgaacgttct gaaaagagct atgaattgca
1021 gacacctttt gaaattaaat tggcaaagaa atttgacacc ttcaatgggg aatgtccaaa
1081 ttttgtattt cccttaaatt ccataatcaa gactattcaa ccaagggttg aaaagaaaaa
1141 gcttgatggc tttatgggta gaattcgatc tgtctatcca gttgcgtcac caaatgaatg
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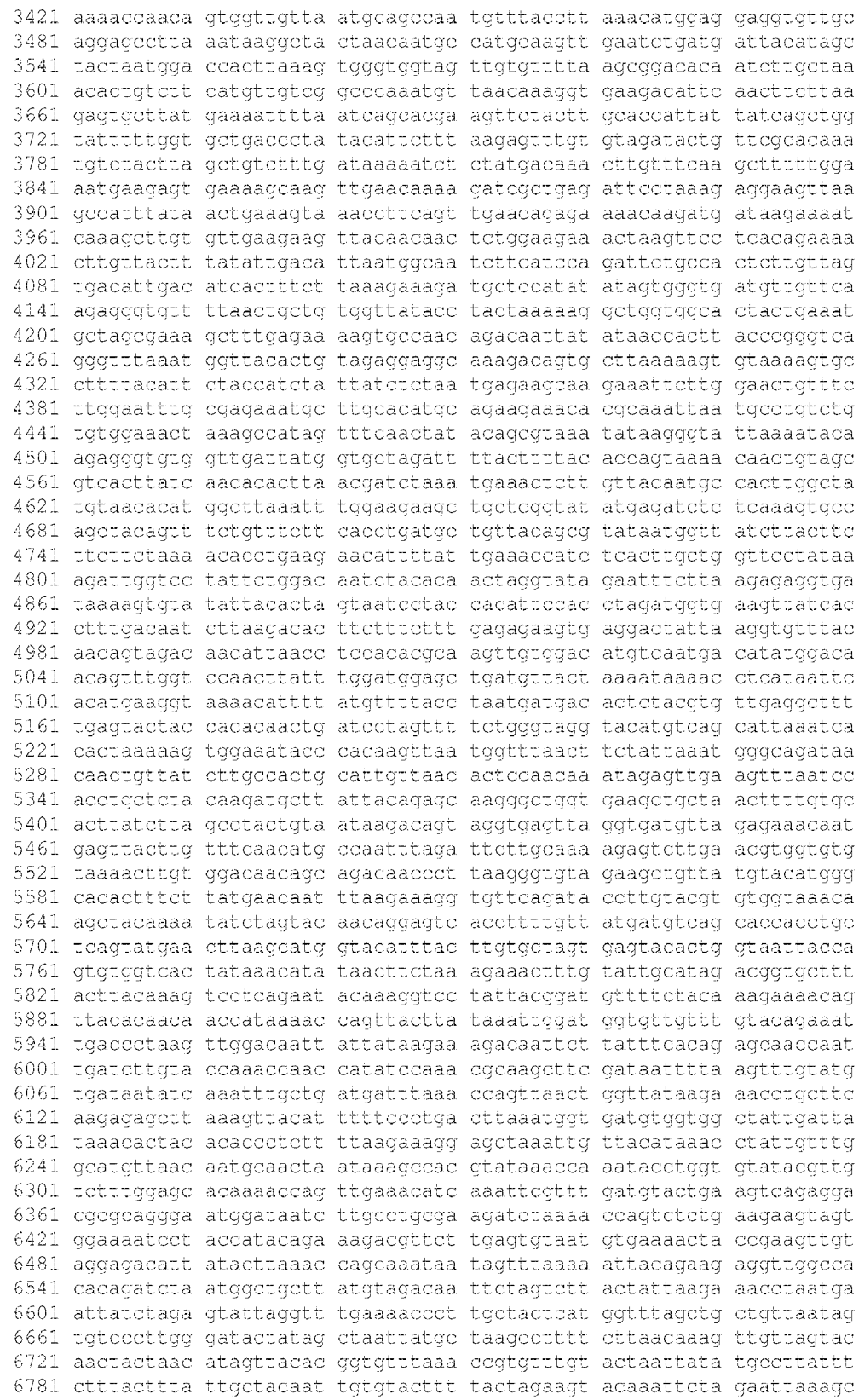
FIG. 35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6781 ctttacttta ttgctacaat tgtgtacttt tactagaagt acaaattcta gaattaaagc

FIG. 35

```
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```

FIG. 35

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FIG. 35

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13681 caacatgaag aaacaattta taatttactt aaggattgtc cagctgttgc taaacatgac
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14881 gattgttacg atggtggctg tattaatgct aaccaagtca tcgtcaacaa cctagacaaa
14941 tcagctggtt ttccatttaa taaatggggt aaggctagac tttattatga ttcaatgagt
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15061 caaatgaatc ttaagtatgc cattagtgca aagaatagag ctcgcaccgt agctggtgtc
15121 tctatctgta gtactatgac caatagacag tttcatcaaa aattattgaa atcaatagcc
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15541 acggccaatg ttaatgcact tttatctact catggtaaca aaattgccga taagtatgtc
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```

FIG. 35

```
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17641 gctcaatgct ttaaaatgtt ttataagggt cttatcacgc atgatgtttc atctgcaatt
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FIG. 35

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FIG. 35

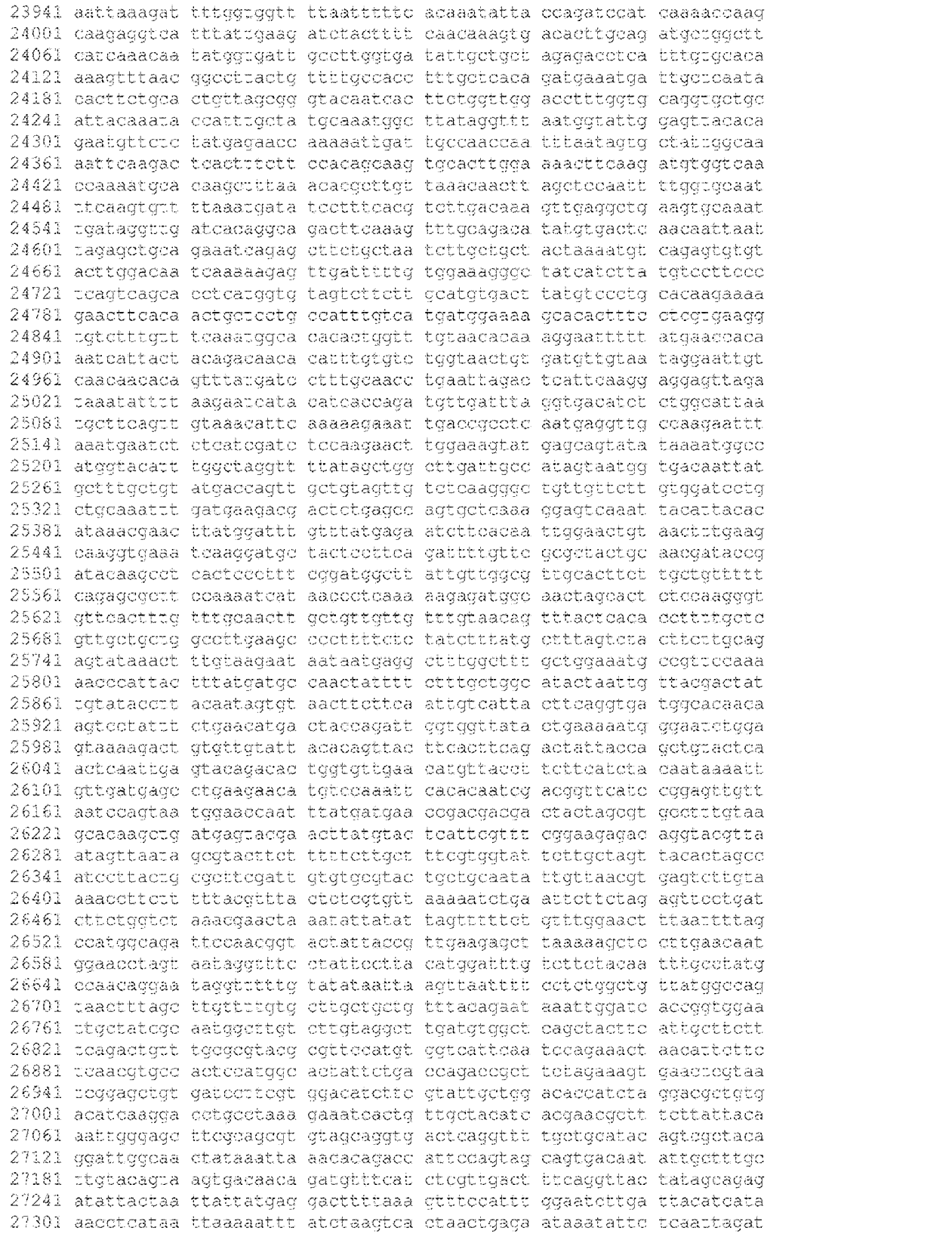
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FIG. 35

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27721 ataacacttt gcttcacact caaaagaaag acagaatgat tgaactttca ttaattgact
27781 tctatttgtg ctttttagcc tttctgctat tccttgtttt aattatgctt attatctttt
27841 ggttctcact tgaactgcaa gatcataatg aaacttgtca cgcctaaacg aacatgaaat
27901 ttcttgtttt cttaggaatc atcacaactg tagctgcatt tcaccaagaa tgtagtttac
27961 agtcatgtac tcaacatcaa ccatatgtag ttgatgaccc gtgtcctatt cacttctatt
28021 ctaaatggta tattagagta ggagctagaa aatcagcacc tttaattgaa ttgtgcgtgg
28081 atgaggctgg ttctaaatca cccattcagt acatcgatat cggtaattat acagtttcct
28141 gtttaccttt tacaattaat tgccaggaac ctaaattggg tagtcttgta gtgcgttgtt
28201 cgttctatga agacttttta gagtatcatg acgttcgtgt tgttttagat ttcatctaaa
28261 cgaacaaact aaaatgtctg ataatggacc ccaaaatcag cgaaatgcac ccgcattac
28321 gtttggtgga ccctcagatt caactggcag taaccagaat ggagaacgca gtgggcgcgg
28381 atcaaaacaa cgtcggcccc aaggtttacc caataatact gcgtcttggt tcaccgctct
28441 cactcaacat ggcaaggaag accttaaatt ccctcgagga caaggcgttc caattaacac
28501 caatagcagt ccagatgacc aaattggcta ctaccgaaga gctaccagac gaattcgtgg
28561 tggtgacggt aaaatgaaag atctcagtcc aagatggtat ttctactacc taggaactgg
28621 gccagaagct ggacttccct atggtgctaa caaagacggc atcatatggg ttgcaactga
28681 gggagccttg aatacaccaa aagatcacat tggcacccgc aatcctgcta acaatgctgc
28741 aatcgtgcta caacttcctc aaggaacaac attgccaaaa ggcttctacg cagaagggag
28801 cagaggcggc agtcaagcct cttctcgttc ctcatcacgt agtcgcaaca gttcaagaaa
28861 ttcaactcca ggcagcagta ggggaacttc tcctgctaga atggctggca atggcggtga
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29101 acgtggtcca gaacaaaccc aaggaaattt tggggaccag gaactaatca gacaaggaac
29161 tgattacaaa cattggccgc aaattgcaca atttgccccc agcgcttcag cgttcttcgg
29221 aatgtcgcgc attggcatgg aagtcacacc ttcgggaacg tggttgacct acacaggtgc
29281 catcaaattg gatgacaaag atccaaattt caaagatcaa gtcattttgc tgaataagca
29341 tattgacgca tacaaaacat tcccaccaac agagcctaaa aaggacaaaa agaagaaggc
29401 tgatgaaact caagccttac cgcagagaca gaagaaacag caaactgtga ctcttcttcc
29461 tgctgcagat ttggatgatt tctccaaaca attgcaacaa tccatgagca gtgctgactc
29521 aactcaggcc taaactcatg cagaccacac aaggcagatg ggctatataa acgttttcgc
29581 ttttccgttt acgatatata gtctactctt gtgcagaatg aattctcgta actacatagc
29641 acaagtagat gtagttaact ttaatctcac atagcaatct ttaatcagtg tgtaacatta
29701 gggaggactt gaaagagcca ccacattttc accgaggcca cgcggagtac gatcgagtgt
29761 acagtgaaca atgctaggga gagctgccta tatggaagag ccctaatgtg taaaattaat
29821 ttagtagtg ctatccccat gtgattttaa tagcttctta ggagaatgac aaaaaaaaaa
29881 aaaaaaaaaa aaaaaaaaaa aaa
```

Fig. 36

SARS CoV-2 genome (Delta variant)(SEQ ID NO:3)

```
1 caaaccaacc aactttcgat ctcttgtaga tctgttctct aaacgaactt taaaatctgt
       61 gtggctgtca ctcggctgca tgcttagtgc actcacgcag tataattaat aactaattac
      121 tgtcgtttgac aggacacgag taactcgtct atctttctgca ggctgcttac ggtttcgtcc
      181 gttttgcagc cgatcatcag cacatctagg ttttgtccgg gtgtgaccga aaggtaagat
      241 ggagagcctt gtcccttggtt tcaacgagaa aacacacgtc caactcagtt tgcctgtttt
      301 acaggttcgc gacgtgctcg tacgtggctt tggagactcc gtggaggagg tcttatcaga
      361 ggcacgtcaa catcttaaag atggcacttg tggcttagta gaagttgaaa aaggcgtttt
      421 gcctcaactt gaacagccct atgtgttcat caaacgttcg gatgctcgaa ctgcacctca
      481 tggtcatgtt atggttgagc tggtagcaga actcgaaggc attcagtacg gtcgtagtgg
      541 tgagacactt ggtgtccttg tcctcatgt cggcgaaata ccagtggctt accgcaaggt
      601 tcttcttcgt aagaacggta ataaaggagc tggtggccat agttacggcg ccgatctaaa
      661 gtcatttgac ttaggcgacg agcttggcac tgatccttat gaagattttc aagaaaactg
      721 gaacactaaa catagcagtg gtgttacccg tgaactcatg cgtgagctta acggaggggc
      781 atacactcgc tatgtcgata acaacttctg tggccctgat ggctaccctc ttgagtgcat
      841 taaagaccct ctagcacgtg ctggtaaagc ttcatgcact ttgtccgaac aactggactt
      901 tattgacact aagaggggtg tatactgctg ccgtgaacat gagcatgaaa ttgcttggta
      961 cacggaacgt tctgaaaaga gctatgaatt gcagacacct tttgaaatta aattggcaaa
     1021 gaaatttgac accttcaatg gggaatgtcc aaattttgta tttcccttaa attccataat
     1081 caagactatt caaccaaggg ttgaaaagaa aaagcttgat ggctttatgg gtagaattcg
     1141 atctgtctat ccagttgcgt caccaaatga atgcaaccaa atgtgccttt caactctcat
     1201 gaagtgtgat cattgtggtg aaacttcatg gcagacgggc gattttgtta aagccacttg
     1261 cgaatttttgt ggcactgaga atttgactaa agaaggtgcc actacttgtg gttacttacc
     1321 ccaaaatgct gttgttaaaa tttattgtcc agcatgtcac aattcagaag taggacctga
     1381 gcatagtcct gccgaatacc ataatgaatc tggcttgaaa accattcttc gtaagggtgg
     1441 tcgcactatt gccttggag gctgtgtgtt ctcttatgtt ggttgccata acaagtgtgc
     1501 ctattgggct ccacgtgcta gcgctaacat aggttgtaac catacaggtg ttgttggaga
     1561 aggttccgaa ggtcttaatg acaaccttct tgaaatactc caaaaagaga aagtcaacat
     1621 caatattgtt ggtgacttta aacttaatga agagatcgcc attattttgg catcttttc
     1681 tgcttccaca agtgctttg tggaaactgt gaaaggtttg gattataaag cattcaaaca
     1741 aattgttgaa tctgtggta attttaaagt tacaaaagga aaagctaaaa aaggtgcctg
     1801 gaatattggt gaacagaaat caatactgag tcctctttat gcatttgcat cagaggctgc
     1861 tcgtgttgta cgatcaattt tctcccgcac tcttgaaact gctcaaaatt ctgtgcgtgt
     1921 tttacagaag gccgctataa caatactaga tggaatttca cagtattcac tgagactcat
     1981 tgatgctatg atgttcacat ctgatttggc tactaacaat ctagttgtaa tggcctacat
     2041 tacaggtggt gttgttcagt tgacttcgca gtggctaact aacatctttg gcactgttta
     2101 tgaaaaactc aaacccgtcc ttgattggct tgaagagaag tttaaggaag gtgtagagtt
     2161 tcttagagac ggttgggaaa ttgttaaatt tatctcaacc tgtgcttgtg aaattgtcgg
     2221 tggacaaatt gtcacctgtg caaaggaaat taaggagagt gttcagacat tctttaagct
     2281 tgtaaataaa ttttttggctt tgtgtgctga ctctatcatt attggtggag ctaaacttaa
     2341 agccttgaat ttaggtgaaa catttgtcac gcactcaaag ggattgtaca gaaagtgtgt
     2401 taaatccaga gaagaaactg gcctactcat cgctctaaaa gccccaaaag aaattatctt
     2461 cttagaggga gaaacacttc ccacagaagt gttaacagag gaagttgtct tgaaaactgg
     2521 tgatttacaa ccattagaac aacctactag tgaagctgtt gaagctccat tggttggtac
     2581 accagtttgt attaacgggc ttatgttgct cgaaatcaaa gacacagaaa agtactgtgc
     2641 ccttgcacct aatatgatgg taacaaacaa taccttcaca ctcaaaggcg gtgcaccaac
     2701 aaaggttact tttggtgatg acactgtgat agaagtgcaa ggttacaaga gtgtgaatat
     2761 cacttttgaa cttgatgaaa ggattgataa agtacttaat gagaagtgct ctgcctatac
     2821 agttgaactc ggtacagaag taaatgagtt cgcctgtgtt gtggcagatg ctgtcataaa
     2881 aactttgcaa ccagtatctg aattacttac accactgggc attgatttag atgagtggag
     2941 tatggctaca tactacttat ttgatgagtc tggtgagttt aaattggctt cacatatgta
     3001 ttgttctttt taccctccag atgaggatga agaagaaggt gattgtgaag aagaagagtt
     3061 tgagccatca actcaatatg agtatggtac tgaagatgat taccaaggta aacctttgga
     3121 atttggtgcc acttctgctg ctcttcaacc tgaagaagag caagaagaag attggttaga
     3181 tgatgatagt caacaaactg ttggtcaaca agacggcagt gaggacaatc agacaactac
     3241 tattcaaaca attgttgagg ttcaacctca attagagatg gaacttacac cagttgttca
```

Fig. 36

```
3301 gactattgaa gtgaatagtt ttagtggtta tttaaaactt actgacaatg tatacattaa
3361 aaatgcagac attgtggaag aagctaaaaa cgtaaaacca acagtggttg ttaatgcagc
3421 caatgtttac cttaaacatg gaggaggtgt tgcaggagcc ttaaataagg ctactaacaa
3481 tgccatgcaa gttgaatctg atgattacat agctactaat ggaccactta aagtgggtgg
3541 tagttgtgtt ttaagcggac acaatcttgc taaacactgt cttcatgttg tcggccccaaa
3601 tgttaacaaa ggtgaagaca ttcaacttct taagagtgct tatgaaaatt ttaatcagca
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3721 tttaagagtt tgtgtagata ctgttcgcac aaatgtctac ttagctgtct ttgataaaaa
3781 tctctatgac aaactgtttt caagcttttt cgaaatgaag agtgaaaagc aagttgaaca
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4141 acctactaaa aagtctggtg gcactactga aatgctagcg aaagctttga gaaaagtgcc
4201 aacagacaat tatataacca cttaccccgg tcagggttta aatggttaca ctgtagagga
4261 ggcaaagaca gtgcttaaaa agtgtaaaag tgccttttac attctaccat ctattatctc
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6601 ccttgctact catggtttag ctgctgttaa tagtgtcccc tgggatacta tagctaatta
6661 tgctaagcct tttcttaaca aagttgttag tacaactact aacatagtta cacggtgttt
```

Fig. 36

```
6721 aaaccgtgtt tgtactaatt atatgcctta tttctttact ttattgctac aattgtgtac
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6841 tactgttaag agtgtcggta aattttgtct agaggcttca tttaattatt tgaagtcacc
6901 taatttttct aaactgataa atattataat ttggttttta ctattaagtg tttgcctagg
6961 ttctttaatc tactcaaccg ctgctttagg tgttttaatg tctaatttag gcatgccttc
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7081 ctgtactggt tctatatctt gtagtgtttg tcttagtggt ttagattctt tagacaccta
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7201 tggcttagtt gcagagtggt ttttggcata tattcttttc actaggtttt tctatgtact
7261 tggattggct gcaatcatgc aattgttttt cagctatttt gcagtacatt ttattagtaa
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7441 agacggttgt aattcatcaa cttgtatgat gtgttacaaa cgtaatagag caacaagagt
7501 cgaatgtaca actattgtta atggtgttag aaggtccttt tatgtctatg ctaatggagg
7561 taaaggcttt tgcaaactac acaattggaa ttgtgttaat tgtgatacat tctgtgctgg
7621 tagtacattt attagtgatg aagttgcgag agacttgtca ctacagttta aaagaccaat
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10081 acaagtaact tgtggtacaa ctacacttaa cggtctttgg cttgatgacg tagtttactg
```

Fig. 36

```
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10261 tggacattct atgcaaaatt gtgtacttaa gcttaaggtt gatacagcca atcctaagac
10321 acctaagtat aagtttgttc gcattcaacc aggacagact ttttcagtgt tagcttgtta
10381 caatggttca ccatcggtg tttaccaatg tgctatgagg cccaatttca ctattaaggg
10441 ttcattcctt aatggttcat gtggtagtgt tggttttaac atagattatg actgtgtctc
10501 tttttgttac atgcaccata tggaattacc aactggagtt catgctggca cagacttaga
10561 aggtaacttt tatggacctt ttgttgacag gcaaacagca caagcagctg gtacggacac
10621 aactattaca gttaatgttt tagcttggtt gtacgctgct gttataaatg gagacaggtg
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10741 ttatgaacct ctaacacaag accatgttga catactagga cctcttctg ctcaaactgg
10801 aattgccgtt ttagatatgt gtgcttcatt aaaagaatta ctgcaaaatg gtatgaatgg
10861 acgtaccata ttgggtagtg ctttattaga agatgaattt acaccttttg atgttgttag
10921 acaatgctca ggtgttactt tccaaagtgc agtgaaaaga acaatcaagg gtacacacca
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11161 accttctctt gccgctgtag cttattttaa tatggtctat atgcctgcta gttgggtgat
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Fig. 36

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Fig. 36

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Fig. 36

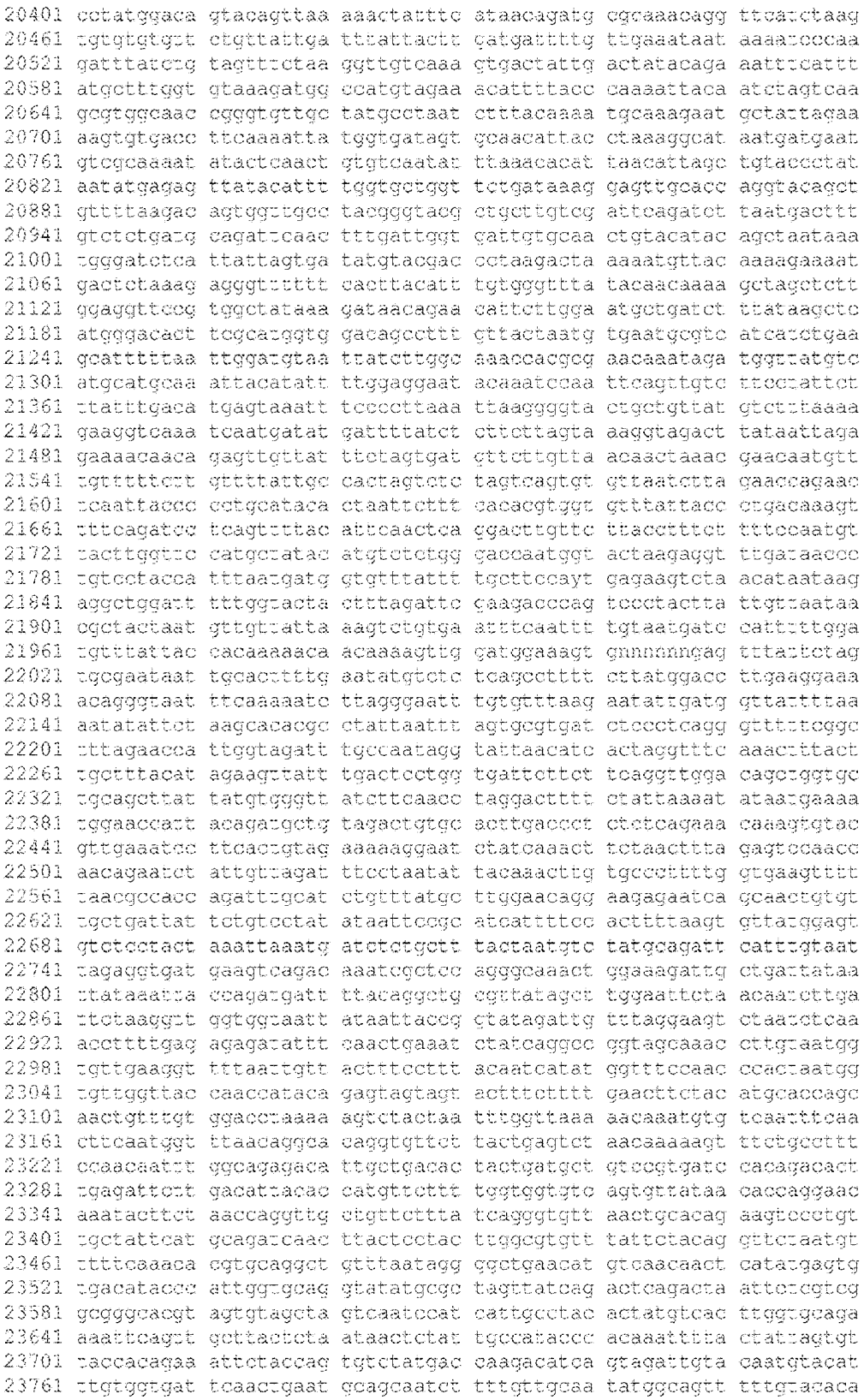
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Fig. 36

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24961 acctgaatta gactcattca aggaggagtt agataaatat tttaagaatc atacatcacc
25021 agatgtttgat ttaggtgaca tctctggcat taatgcttca gttgtaaaca ttcaaaaaga
25081 aattgaccgc ctcaatgagg ttgccaagaa tttaaatgaa tctctcatcg atcttccaaga
25141 acttggaaag tatgagcagt atataaaatg gccatggtac atttggctag gttttatagc
25201 tggcttgatt gccatagtaa tggtgacaat tatgctttgc tgtatgacca gttgctgtag
25261 ttgtctcaag ggctgttgtt cttgtggatc ctgctgcaaa tttgatgaag acgactctga
25321 gccagtgctc aaaggagtca aattacatta cacataaacg aacttatgga tttgtttatg
25381 agaatcttca caattggaac tgtaaccttg aagcaaggtg aaatcaagga tgctactcct
25441 ttagattttg ttcgcgctac tgcaacgata ccgatacaag cctcactccc ttteggatgg
25501 cttattgttg gcgttgcact tcttgctgtt tttcagagcg cttccaaaat cataaccctc
25561 aaaaagagat ggcaactagc acttctccaag cgtgttcact ttgtttgcaa cttgctgttg
25621 ttgtttgtaa cagtttacic acaccttttg ctcgttgctg ctggccttga agccccttit
25681 ctctatcttt atgctttagt ctacttcttg cagagtataa actttgtaag aataataatg
25741 aggctttggc tttgcrggaa atgccgttcc aaaaacccat tactttatga tgccaactat
25801 tttctttgct ggcatactaa ttgttacgac tattgtatac cttacaatag tgtaacttct
25861 tcaattgtca ttacttcagg tgatggcaca acaagtccta tttctgaaca tgactaccag
25921 attggtggrt atactgaaaa atgggaatct ggagtaaaag actgtgttgt attacacagt
25981 tacttcactt cagactatta ccagctgtac tcaactcaat tgagtacaga cactggtgtt
26041 gaacatgtta ccttcttcat ctacaataaa attgttgatg agcctgaaga acatgtccaa
26101 attcacacaa tcgacggttc atccggagtt cttaatccag taatggaacc aatttatgat
26161 gaaccgacga cgactactag cgtgcctttg taagcacaag crgatgagta cgaacttatg
26221 tactcattcg tttcggaaga gacaggtacg ttaatagtta atagcgtact tcttittctt
26281 gctttcgtgg tattcttgct agttacacta ccccatctta ctgcgcttcg attgrgtgcg
26341 tactgctgca atattgttaa cgtgagtctt ctaaaacctt cttttacgt ttactctcgt
26401 gttaaaaatc tgaattcttc tagagttcct gatcttctgg tctaaacgaa ctaaatatta
26461 tattagtttt tctgttggga actttaattt tagccatggc agattccaac ggtactatta
26521 ccgttgaaga gcttaaaaag ctccttgaac aatggaacct agtaaraggt ttcctattcc
26581 ttacatggat ttgtcttcta caatttgcct atgccaacag gaataggttt ttgtatataa
26641 ttaagttaat ttttcrctgg ctgttatggc cagtaacttt agcttgtttt gtgcttgctg
26701 ctgtttacag aataaattgg atcaccggtg gaattgctay cgcaatggct tgtcttgtag
26761 gcttgatgtg gctcagctac ttcattgctt ctttcagact gtttgcgcgt acgcgttcca
26821 tgtggtcatt caatccagaa actaacattc ttctcaacgt gccactccat ggcactattc
26881 tgaccagacc gcttctagaa agtgaactcg taatcggagc tgtgatcctt cgtggacatc
26941 ttcgtattgc tggacaccat ctaggacgct ctgacatcaa ggacctgcct aaagaaatca
27001 ctgttgctac atcacgaacg ctttcttatt acaaattggg agcttcgcag cgtgtagcag
27061 gtgactcagg ttttgctgca tacagtcgct acaggattgg caactataaa ttaaacacag
27121 accattccag tagcagtgac aatattgctt tgcttgtaca gtaagtgaca acagatgttt
27181 catctcgttg actttcaggt tactatagca gagatattac taattattat gaggactttt
```

Fig. 36

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27241 aaagtttcca tttggaatct tgattacatc ataaacctca taattaaaaa tttatctaag
27301 tcactaactg agaataaata ttctcaatta catgaagagc aaccaatgga gattgattaa
27361 acgaacatga aaattattct tttcttggca ctgataacac tcgctacttg tgagctttat
27421 cactaccaag agtgtgttag aggtacaaca gtacttttaa aagaaccttg ctcttctgga
27481 acatacgagg gcaattcacc atttcatcct ctagctgata acaaatttgc actgacttgc
27541 tttagcactc aatttgcttt tgcttgtcct gacggcgtaa aacacgtcta tcagttacgt
27601 gccagatcag cttcacctaa actgttcatc agacaagagg aagttcaaga actttactct
27661 ccaatttttc ttattgttgc ggcaatagtg tttataacac tttgcttcac actcaaaaga
27721 aagacagaat gattgaactt tcattaattg acttctattt gtgcttttta gcctttctgc
27781 tattccttgt tttaattatg cttattatct tttggttctc acttgaactg caagatcata
27841 atgaaatttg tcacgcctaa acgaacatga aatttcttgt tttcttagga atcatcacaa
27901 ctgtagctgc atttcaccaa gaatgtagtt tacagtcatg tactcaacat caaccatatg
27961 tagttgatga cccgtgtcct attcacttct attctaaatg gtatattaga gtaggagcta
28021 gaaaatcagc accttttaatt gaattgtgcg tggatgaggc tggttctaaa tcacccattc
28081 agtacatcga tatcggtaat tatacagttt cctgtttacc ttttacaatt aattgccagg
28141 aacctaaatt gggtagtctt gtagtgcgtt gttcgttcta tgaagacttt ttagagtatc
28201 atgacgttcg tgttgtttta gatttaatct aaacgaacaa actaaaatgt ctgataatgg
28261 accccaaaat cagcgaaatg cacccgcat tacgtttggt ggacctcag attcaactgg
28321 cagtaaccag aatggagaac gcagtggggc gcgatcaaaa caacgtcggc cccaaggttt
28381 acccaataat actgcgtctt ggttcaccgc tctcactcaa catggcaagg aaggccttaa
28441 attcctcga ggacaaggcg ttccaattaa caccaatagc agtccagatg accaaattgg
28501 ctactaccga agagctacca gacgaattcg tggtggtgac ggtaaaatga aagatctcag
28561 tccaagatgg tatttctact acctaggaac tgggccagaa gctggacttc cctatggtgc
28621 taacaaagac ggcatcatat gggttgcaac tgagggagcc ttgaatacac caaaagatca
28681 cattggcacc cgcaatcctg ctaacaatgc tgcaatcgtg ctacaacttc ctcaaggaac
28741 aacattgcca aaaggcttct acgcagaagg gagcagaggc ggcagtcaag cctcttctcg
28801 ttcctcatca cgtagtcgca acagttcaag aaattcaact ccaggcagca gtatgggaac
28861 ttctcctgct agaatggctg gcaatggctg tgatgctgct cttgctttgc tgctgcttga
28921 cagattgaac cagcttgaga gcaaaatgtc tggtaaaggc caacaacaac aaggccaaac
28981 tgtcactaag aaatcatgctg ctgaggcttc taagaagcct cggcaaaaac gtactgccac
29041 taaagcatac aatgtaacac aagctttcgg cagacgtggt ccagaacaaa cccaaggaaa
29101 ttttggggac caggaactaa tcagacaagg aactgattac aaacattggc cgcaaattgc
29161 acaatttgcc cccagcgctt cagcgttctt cggaatgtcg cgcattggca tggaagtcac
29221 accttcggga acgtggttga cctacacagg tgccatcaaa ttggatgaca aagatccaaa
29281 tttcaaagat caagtcattt tgctgaataa gcatattgac gcatacaaaa cattcccacc
29341 aacagagcct aaaaaggaca aaaagaagaa ggcttatgaa actcaagcct taccgcagag
29401 acagaagaaa cagcaaactg tgactcttct tcctgctgca gatttggatg atttctccaa
29461 acaattgcaa caatccatga gcagtgctga ctcaactcag gcctaaactc atgcagacca
29521 cacaaggcag atgggctata taaacgtttt cgcttttccg tttacgatat atagtctact
29581 cttgtgcaga atgaattctc gtaactacat agcacaagta gatgtagtta actttaatct
29641 cacatagcaa tctttaatca gtgtgtaaca ttagggagga cttgaaagag ccaccacatt
29701 ttcaccgagg ccactcggag tacgatcgag tgtacagtga acaatgctag ggagagctgc
29761 ctatatggaa gagccctaat gtgtaaaatt aattttagta gtgctatccc catgtgattt
29821 taatagcttc ttagga
```

FIG. 37

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1 ttcccaggta acaaaccaac caactttcga tctcttgtag atctgttctc taaacgaact
61 ttaaaatctg tgtggctgtc actcggctgc atgcttagtg cactcacgca gtataattaa
121 taactaatta ctgtcgttga caggacacga gtaactcgtc tatctttgtc aggctgctta
181 cggtttcgtc cgtgttgcag ccgatcatca ncacatctag gttttgtccg ggtgtgaccg
241 aaaggtaaga tggagagcct tgtccctggt ttcaacgaga aaacacacgt ccaactcagt
301 ttgcctgttt tacaggttcg cgacgtgctc gtacgtggct ttggagactc cgtggaggag
361 gtcttatcag aggcacgtca acatcttaaa gatggcactt gtggcttagt agaagttgaa
421 aaaggcgttt tgcctcaact tgaacagccc tatgtgttca tcaaacgttc ggatgctcga
481 actgcaccta atggtcatgt tatggttgag ctggtagcag aactcgaagg cattcagtac
541 ggtcgtagtg gtgagacact tggtgtcctt gtccctcatg tgggcgaaat accagtggct
601 taccgcaagg ttcttcttcg taagaacggt aataaaggag ctggtggcca tagttacggc
661 gccgatctaa agtcaatttga cttaggcgac gagcttggca ctgatcctta tgaagatttt
721 caagaaaact ggaacactaa acatagcagt cgtgttaccc gtgaactcat gcgtgagctt
781 aacggagggg catacactcg ctatgtcgat aacaacttct gtggccctga tggctaccct
841 cttgagtgca ttaaagacct tctagcacgt gctggtaaag cttcatgcac tttgtccgaa
901 caactggact ttattgacac taagaggggt gtatactgct gccgtgaaca tgagcatgaa
961 attgcttggt acacggaacg ttctgaaaag agctatgaat tgcagacacc ttttgaaatt
1021 aaattggcaa agaaatttga caccttcaat cgggaatgtc caaattttgt atttcccttа
1081 aattccataa tcaagactat tcaaccaagg gttgaaaaga aaaagcttga tggctttatg
1141 ggtagaattc gatctgtcta tccagttgcg tcaccaaatg aatgcaacca aatgtgcctt
1201 tcaactctca tgaagtgtga tcattgtggt gaaacttcat ggcagacggg cgattttgtt
1261 aaagccactt gcgaattttg tggcactgag aatttgacta aagaaggtgc cactacttgt
1321 ggttacttac cccaaaatgc tgttgttaaa atttattgtc cagcatgtca caattcagaa
1381 gtaggacctg agcatagtct tgccgaatac cataatgaat ctggcttgaa aaccattctt
1441 cgtaagggtg gtcgcactat tgcctttgga cgctgtgtgt tctcttatgt tggttgccat
1501 aacaagtgtg cctattgggt tccacgtgct agcgctaaca taggttgtaa ccatacaggt
1561 gttgttggag aaggttccga aggtcttaat cacaaccttc ttgaaatact ccaaaaagag
1621 aaagtcaaca tcaatattgt tggtgacttt aaacttaatg aagagatcgc cattattttg
1681 gcatcttttt ctgcttccac aagtgctttt gtggaaactg tgaaaggttt ggattataaa
1741 gcattcaaac aaattgttga atcctgtggt aattttaaag ttacaaaagg aaaagctaaa
1801 aaaggtgcct ggaatattgg tgaacagaaa tcaatactga gtcctcttta tgcatttgca
1861 tcagaggctg ctcgtgttgt acgatcaatt ttctcccgca ctcttgaaac tgctcaaaat
1921 tctgtgcgtg ttttacagaa ggccgctata acaatactag atggaatttc acagtattca
1981 ctgagactca ttgatgctat gatgttcaca tctgatttgg ctactaacaa tctagttgta
2041 atggcctaca ttacaggtgg tgttgttcag ttgacttcgc agtggctaac taacatcttt
2101 ggcactgttt atgaaaaact caaacccgtc cttgattggc ttgaagagaa gtttaaggaa
2161 ggtgtagagt ttcttagaga cggttgggaa attgttaaat ttatctcaac ctgtgcttgt
2221 gaaattgtcg gtggacaaat tgtcacctgt gcaaaggaaa ttaaggagag tgttcagaca
2281 ttctttaagc ttgtaaataa atttttggct ttgtgtgctg actctatcat tattggtgga
2341 gctaaactta aagccttgaa tttaggtgaa acatttgtca cgcactcaaa gggattgtac
2401 agaaagtgtg ttaaatccag agaagaaact cgcctactca tgcctctaaa agccccaaaa
2461 gaaattatct tcttagaggg agaaacactt cccacagaag tgttaacaga ggaagttgtc
2521 ttgaaaactg gtgatttaca accattagaa caacctacta gtgaagctgt tgaagctcca
2581 ttggttggta caccagtttg tattaacggg cttatgttgc tcgaaatcaa agacacagaa
2641 aagtactgtg cccttgcacc taatatgatg gtaacaaaca ataccttcac actcaaaggc
2701 ggtgcaccaa caaaggttac ttttggtgat cacactgtga tagaagtgca aggttacaag
2761 agtgtgaata tcacttttga acttgatgaa aggattgata aagtacttaa tgagaggtgc
2821 tctgcctata cagttgaact cggtacagaa gtaaatgagt tcgcctgtgt tgtggcagat
2881 gctgtcataa aaacttтgca accagtatct gaattactta caccactggg cattgattta
2941 gatgagtgga gtatggctac atactactta tttgatgagt ctggtgagtt taaattggct
3001 tcacatatgt attgttcttt ttaccctcca gatgaggatg aagaagaagg tgattgtgaa
3061 gaagaagagt ttgagccatc aactcaatat gagtatggta ctgaagatga ttaccaaggt
3121 aaacctttgg aatttggtgc cacttctgct gctcttcaac ctgaagaaga gcaagaagaa
3181 gattggttag atgatgatag tcaacaaact gttggtcaac aagacggcag tgaggacaat
3241 cagacaacta ctattcaaac aattgttgag gttcaacctc aattagagat ggaacttaca
```

FIG. 37 continued

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3301 ccagttgttc agactattga agtgaatagt tttagtggtt atttaaaact tactgacaat
3361 gtatacatta aaaatgcaga cattgtggaa caagctaaaa aggtaaaacc aacagtggtt
3421 gttaatgcag ccaatgttta ccttaaacat cgaggaggtg ttgcaggagc cttaaataag
3481 gctactaaca atgccatgca agttgaatct catgattaca tagctactaa tggaccactt
3541 aaagtgggtg gtagttgtgt tttaagcgga cacaatcttg ctaaacactg tcttcatgtt
3601 gtcggcccaa atgttaacaa aggtgaagac attcaacttc ttaagagtgc ttatgaaaat
3661 tttaatcagc acgaagttct accttgcacca ttattatcag ctggtatttt tggtgctgac
3721 cctatacatt ctttaagagt ttgtgtagat actgttcgca caaatgtcta cttagctgtc
3781 tttgataaaa atctctatga caaacttgtt tcaagctttt tggaaatgaa gagtgaaaag
3841 caagttgaac aaaagatcgc tgagattcct aaagaggaag ttaagccatt tataactgaa
3901 agtaaacctt cagttgaaca gagaaaacaa catgataaga aaatcaaagc ttgtgttgaa
3961 gaagttacaa caactctgga agaaactaag ttcctcacag aaaacttgtt actttatatt
4021 gacattaatg gcaatcttca tccagattct gccactcttg ttagtgacat tgacatcact
4081 ttcttaaaga aagatgctcc atatatagtg cgtgatgttg ttcaagaggg tgttttaact
4141 gctgtggtta tacctactaa aaaggctggt cgcactactg aaatgctagc gaaagctttg
4201 agaaaagtgc caacagacaa ttatataacc acttacccgg gtcagggttt aaatggttac
4261 actgtagagg aggcaaagac agtgcttaaa aagtgtaaaa gtgcctttta cattctacca
4321 tctattatct ctaatgagaa gcaagaaatt cttggaactg tttcttggaa tttgcgagaa
4381 atgcttgcac atgcagaaga aacacgcaaa ttaatgcctg tctgtgtgga aactaaagcc
4441 atagtttcaa ctatacagcg taaatataag cgtattaaaa tacaagaggg tgtggttgat
4501 tatggtgcta gattttactt ttacaccagt aaaacaactg tagcgtcact tatcaacaca
4561 cttaacgatc taaatgaaac tcttgttaca atgccacttg gctatgtaac acatggctta
4621 aatttggaag aagctgctcg gtatatgaga tctctcaaag tgccagctac agtttctgtt
4681 tcttcaccta atgctgttac agcgtataat ggttatctta cttcttcttc taaaacacct
4741 gaagaacatt ttattgaaac catctcactt cctggttcct ataaagattg gtcctattct
4801 ggacaatcta cacaactagg tatagaattt cttaagagag gtgataaaag tgtatattac
4861 actagtaatc ctaccacatt ccacctagat cgtgaagtta tcacctttga caatcttaag
4921 acacttcttt ctttgagaga agtgaggact attaaggtgt ttacaacagt agacaacatt
4981 aacctccaca cgcaagttgt ggacatgtca atgacatatg gacaacagtt tggtccaact
5041 tatttggatg gagctgatgt tactaaaata aaacctcata attcacatga aggtaaaaca
5101 ttttatgttt tacctaatga tgacactcta cgtgttgagg cttttgagta ctaccacaca
5161 actgatccta gtttttctggg taggtacatg tcagcattaa atcacactaa aaagtggaaa
5221 tacccacaag ttaatggttt aacttctatt aaatgggcag ataacaactg ttatcttgcc
5281 actgcattgt taacactcca acaaatagag ttgaagttta atccacctgc tctacaagat
5341 gcttattaca gagcaagggc tggtgaagcn cctaactttt gtgcacttat cttagcctac
5401 tgtaataaga cagtaggtga gttaggtgat gttagagaaa caatgagtta cttgtttcaa
5461 catgccaatt tagattcttg caaaagagtc ttgaacgtgg tgtgtaaaac ttgtggacaa
5521 cagcagacaa ccctaaggg tgtagaagct cttatgtaca tgggcacact ttcttatgaa
5581 caatttaaga aaggtgttca gataccttgt acgtgtggta aacaagctac aaaatatcta
5641 gtacaacagg agtcaccttt tgttatgatg tcagcaccac ctgctcagta tgaacttaag
5701 catggtacat ttacttgtgc tagtgagtac actggtaatt accagtgtgg tcactataaa
5761 catataactt ctaaagaaac tttgtattgc atagacggtg ctttacttac aaagtcctca
5821 gaatacaaag gtcctattac ggatgttttc tacaaagaaa acagttacac aacaaccata
5881 aaaccagtta cttataaatt ggatggtgtt ctttgtacag aaattgaccc taagttggac
5941 aattattata agaaagacaa ttcttatttc acagagcaac caattgatct tgtaccaaac
6001 caaccatatc caaacgcaag cttcgataat tttaagtttg tatgtgataa tatcaaattt
6061 gctgatgatt taaaccagtt aactggttat aagaaacctg cttcaagaga gcttaaagtt
6121 acatttttcc ctgacttaaa tggtgatgtg ctggctattg attataaaca ctacacaccc
6181 tcttttaaga aaggagctaa attgttacat aaacctattg tttggcatgt taacaatgca
6241 actaataaag ccacgtataa accaaatacc tggtgtatac gttgtctttg gagcacaaaa
6301 ccagttgaaa catcaaattc gttttgatgta ctgaagtcag aggacgcgca gggaatggat
6361 aatcttgcct gcgaagatct aaaaccagtc tctgaagaag tagtggaaaa tcctaccata
6421 cagaaagacg ttcttgagtg taatgtgaaa actaccgaag ttgtaggaga cattatactt
6481 aaaccagcaa ataatataaa aattacagaa gaggttggcc acacagatct aatggctgct
6541 tatgtagaca attctagtct tactattaag aaacctaatg aattatctag agtattaggt
6601 ttgaaaaccc ttgctactca tggtttagct gctgttaata gtgtcccttg ggatactata
6661 gctaattatg ctaagccttt tcttaacaaa gttgttagta caactactaa catagttaca
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FIG. 37 continued

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 6721 cggtgtttaa accgtgtttg tactaattat atgccttatt tctttacttt attgctacaa
 6781 ttgtgtactt ttactagaag tacaaatttct agaattaaag catctatgcc gactactata
 6841 gcaaagaata ctgttaagag tgtcggtaaa ttttgtctag aggcttcatt taatttatttg
 6901 aagtcaccta attttttctaa actgataaat attataattt ggtttttact attaagtgtt
 6961 tgcctaggtt ctttaatcta ctcaaccgct gctttaggtg ttttaatgtc taatttaggc
 7021 atgccttctt actgtactgg ttacagagaa ggctatttga actctactaa tgtcactatt
 7081 gcaacctact gtactggttc tataccttgt agtgtttgtc ttagtggttt agatttttta
 7141 gacacctatc cttctttaga aactatacaa attaccattt catcttttaa atgggattta
 7201 actgcttttg gcttagttgc agagtggttt ttggcatata ttcttttcac taggttttte
 7261 tatgtacttg gattggctgc aatcatgcaa ttgtttttca gctattttgc agtacatttt
 7321 attagtaatt cttggcttat gtggttaata attaatcttg tacaaatggc cccgatttca
 7381 gctatggtta gaatgtacat cttctttgca tcatttttatt atgtatggaa aagttatgtg
 7441 catgttgtag acggttgtaa ttcatcaact tgtatgatgt gttacaaacg taatagagca
 7501 acaagagtcg aatgtacaac tattgttaat cgtgttagaa ggtccttta tgtcttatgct
 7561 aatggaggta aaggcttttg caaactacac aattggaatt gtgttaattg tgatacattc
 7621 tgtgctggta gtacatttat tagtgatgaa gttgcgagag acttgtcact acagtttaaa
 7681 agaccaataa atcctactga ccagtcttct tacatcgttg atagtgttac agtgaagaat
 7741 ggttccatcc atctttactt tgataaagct cgtcaaaaga cttatgaaag acattctctc
 7801 tctcattttg ttaacttaga caacctgaga gctaataaca ctaaaggttc attgcctatt
 7861 aatgttatag ttttttgatgg taaatcaaaa tgtgaagaat catctgcaaa atcagcgtct
 7921 gtttactaca gtcagcttat gtgtcaacct atactgttac tagatcaggc attagtgtct
 7981 gatgttggtg atagtgcgga agttgcagtt aaaatgtttg atgcttacgt taatacgttt
 8041 tcatcaactt ttaacgtacc aatggaaaaa ctcaaaacac tagttgcaac tgcagaagct
 8101 gaacttgcaa agaatgtgtc cttagacaat gtcttatcta cttttatttc agcagctcgg
 8161 caagggtttg ttgattcaga tgtagaaact aaagatgttg ttgaatgtct taaattgtca
 8221 catcaatcag acatagaagt tactggcgat agttgtaata actatatgct cacctataac
 8281 aaagttgaaa acatgacacc ccgtgacctt cgtgcttgta ttgactgtag tgcgcgtcat
 8341 attaatgcgc aggtagcaaa aagtcacaac attactttga tatggaacgt taaagatttc
 8401 atgtcattgt ctgaacaact acgaaaacaa atacgtagtg ctgctaaaaa gaataactta
 8461 cattttaagt tgacatgtgc aactactaga caagttgtta atgttgtaac aacaaagata
 8521 gcacttaagg gtggtaaaat tgttaataat tggttgaagc agttaattaa agttacactt
 8581 gtgttccttt ttgttgctgc tatttttctat ttaataacac ctgttcatgt catgtctaaa
 8641 catactgact tttcaagtga aatcatagga tacaaggcta ttgatggtgg tgtcactcgt
 8701 gacatagcat ctacagatac ttgttttgct aacaaacatg ctgattttga cacatggttt
 8761 agccagcgtg gtggtagtta tactaatgac aaagcttgcc cattgattgc tgcagtcata
 8821 acaagagaag tgggttttgt cgtgcctggt ttgcctggca cgatattacg cacaactaat
 8881 ggtgactttt tgcatttctt acctagagtt tttagtgcag ttggtaacat ctgttacaca
 8941 ccatcaaaac ttatagagta cactgacttt tcaacatcag cttgtgtttt ggctgctgaa
 9001 tgtacaattt ttaaagatgc ttctggtaag ccagtaccat attgttatga taccaatgta
 9061 ctagaaggtt ctgttgctta tgaaagttta cgccctgaca cacgttatgt gctcatggat
 9121 ggctctattt ttcaatttcc taacacctac cttgaaggtt ctgttagagt ggtaacaact
 9181 tttgattctg agtacttgtag gcacggcact tgtgaaagat cagaagctgg tgtttgtgta
 9241 tctactagtg gtagatgggt acttaacaat cattattaca gatctttacc aggagttttc
 9301 tgtggtgtag atgctgtaaa tttacttact aatatgttta caccactaat tcaacctatt
 9361 ggtgctttgg acatattcagc atctatagta cctggtggta ttgtagctat cgtagtaaca
 9421 tgccttgcct actatttttat gaggtttaga agagcttttg gtgaatacag tcatgtagtt
 9481 gcctttaata ctttactatt ccttatgtca ttcactgtac tctgtttaac accagtttac
 9541 tcattcttac ctggtgttta ttctgttatt tacttgtact tgacatttta tcttactaat
 9601 gatgtttctt ttttagcaca tattcagtgg atggttatgt tcacaccttt agtacctttc
 9661 tggataacaa ttgcttatat catttgtatt tccacaaagc atttctattg gttcttagt
 9721 aattacctaa agagacgtgt agtctttaat cgtgtttcct ttagtacttt tgaagaagct
 9781 gcgctgtgca cctttttgtt aaataaagaa atgtatctaa agttgcgtag tgatgtgcta
 9841 ttacctctta cgcaatataa tagatactta gctctttata ataagtacaa gtattttagt
 9901 ggagcaatgg atacaactag ctacagagaa gctgcttgtt gtcatctcgc aaaggctctc
 9961 aatgacttca gtaactcagg ttctgatgtt ctttaccaac caccacaaat ctctatcacc
10021 tcagctgttt tgcagagtgg ttttagaaaa atggcattcc catctggtaa agttgagggt
10081 tgtatggtac aagtaacttg tggtacaact acacttaacg gtctttggct tgatgacgta
```

FIG. 37 continued

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10141 gtttactgtc caagacatgt gatctgcacc tctgaagaca tgcttaaccc taattatgaa
10201 gatttactca ttcgtaagtc taatcataat ttcttggtac aggctggtaa tgttcaactc
10261 agggttattg gacattctat gcaaaattgt ctacttaagc ttaaggttga tacagccaat
10321 cctaagacac ctaagtataa gtttgttcgc attcaaccag gacagacttt ttcagtgtta
10381 gcttgttaca atggttcacc atctggtgtt taccaatgtg ctatgaggca caatttcact
10441 attaagggat cattccttaa tggttcatgt ggtagtgttg gttttaacat agattatgac
10501 tgtgtctctt tttgttacat gcaccatatg caattaccaa ctggagttca tgctggcaca
10561 gacttagaag gtaactttta tggacctttt gttgacaggc aaacagcaca agcagctggt
10621 acggacacaa ctattacagt taatgtttta gcttggttgt acgctgctgt tataaatgga
10681 gacaggtggt ttctcaatcg atttaccaca actcttaatg actttaacct tgtggctatg
10741 aagtacaatt atgaacctct aacacaagac catgttgaca tactaggacc tctttctgct
10801 caaactggaa ttgccgtttt agatatgtgt gcttcattaa aagaattact gcaaaatggt
10861 atgaatggac gtaccatatt gggtagtgct ttattagaag atgaatttac acctttgat
10921 gttgttagac aatgctcagg tgttactttc caaagtgcag tgaaaagaac aatcaagggt
10981 acacaccact ggttgttact cacaattttg acttcacttt tagttttagt ccagagtact
11041 caatggtctt tgttcttttt tttgtatgaa aatgcctttt taccttttgc tatgggtatt
11101 attgctatgt ctgctttgc aatgatgttt gtcaaacata agcatgcatt tctcgtttg
11161 ttttgttac cttctcttgc cactgtagct tattttaata tggtctatat gcctgctagt
11221 tgggtgatgc gtattatgac atggttggat atggttgata ctagttttaa gctaaaagac
11281 tgtgttatgt atgcatcagc tgtagtgtta ctaatccttа tgacagcaag aactgtgtat
11341 gatgatgggg ctaggagagt gtggacactt atgaatgtct tgacactcgt ttataaagtt
11401 tattatggga atgctttaga tcaagccatt tccatgtggg ctcttataat ctctgttact
11461 tctaactact caggtgtagt tacaactgtc atgtttttgg ccagaggtgt tgttttatg
11521 tgtgttgagt attgccctat tttcttcata actggtaata cacttcagtg tataatgcta
11581 gtttattgtt tcttaggcta tttttgtact tgttactttg gcctcttttg tttactcaac
11641 cgctactttа gactgactct tggtgtttat gattacttag tttctacaca ggagtttaga
11701 tatatgaatt cacagggact actcccaccc aagaatagca tagatgcctt caaactcaac
11761 attaaattgt tgggtgttgg tggcaaacct tgtatcaaag tagccactgt acagtctaaa
11821 atgtcagatg taaagtgcac atcagtagtc ttactctcag ttttgcaaca actcagagta
11881 gaatcatcat ctaaattgtg ggctcaatgt ctccagttac acaatgacat tctcttagct
11941 aaagatacta ctgaagcctt tgaaaaaatg gtttcactac tttctgtttt gctttccatg
12001 cagggtgctg tagacataaa caagctttgt caagaaatgc tggacaacag ggcaaccttа
12061 caagctatag cctcagagtt tagttccctt ccatcatatg cagcttttgc tactgctcaa
12121 gaagcttatg agcaggctgt tgctaatggt cattctgaag ttgttcttaa aaagttgaag
12181 aagtctttga atgtggctaa atctgaattt caccgtgatg cagccatgca acgtaagttg
12241 gaaaagatgg ctgatcaagc tatgacccaa atgtataaac aggctagatc tgaggacaag
12301 agggcaaaag ttactagtgc tatgcagaca atgcttttca ctatgcttag aaagttggat
12361 aatgatgcac tcaacaacat tatcaacaat ccaagagatg gttgtgttcc cttgaacata
12421 atacctctta caacagcagc caaactaatg cttgtcatac cagactataa cacatataaa
12481 aatacgtgtg atggtacaac atttacttat ccatcagcat tgtgggaaat ccaacaggtt
12541 gtagatgcag atagtaaaat tgttcaactt agtgaaatta gtatggacaa ttcacctaat
12601 ttagcatggc ctcttattgt aacagcttta agggccaatt ctgctgtcaa attacagaat
12661 aatgagctta gtcctgttgc actacgacag atgtcttgtg ctgccggtac tacacaaact
12721 gcttgcactg atgacaatgc gttagcttac tacaacacaa caaagggagg taggtttgta
12781 cttgcactgt tatccgattt acaggatttg aaatgggcta gattccctaa gagtgatgga
12841 actggtacta tctatacaga actggaacca ccttgtaggt ttgttacaga cacacctaaa
12901 ggtcctaaag tgaagtattt atactttatt aaaggattaa acaacctaaa tagaggtatg
12961 gtacttggta gtttagctgc cacagtacgt ctacaagctg gtaatgcaac agaagtgcct
13021 gccaattcaa ctgtattatc tttctgtgct tttgctgtag atgctgctaa agcttacaaa
13081 gattatctag ctagtggggg acaaccaatc actaattgtg ttaagatgtt gtgtacacac
13141 actggtactg gtcaggcaat aacagtcaca ccggaagcca atatggatca agaatccttt
13201 ggtggtgcat cgtgttgtct gtactgccgt tgccacatag atcatccaaa tcctaaagga
13261 ttttgtgact taaaaggtaa gtatgtacaa atacctacaa cttgtgctaa tgaccctgtg
13321 ggttttacac ttaaaaacac agtctgtacc gtctgcggta tgtggaaagg ttatggctgt
13381 agttgtgatc aactccgcga acccatgctt cagtcagctg atgcacaatc gtttttaaac
13441 gggtttgcgg tgtaagtgca gcccgtctta caccgtgcgg cacaggcact agtactgatg
13501 tcgtatacag ggcttttgac atctacaatg ataaagtagc tggttttgct aaattcctaa
```

FIG. 37 continued

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13561 aaactaattg ttgtcgcttc caagaaaagg acgaagatga caatttaatt gattcttact
13621 ttgtagttaa gagacacact ttctctaact accaacatga agaaacaatt tataatttac
13681 ttaaggattg tccagctgtt gctaaacatg acttctttaa gtttagaata gacggtgaca
13741 tggtaccaca tatatcacgt caacgtctta ctaaatacac aatggcagac ctcgtctatg
13801 ctttaaggca ttttgatgaa ggtaattgtg acacattaaa agaaatactt gtcacataca
13861 attgttgtga tgatgattat ttcaataaaa aggactggta tgattttgta gaaaacccag
13921 atatattacg cgtatacgcc aacttaggtg aacgtgtacg ccaagctttg ttaaaaacag
13981 tacaattctg tgatgccatg cgaaatgctg gtattgttgg tgtactgaca ttagataatc
14041 aagatctcaa tggtaactgg tatgatttcg gtgatttcat acaaaccacg ccaggtagtg
14101 gagttcctgt tgtagattct tattattcat tgttaatgcc tatattaacc ttgaccaggg
14161 ctttaacttgc agagtcacat gttgacactg acttaacaaa gccttacatt aagtgggatt
14221 tgttaaaata tgacttcacg gaagagaggt taaaactctt tgaccgttat tttaaatatt
14281 gggatcagac ataccaccca aattgtgtta actgtttgga tgacagatgc attctgcatt
14341 gtgcaaactt taatgtttta ttctctacag tgttcccact tacaagtttt ggaccactag
14401 tgagaaaaat atttgttgat ggtgttccat ttgtagtttc aactggatac cacttcagag
14461 agctaggtgt tgtacataat caggatgtaa acttacatag ctctagactt agttttaagg
14521 aattacttgt gtatgctgct gaccctgcta tgcacgctgc ttctggtaat ctattactag
14581 ataaacgcac tacgtgcttt tcagtagctg cacttactaa caatgttgct tttcaaactg
14641 tcaaacccgg taatttttaac aaagacttct atgactttgc tgtgtctaag ggtttcttta
14701 aggaaggaag ttctgttgaa ttaaaacact tcttctttgc tcaggatggt aatgctgcta
14761 tcagcgatta tgactactat cgttataatc taccaacaat gtgtgatatc agacaactac
14821 tatttgtagt tgaagttgtt gataagtact ttgattgtta cgatggtggc tgtattaatg
14881 ctaaccaagt catcgtcaac aacctagaca aatcagctgg ttttccattt aataaatggg
14941 gtaaggctag actttattat gattcaatga gttatgagga tcaagatgca cttttcgcat
15001 atacaaaacg taatgtcatc cctactataa ctcaaatgaa tcttaagtat gccattagtg
15061 caaagaatag agctcgcacc gtagctggtg tctctatctg tagtactatg accaatagac
15121 agtttcatca aaaattattg aaatcaatag ccgccactag aggagctact gtagtaattg
15181 gaacaagcaa attctatggt ggttggcaca atatgttaaa aactgtttat agtgatgtag
15241 aaaaccctca ccttatgggt tgggattatc ctaaatgtga tagagccatg cctaacatgc
15301 ttagaattat ggcctcactt gttcttgctc gcaaacatac aacgtgttgt agcttgtcac
15361 accgtttcta tagattagct aatgagtgtg ctcaagtatt gagtgaaatg gtcatgtgtg
15421 gcggttcact atatgttaaa ccaggtggaa cctcatcagg agatgccaca actgcttatg
15481 ctaatagtgt ttttaacatt tgtcaagctg tcacggccaa tgttaatgca cttttatcta
15541 ctgatggtaa caaaattgcc gataagtatg tccgcaattt acaacacaga ctttatgagt
15601 gtctctatag aaatagagat gttgacacag actttgtgaa tgagttttac gcatatttgc
15661 gtaaacattt ctcaatgatg atactctctg acgatgctgt tgtgtgtttc aatagcactt
15721 atgcatctca aggtctagtg gctagcataa agaactttaa gtcagttctt tattatcaaa
15781 acaatgtttt tatgtctgaa gcaaaatgtt cgactgagac tgaccttact aaaggacctc
15841 atgaattttg ctctcaacat acaatgctag ttaaacaggg tgatgattat gtgtaccttc
15901 cttacccaga tccatcaaga atcctagggg ccggctgttt tgtagatgat atcgtaaaaa
15961 cagatggtac acttatgatt gaacggttcg tgtctttagc tatagatgct tacccactta
16021 ctaaacatcc taatcaggag tatgctgatg tctttcattt gtacttacaa tacataagaa
16081 agctacatga tgagttaaca ggacacatgt tagacatgta ttctgttatg cttactaatg
16141 ataacacttc aaggtattgg gaacctgagt tttatgaggc tatgtacaca ccgcatacag
16201 tcttacaggc tgttggggct tgtgttcttt gcaattcaca gacttcatta agatgtggtg
16261 cttgcatacg tagaccattc ttatgttgta aatgctgtta cgaccatgtc atatcaacat
16321 cacataaatt agtcttgtct gttaatccgt atgtttgcaa tgctccaggt tgtgatgtca
16381 cagatgtgac tcaactttac ttaggaggta tgagctatta ttgtaaatca cataaaccac
16441 ccattagttt tccattgtgt gctaatggac aagtttttgg tttatataaa aatacatgtg
16501 ttggtagcga taatgttact gactttaatg caattgcaac atgtgactgg acaaatgctg
16561 gtgattacat tttagctaac acctgtactg aaagactcaa gcttttgca gcagaaacgc
16621 tcaaagctac tgaggagaca tttaaactgt cttatggtat tgctactgta cgtgaagtgc
16681 tgtctgacag agaattacat ctttcatggg aagttggtaa acctagacca ccacttaacc
16741 gaaattatgt ctttactggt tatcgtgtaa ctaaaaacag taaagtacaa ataggagagt
16801 acacctttga aaaaggtgac tatggtgatg ctgttgttta ccgaggtaca acaacttaca
16861 aattaaatgt tggtgattat tttgtgctga catcacatac agtaatgcca ttaagtgcac
16921 ctacactagt gccacaagag cactatgtta caattactgg cttataccca acactcaata
```

FIG. 37 continued

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16981 tctcagatga gttttctagc aatgttgcaa attatcaaaa ggttggtatg caaaagtatt
17041 ctacactcca gggaccacct ggtactggta agagtcattt tgctattggc ctagctctct
17101 actacccttc tgctcgcata gtgtatacag cttgctctca tgccgctgtt gatgcactat
17161 gtgagaaggc attaaaatat ttgcctatag ataaatgtag tagaattata cctgcacgtg
17221 ctcgtgtaga gtgttttgat aaattcaaag tgaattcaac attagaacag tatgtctttt
17281 gtactgtaaa tgcattgcct gagacgacag cagatatagt tgtctttgat gaaatttcaa
17341 tggccacaaa ttatgatttg agtgttgtca atgccagatt acgtgctaag cactatgtgt
17401 acattggcga ccctgctcaa ttacctgcac cacgcacatt gctaactaag ggcacactag
17461 aaccagaata tttcaattca gtgtgtagac ttatgaaaac tataggtcca gacatgttcc
17521 tcggaacttg tcggcgttgt cctgctgaaa ttgttgacac tgtgagtgct ttggtttatg
17581 ataataagct taaagcacat aaagacaaat cagctcaatg ctttaaaatg ttttataagg
17641 gtgttatcac gcatgatgtt tcatctgcaa ttaacaggcc acaaataggc gtggtaagag
17701 aattccttac acgtaaccct gcttggagaa aagctgtctt tatttcacct tataattcac
17761 agaatgctgt agcctcaaag attttgggac taccaactca aactgttgat tcatcacagg
17821 gctcagaata tgactatgtc atattcactc aaaccactga aacagctcac tcttgtaatg
17881 taaacagatt taatgttgct attaccagag caaaagtagg catactttgc ataatgtctg
17941 atagagacct ttatgacaag ttgcaattta caagtcttga aattccacgt aggaatgtgg
18001 caactttaca agctgaaaat gtaacaggac tctttaaaga ttgtagtaag gtaatcactg
18061 ggttacatcc tacacaggca cctacacacc tcagtgttga cactaaattc aaaactgaag
18121 gtttatgtgt tgacgtacct ggcataccta aggacatgac ctatagaaga ctcatctcta
18181 tgatgggttt taaaatgaat tatcaagtta atggttaccc taacatgttt atcacccgcg
18241 aagaagctat aagacatgta cgtgcatgga ttggcttcga tgtcgagggg tgtcatgcta
18301 ctagagaagc tgttggtacc aatttacctt tacagctagg tttttctaca ggtgttaacc
18361 tagttgctgt acctacaggt tatgttgata cacctaataa tacagatttt tccagagtta
18421 gtgctaaacc accgcctgga gatcaattta aacacctcat accacttatg tacaaaggac
18481 ttccttggaa tgtagtgcgt ataaagattg tacaaatgtt aagtgacaca cttaaaaatc
18541 tctctgacag agtcgtattt gtcttatggg cacatggctt tgagttgaca tctatgaagt
18601 attttgtgaa aataggacct gagcgcacct cttgtctatg tgatagacgt gccacatgct
18661 tttccactgc ttcagacact tatgcctgtt cgcatcattc tattggattt gattacgtct
18721 ataatccgtt tatgattgat gttcaacaat cgggttttac aggtaaccta caaagcaacc
18781 atgatctgta ttgtcaagtc catggtaatg cacatgtagc tagttgtgat gcaatcatga
18841 ctaggtgtct agctgtccac gagtgctttg ttaagcgtgt tgactggact attgaatatc
18901 ctataattgg tgatgaactg aagattaatg cggcttgtag aaaggttcaa cacatggttg
18961 ttaaagctgc attattagca gacaaattcc cagttcttca cgacattggt aaccctaaag
19021 ctattaagtg tgtacctcaa gctgatgtag aatggaagtt ctatgatgca cagccttgta
19081 gtgacaaagc ttataaaata gaagaattat tctattctta tgccacacat tctgacaaat
19141 tcacagatgg tgtatgccta ttttggaatt gcaatgtcga tagatatcct gctaattcca
19201 ttgtttgtag atttgacact agagtgctat ctaaccttaa cttgcctggt tgtgatggtg
19261 gcagtttgta tgtaaataaa catgcattcc acacaccagc ttttgataaa agtgcttttg
19321 ttaatttaaa acaattacca ttttttctatt actctgacag tccatgtgag tctcatggaa
19381 aacaagtagt gtcagatata gattatgtac cactaaagtc tgctacgtgt ataacacgtt
19441 gcaatttagg tggtgctgtc tgtagacatc atgctaatga gtacagattg tatccgatg
19501 cttataacat gatgatctca gctggcttta gcttgtgggt ttacaaacaa tttgatactt
19561 ataacctctg gaacactttt acaagacttc agagtttaga aaatgtggct tttaatgttg
19621 taaataaggg acactttgat ggacaacagg ctgaagtacc agtttctatc attaataaca
19681 ctgtttacac aaaagttgat ggtgttgatg tagaattgtt tgaaaataaa acaacattac
19741 ctgttaatgt agcatttgag ctttgggcta agcgcaacat taaaccagta ccagaggtga
19801 aaatactcaa taatttgggt gtggacattg ctgctaatac tgtgatctgg gactacaaaa
19861 gagatgctcc agcacatata tctactattg gtgtttgttc tatgactgac atagccaaga
19921 aaccaactga aacgatttgt gcaccactca ctgtcttttt tgatggtaga gttgatggtc
19981 aagtagactt atttagaaat gcccgtaatg gtgttcttat tacagaaggt agtgttaaag
20041 gtttacaacc atctgtaggt cccaaacaag ctagtcttaa tggagtcaca ttaattggag
20101 aagccgtaaa aacacagttc aattattata agaaagttga tggtgttgtc caacaattac
20161 ctgaaactta ctttactcag agtagaaatt tacaagaatt taaacccagg agtcaaatgg
20221 aaattgattt cttagaatta gctatggatg aattcattga acggtataaa ttagaaggct
20281 atgccttcga acatatcgtt tatggagatt ttagtcatag tcagttaggt ggtttacatc
20341 tactgattgg actagctaaa cgttttaagg aatcaccttt tgaattagaa gattttattc
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FIG. 37 continued

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20401 ctatggacag tacagttaaa aactatttca taacagatgc gcaaacaggt tcatctaagt
20461 gtgtgtgttc tgttattgat ttattacttg atgattttgt tgaaataata aaatcccaag
20521 atttatctgt agtttctaag gttgtcaaag tgactattga ctatacagaa atttcattta
20581 tgctttggtg taaagatggc catgtagaaa cattttaccc aaaattacaa tctagtcaag
20641 cgtggcaacc gggtgttgct atgcctaatc tttacaaaat gcaaagaatg ctattagaaa
20701 agtgtgacct tcaaaattat ggtgatagtg caacattacc taaaggcata atgatgaatg
20761 tcgcaaaata tactcaactg tgtcaatatt taaacacatt aacattagct gtaccctata
20821 atatgagagt tatacatttt ggtgctggtt ctgataaagg agttgcacca ggtacagctg
20881 ttttaagaca gtggttgcct acgggtacgc tgcttgtcga ttcagatctt aatgactttg
20941 tctctgatgc agattcaact ttgattggtg attgtgcaac tgtacataca gctaataaat
21001 gggatttcat tattagtgat atgtacgacc ctaagactaa aaatgttaca aaagaaaatg
21061 actctaaaga gggttttttc acttacattt ctgggtttat acaacaaaag ctagctcttg
21121 gaggttccgt ggctatgaag ataacagaac attcttggaa tgctgatctt tataagctca
21181 tgggacactt cgcatggtgg acagcctttg ttactaatgt gaatgcgtca tcatctgaag
21241 catttttaat tggatgtaat tatcttggca aaccacgcga acaaatagat ggttatgtca
21301 tgcatgcaaa ttacatattt tggaggaata caaatccaat tcagttgtct tcctattctt
21361 tatttgacat gagtaaattt ccccttaaat taaggggtac tgctgttatg tctttaaaag
21421 aaggtcaaat caatgatatg attttatctc ttcttagtaa aggtagactt ataattagag
21481 aaaacaacag agttgttatt tctagtgatg ttcttgttaa caactaaacg aacaatgttt
21541 gttttttctg ttttattgcc actagtctct agtcagtgtg ttaatcttac aaccagaact
21601 caattacccc ctgcatacac taattctttc acacgtggtg tttattaccc tgacaaagtt
21661 ttcagatcct cagtttttaca ttcaactcag gacttgttct taccttttctt ttccaatgtt
21721 acttggttcc atgttatctc tgggaccaat ggtactaaga ggtttgataa ccctgtccta
21781 ccatttaatg atggtgttta ttttgcttcc attgagaagt ctaacataat aagaggctgg
21841 atttttggta ctactttaga ttcgaagacc cagtccctac ttattgttaa taacgctact
21901 aatgttgtta ttaaagtctg tgaatttcaa ttttgtaatg atccattttt ggaccacaaa
21961 aacaacaaaa gttggatgga aagtgagttc agagtttatt ctagtgcgaa taattgcact
22021 tttgaatatg tctctcagcc ttttcttatg gaccttgaag gaaaacaggg taatttcaaa
22081 aatcttaggg aatttgtgtt taagaatatt gatggttatt ttaaaatata ttctaagcac
22141 acgcctatta tagtgcgtga gccagaagat ctccctcagg gtttttcggc tttagaacca
22201 ttggtagatt tgccaatagg tattaacatc actaggtttc aaactttact tgctttacat
22261 agaagtttat tgactcctgg tgattcttct tcaggttgga cagctggtgc tgcagcttat
22321 tatgtgggtt atcttcaacc taggactttt ctattaaaat ataatgaaaa tggaaccatt
22381 acagatgctg tagactgtgc acttgaccct ctctcagaaa caaagtgtac gttgaaatcc
22441 ttcactgtag aaaaaggaat ctatcaaact tctaacttta gagtccaacc aacagaatct
22501 attgttagat ttcctaatat tacaaacttg tgccctttg atgaagtttt taacgccacc
22561 agatttgcat ctgtttatgc ttggaacagg aagagaatca gcaactgtgt tgctgattat
22621 tctgtcctat ataatctcgc accatttttc actttaaagt gttatggagt gtctcctact
22681 aaattaaatg atctctgctt tactaatgtc tatgcagatt catttgtaat tagaggtgat
22741 gaagtcagac aaatcgctcc agggcaaact cgaaatattg ctgattataa ttataaatta
22801 ccagatgatt ttacaggctg cgttatagct tggaattcta acaagcttga ttctaaggtt
22861 agtggtaatt ataattacct gtatagattg tttaggaagt ctaatctcaa acctttgag
22921 agagatattt caactgaaat ctatcaggcc cgtaacaaac cttgtaatgg tgttgcaggt
22981 tttaattgtt actttctttt acgatcatat agtttccgac ccacttatgg tgttggtcac
23041 caaccataca gagtagtagt actttctttt cgaacttctac atgcaccagc aactgtttgt
23101 ggacctaaaa agtctactaa tttggttaaa aacaaatgtg tcaattttcaa cttcaatggt
23161 ttaaaaggca caggtgttct tactgagtct aacaaaaagt ttctgccttt ccaacaattt
23221 ggcagagaca ttgctgacac tactgatgct ctccgtgatc cacagacact tgagattctt
23281 gacattacac catgttcttt tggtggtgtc agtgttataa caccaggaac aaatacttct
23341 aaccaggttg ctgttctttta tcagggtgtt aactgcacag aagtccctgt tgctattcat
23401 gcagatcaac ttactcctac ttggcgtgtt tattctacag gttctaatgt ttttcaaaca
23461 cgtgcaggct gtttaatagg ggctgaatat gtcaacaact catatgagtg tgacataccc
23521 attggtgcag gtatatgcgc tagttatcag actcagacta agtctcatcg gcgggcacgt
23581 agtgtagcta gtcaatccat cattgcctac actatgtcac ttggtgcaga aaattcagtt
23641 gcttactcta ataactctat tgccataccc acaaatttta ctattagtgt taccacagaa
23701 attctaccag tgtctatgac caagacatca gtagattgta caatgtacat ttgtggtgat
23761 tcaactgaat gcagcaatct tttgttgcaa tatggcagtt tttgtacaca attaaaacgt
```

FIG. 37 continued

```
23821 gctttaactg gaatagctgt tgaacaagac aaaaacaccc aagaagtttt tgcacaagtc
23881 aaacaaattt acaaaacacc accaattaaa tattttggtg gttttaattt ttcacaaata
23941 ttaccagatc catcaaaacc aagcaagagg tcatttattg aagatctact tttcaacaaa
24001 gtgacacttg cagatgctng cttcatcaaa caatatggtg attgccttgg tgatattgct
24061 gctagagacc tcatttgtgc acaaaagttt aaaggcctta ctgttttgcc acctttgctc
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24181 tggacctttg gtgcaggtgc tgcattacaa ataccatttg ctatgcaaat ggcttatagg
24241 tttaatggta ttggagttac acagaatgtt ctctatgaga accaaaaatt gattgccaac
24301 caatttaata gtgctattgg caaaattcaa gactcacttt cttccacagc aagtgcactt
24361 ggaaaacttc aagatgtggt caaccataat gcacaagctt taaacacgct tgttaaacaa
24421 cttagctcca aatttggtgc aatttcaagt gttttaaatg atatcttttc acgtcttgac
24481 aaagttgagg ctgaagtgca aattgatagg ttgatcacag gcagacttca aagttgcag
24541 acatatgtga ctcaacaatt aattagagct gcagaaatca gagcttctgc taatcttgct
24601 gctactaaaa tgtcagagtg tgtacttgga caatcaaaaa gagttgattt ttgtggaaag
24661 ggctatcatc ttatgtcctt cctcagtca gcacctcatg gtgtagtctt cttgcatgtg
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24781 aaagcacact ttcctcgtga aggtgtcttt gtttcaaatg gcacacactg gtttgtaaca
24841 caaaggaatt tttatgaacc acaaatcatt actacagaca acacatttgt gtctggtaac
24901 tgtgatgttg taataggaat tgtcaacaac acagtttatg atcctttgca acctgaatta
24961 gattcattca aggaggagtt agataaatat tttaagaatc atacatcacc agatgttgat
25021 ttaggtgaca tctctggcat taatgcttca gttgtaaaca ttcaaaaaga aattgacgc
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25201 gccatagtaa tggtgacaat tatgctttgc tgtatgacca gttgctgtag ttgtctcaag
25261 ggctgttgtt ctttgtggatc ctgctgcaaa tttgatgaag acgactctga gccagtgctc
25321 aaaggagtca aattacatta cacataaacg aacttatgga tttgttttatg agaatcttca
25381 caattggaac tgtaacttta aagcaaggtg aaatcaagga tgctactcct tcagattttg
25441 ttcgcgctac tgcaacgata ccgatacaag cctcactccc tttcggatgg cttattgttg
25501 gcgttgcact tcttgctgtt tttcagagcg cttccaaaat cataactctc aaaaagagat
25561 ggcaactagc actctccaag ggtgttcact ttgtttgcaa cttgctgttg ttgttttgtaa
25621 cagtttacac acacctttg ctcgttgctg ctggccttga agccccttt ctctatcttt
25681 atgctttagt ctacttcttg cagagtataa actttgtaag aataataatg aggcttggc
25741 tttgctggaa atgccgttcc aaaaacccat tactttatga tgccaactat tttcttgct
25801 ggcatactaa ttgttacgac tattgtatac cttacaatag tgtaacttct tcaattgtca
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25981 cagactatta ccagcttgtac tcaactcaat tgagtacaga cactggtgtt gaacatgtta
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26581 ttgtcttcca caatttgcct atgccaacag gaataggttt ttgtatataa ttaagttaat
26641 tttcctctgg ctgttatggc cagtaacttt aacttgtttt gtgcttgctg ctgtttacag
26701 aataaattgg atcaccggtg gaattgctat cgcaatggct tgtcttgtag gcttgatgtg
26761 gctcagctac ttcattgctt ctttcagact gtttgcgcgt acgcgttcca tgtggtcatt
26821 caatccagaa actaacattc ttctcaacgt gccactccat ggcactattc tgaccagacc
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26941 tggacaccat ctaggacgct gtgacatcaa cgacctgcct aaagaaatca ctgttgctac
27001 atcacgaacg ctttcttatt acaaattggg agcttcgcag cgtgtagcag gtgactcagg
27061 ttttgctgca tacagtcgct acaggattgg caactataaa ttaaacacag accattccag
27121 tagcagtgac aatattgctt tgcttgtaca gtaagtgaca acagatgttt catcttgttg
27181 actttcaggt tactatagca gagatattac taattattat gcggactttt aaagtttcca
```

Fig. 37 continued

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27241 tttggaatct tgattacatc ataaacctca taattaaaaa tttatctaag tcactaactg
27301 agaataaata ttctcaatta gatgaagagc aaccaatgga gattgattaa acgaacatga
27361 aaattattct tttcttggca ctgataacac tcgctacttg tgagctttat cactaccaag
27421 agtgtgttag aggtacaaca gtacttttaa aagaaccttg ctcttctgga acatacgagg
27481 gcaattcacc atttcatcct ctagctgata acaaatttgc actgacttgc tttagcactc
27541 aatttgcttt tgcttgtcct gacggcgtaa aacacgtcta tcagttacgt gccagatcag
27601 tttcacctaa actgttcatc agacaagagg aagttcaaga actttactct ccaatttttc
27661 ttattgttgc ggcaatagtg tttataacac tttgcttcac actcaaaaga aagacagaat
27721 gattgaactt tcattaattg acttctattt ctgcttttta gcctttctgt tatttcttgt
27781 tttaattatg cttattatct tttggttctc acttgaactg caagatcata atgaaacttg
27841 tcacgcctaa acgaacatga aatttcttgt tttcttagga atcatcacaa ctgtagctgc
27901 atttcaccaa gaatgtagtt tacagtcatg tactcaacat caaccatatg tagttgatga
27961 cccgtgttct attcacttct attctaaatg ctatattaga gtaggagcta gaaaatcagc
28021 acctttaatt gaattgtgcg tggatgaggc tggttctaaa tcacccattc agtacatcga
28081 tatcggtaat tatacagttt cctgtttacc ttttacaatt aattgccagg aacctaaatt
28141 gggtagtctt gtagtgcgtt gttcgttcta tgaagacttt ttagagtatc atgacgttcg
28201 tgttgtttta gatttcatct aaacgaacaa acttaaatgt ctgataatgg accccaaaat
28261 cagcgaaatg cactccgcat tacgtttggt cgaccctcag attcaactgg cagtaaccag
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28501 agacgaattc gtggtggtga cggtaaaatg aaagatctca gtccaagatg gtatttctac
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28621 tgggttgcaa ctgagggagc cttgaataca ccaaaagatc acattggcac ccgcaatcct
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28861 ggcaatggcg gtgatgctgc tcttgctttg ctgctgcttg acagattgaa ccagcttgag
28921 agcaaaatgt ctggtaaagg ccaacaacaa caaggccaaa ctgtcactaa gaaatctgct
28981 gctgaggctt ctaagaagcc tcggcaaaaa cgtactgcca ctaaagcata caatgtaaca
29041 caagctttcg gcagacgtgg tccagaacaa acccaaggaa attttggtga ccaggaacta
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29161 tcagcgttct tcggaatgtc gcgcattggc atggaagtca cacctcgggg aacgtggttg
29221 acctacacag gtgccatcaa attggatgac aaagatccaa atttcaaaga tcaagtcatt
29281 ttgctgaata agcatattga cgcatacaaa acattcccac caacagagcc taaaaaggac
29341 aaaaagaaga aggctgatga aactcaagcc ttaccgcaga gacagaagaa acagcaaact
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29461 agcagtgctg actcaactca ggcctaaact catgcagacc acacaaggca gatgggctat
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29581 cgtaactaca tagcacaagt agatgtagtt aactttaatc tcacatagca atctttaatc
29641 agtgtgtaac attagggagg acttgaaaga gccaccacat tttcaccgag gccacgcgga
29701 gtacgatcga gtgtacagtg aacaatgcta cgggagagctg cctatatgga agagccctaa
29761 tgtgtaa
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Fig. 38

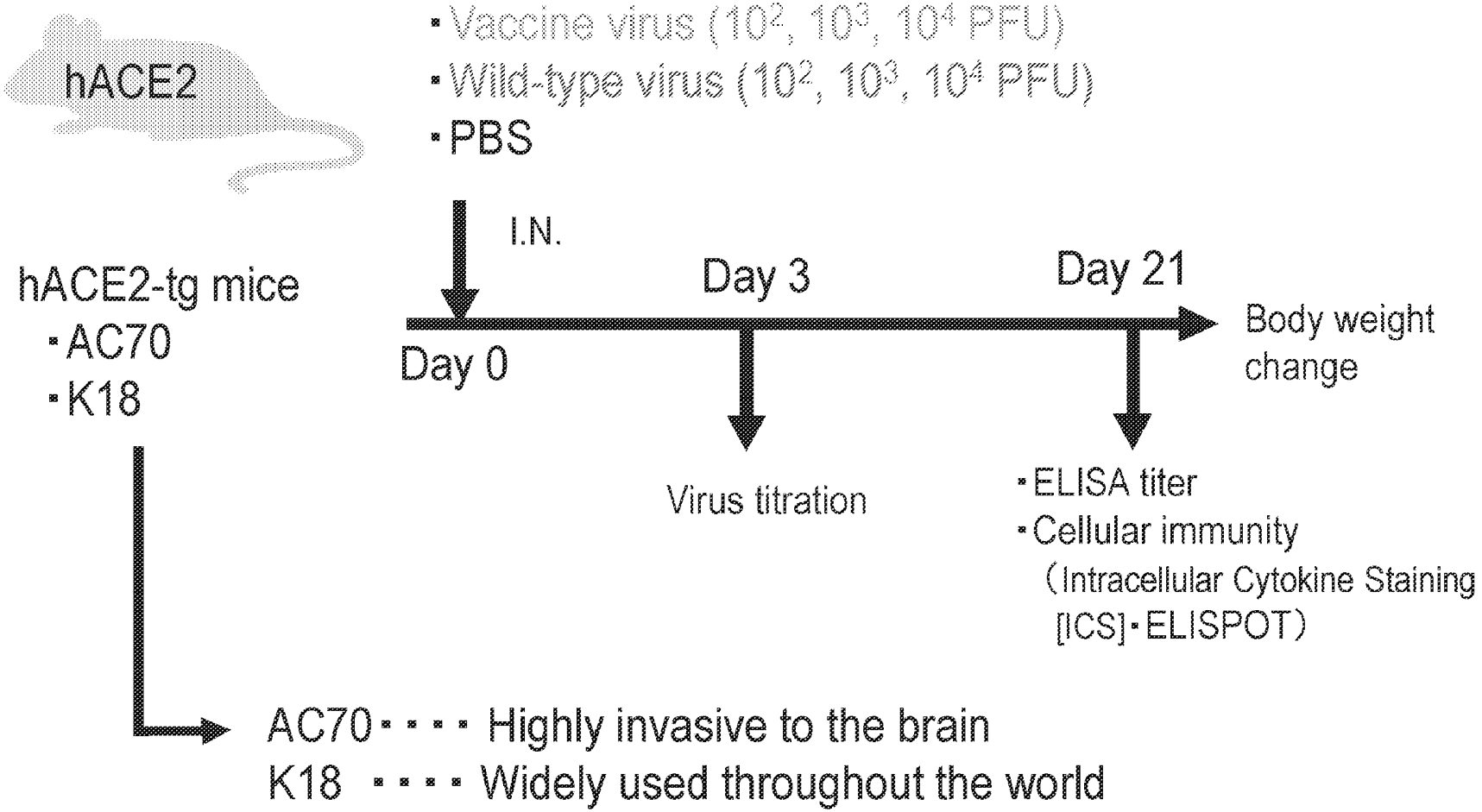
hACE2
▪ Vaccine virus (10^2, 10^3, 10^4 PFU)
▪ Wild-type virus (10^2, 10^3, 10^4 PFU)
▪ PBS
I.N.
hACE2-tg mice
▪ AC70
▪ K18
Day 0
Day 3
Day 21
Body weight change
Virus titration
▪ ELISA titer
▪ Cellular immunity
(Intracellular Cytokine Staining [ICS]▪ELISPOT)
AC70▪▪▪▪ Highly invasive to the brain
K18 ▪▪▪▪ Widely used throughout the world

Fig. 39

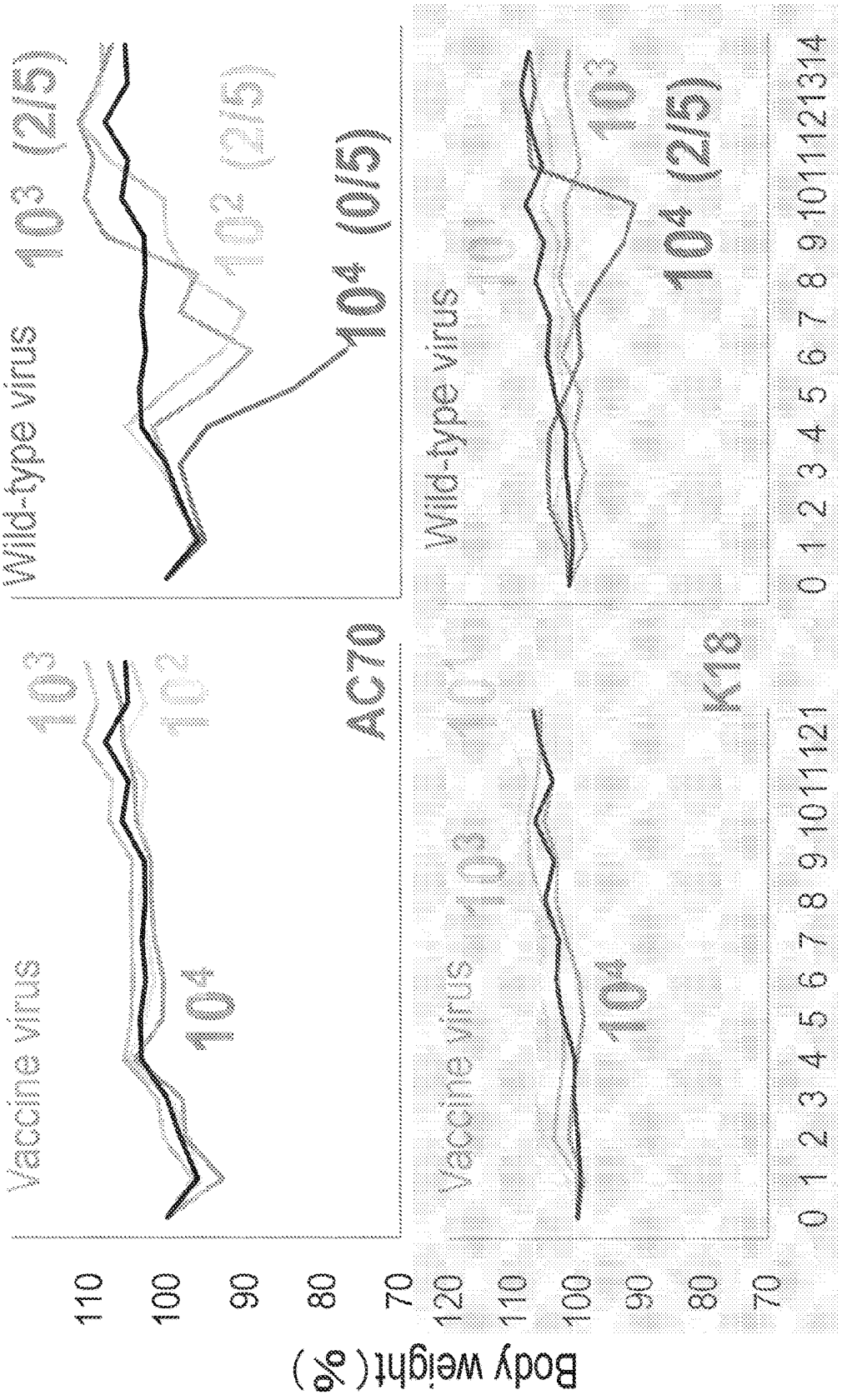
Vaccine virus
Wild-type virus
AC70
K18
10^3
10^2
10^4
10^4 (0/5)
10^3 (2/5)
10^2 (2/5)
10^4 (2/5)
Body weight (%)
Days after infection

Fig. 40

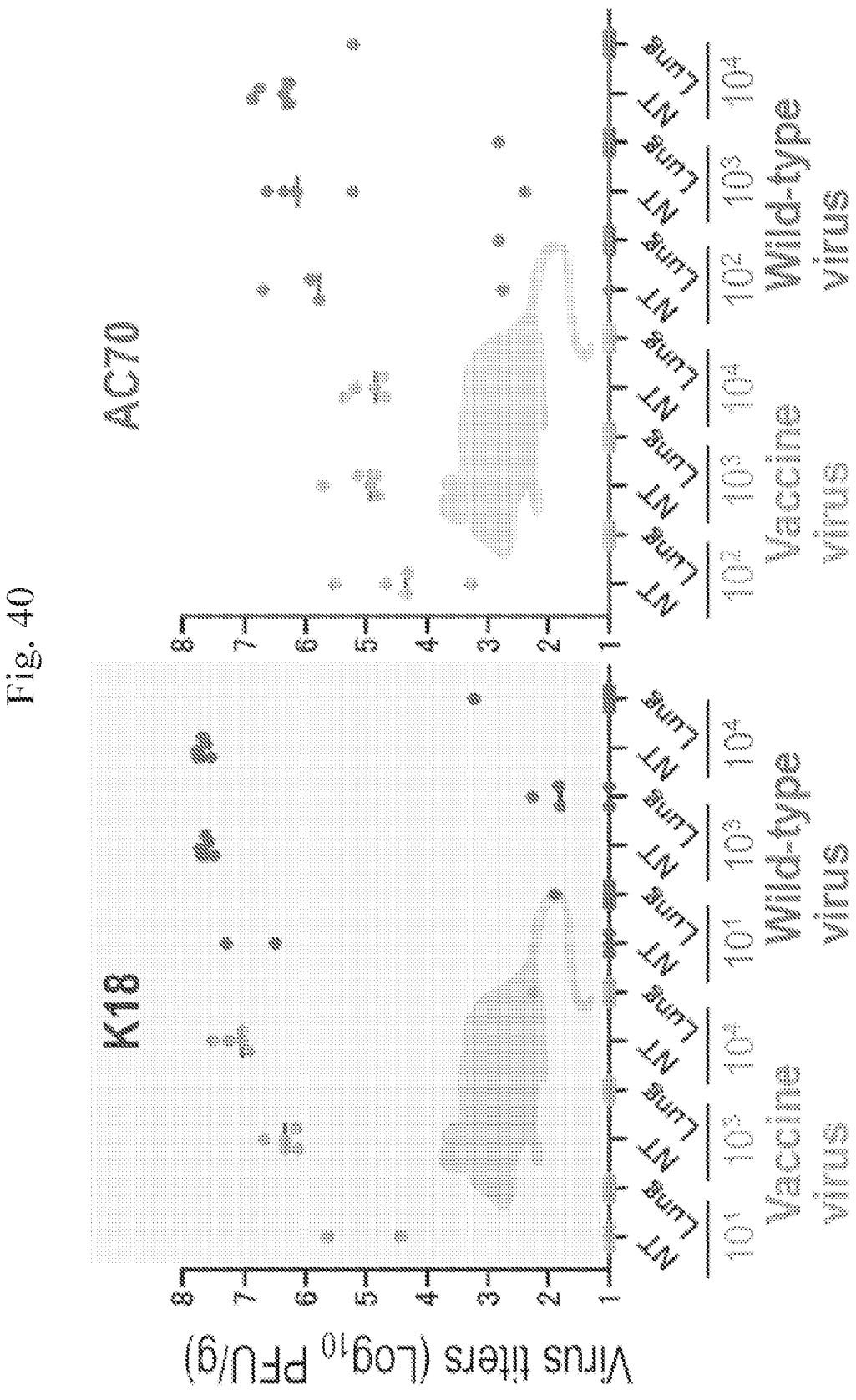
K18
AC70
Virus titers (Log10 PFU/g)
8
7
6
5
4
3
2
1
Vaccine virus
Wild-type virus
10^1
10^2
10^3
10^4
Lung
NT

Fig. 41

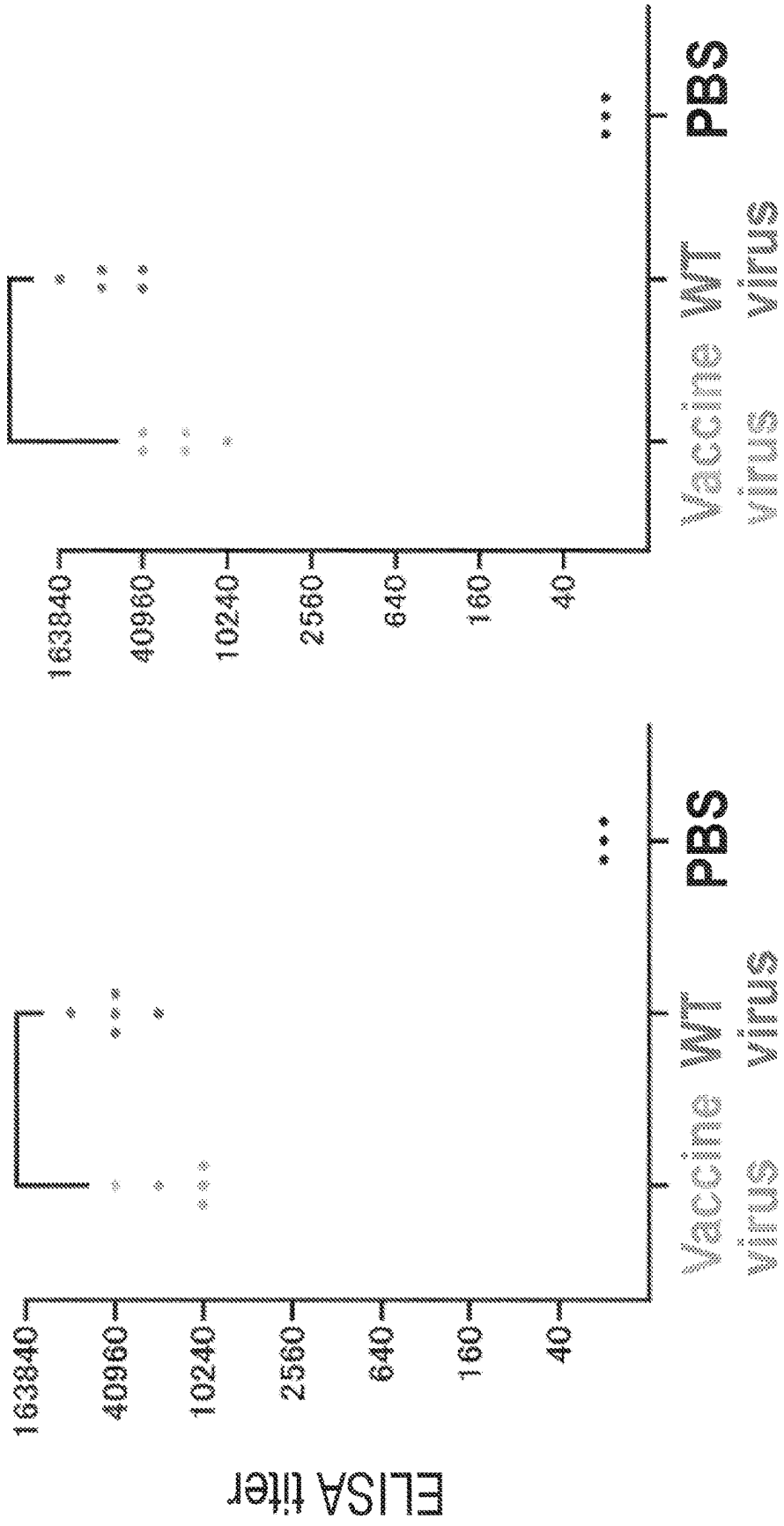
Anti-RBD ELISA titer
ELISA titer
163840
40960
10240
2560
640
160
40
ns
***
Vaccine
virus
WT virus
PBS
Anti-S ELISA titer
*
***

Fig. 42

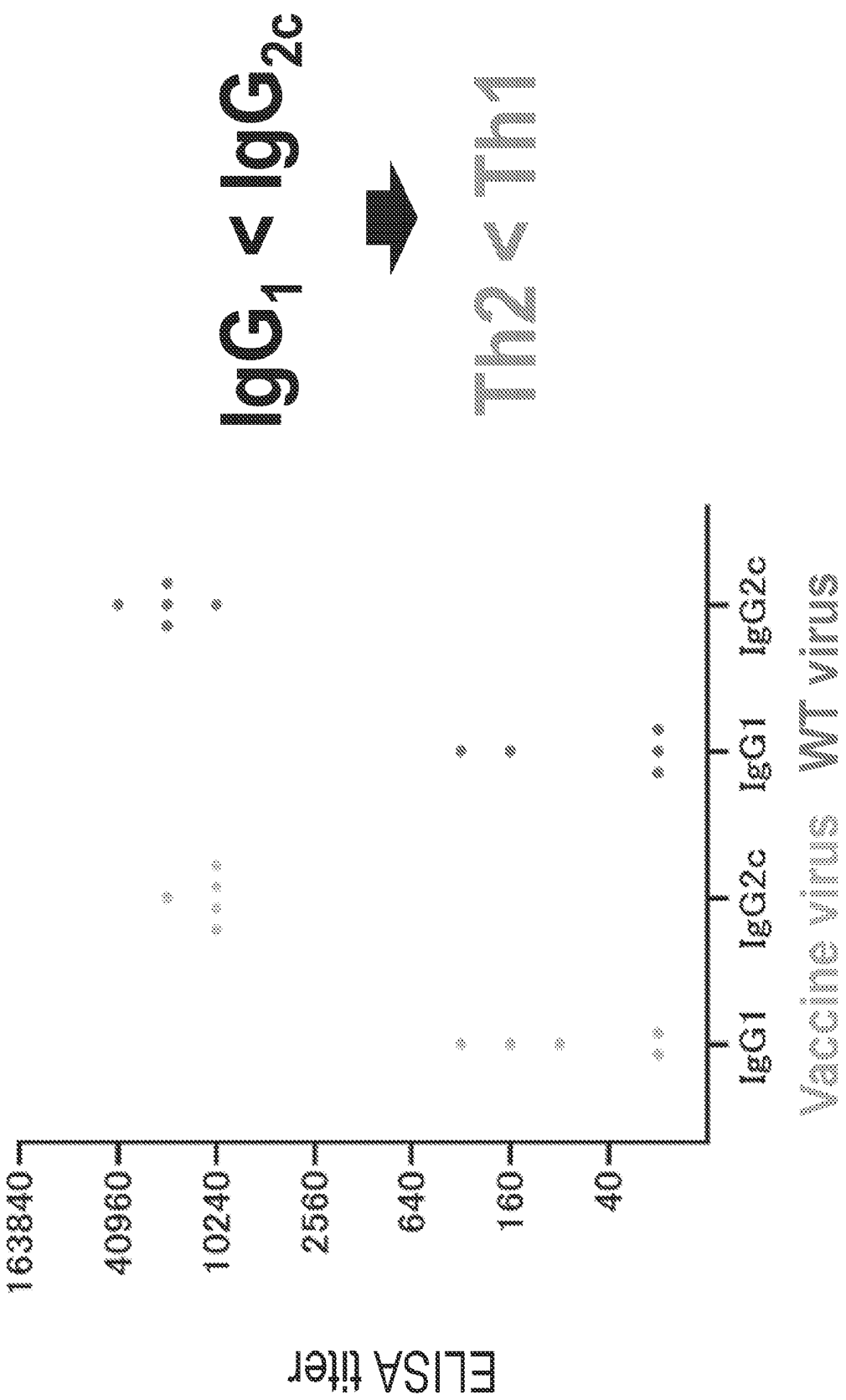
IgG1 < IgG2c
Th2 < Th1
ELISA titer
163840
40960
10240
2560
640
160
40
IgG1
IgG2c
IgG1
IgG2c
Vaccine virus
WT virus

Fig. 43

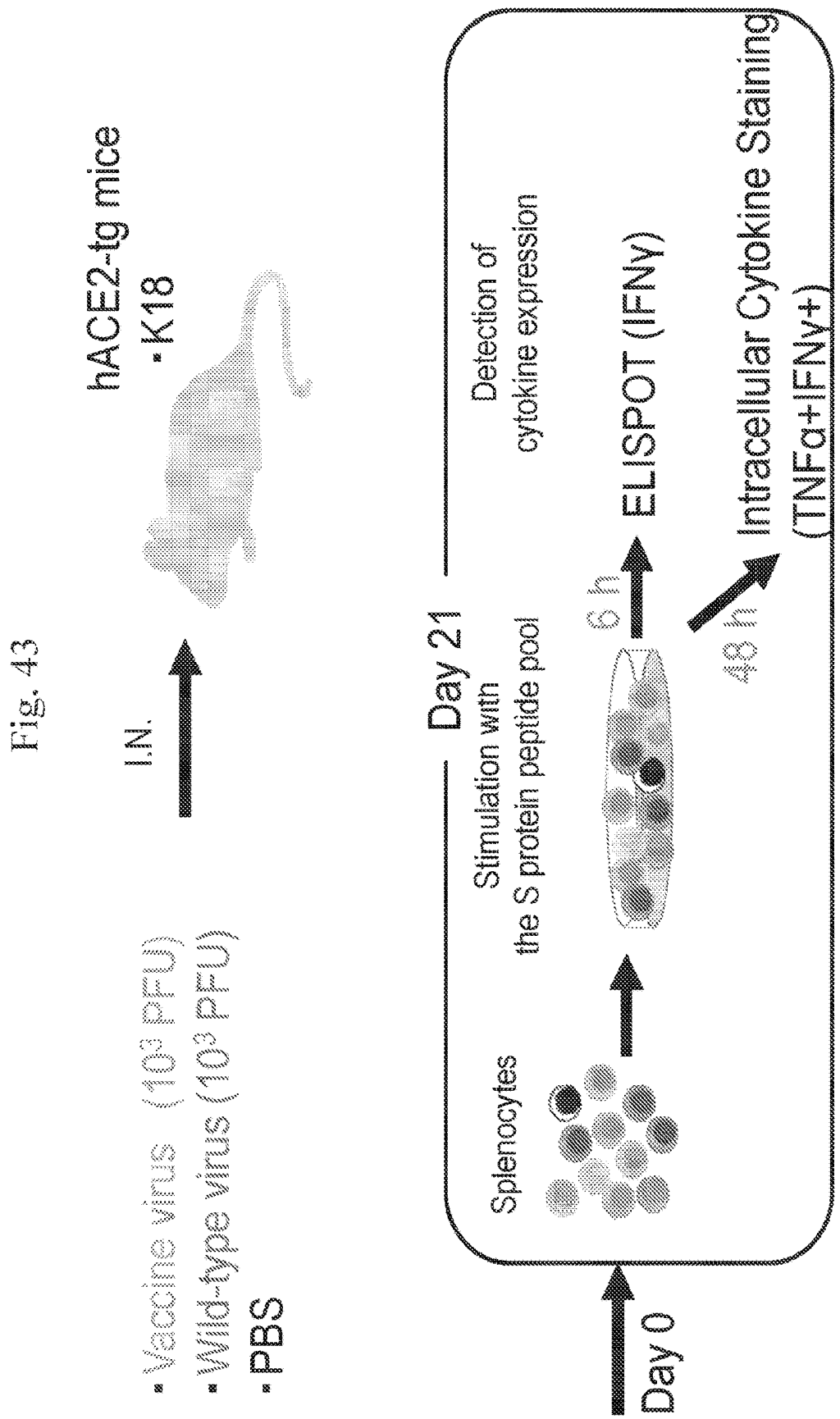
hACE2-tg mice
K18
I.N.
Vaccine virus (10³ PFU)
Wild-type virus (10³ PFU)
PBS
Day 0
Day 21
Splenocytes
Stimulation with the S protein peptide pool
6 h
48 h
ELISPOT (IFNγ)
Intracellular Cytokine Staining (TNFα+IFNγ+)
Detection of cytokine expression

Fig. 44

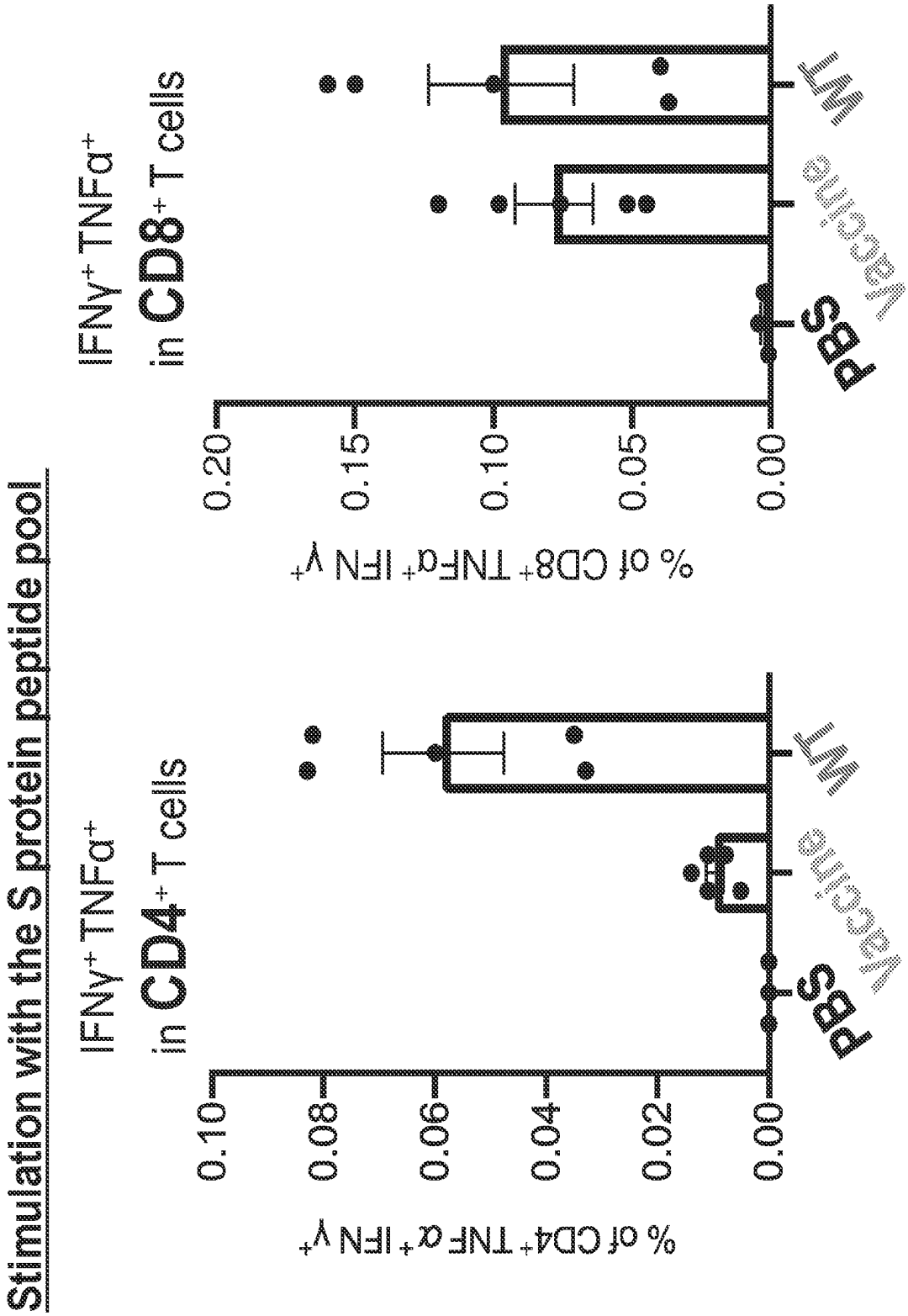
Stimulation with the S protein peptide pool
IFNγ+ TNFα+
in CD4+ T cells
% of CD4+ TNFα+ IFN γ+
0.10
0.08
0.06
0.04
0.02
0.00
PBS
Vaccine
IFNγ+ TNFα+
in CD8+ T cells
% of CD8+ TNFα+ IFN γ+
0.20
0.15
0.10
0.05
0.00
PBS
Vaccine

Fig. 45

Stimulation with the S protein peptide pool

- attenuation of the vaccine virus in hACE2-tg mice was confirmed
- Vaccine virus elicited Th1-dominant immune responses including antibodies and cellular immunity.

Fig. 46

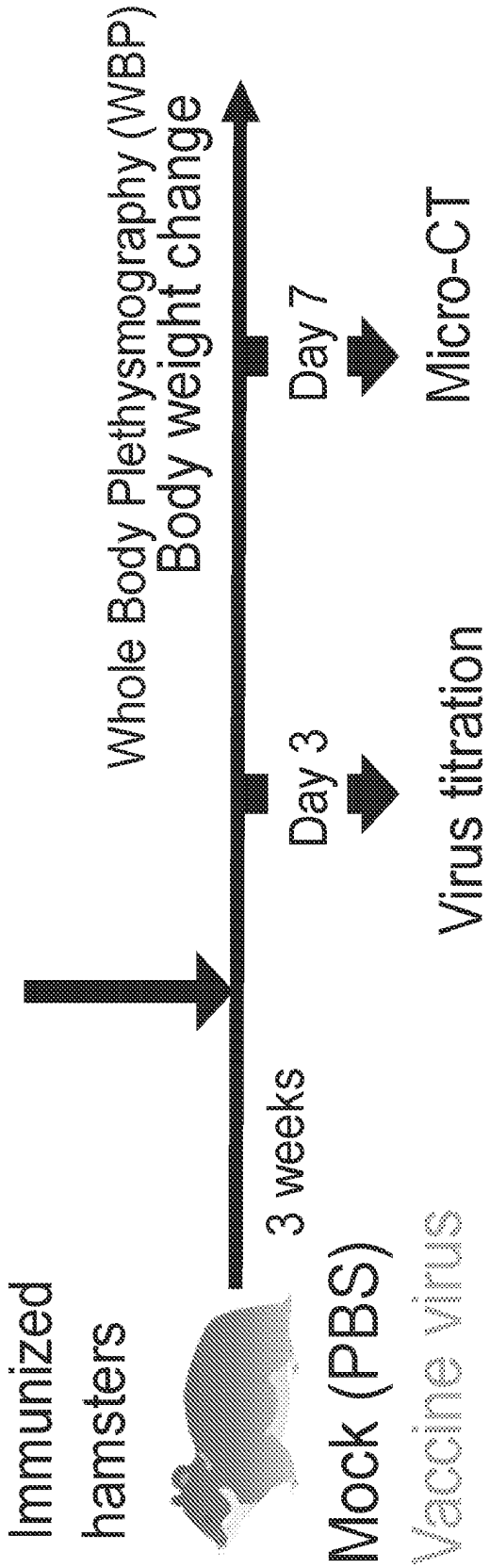
Challenge with 10^5 PFU
Wuhan-like, Delta, or BA.1
Whole Body Plethysmography (WBP)
Body weight change
Day 7
Micro-CT
Day 3
Virus titration
3 weeks
Immunized hamsters
Mock (PBS)
Vaccine virus

Fig. 47

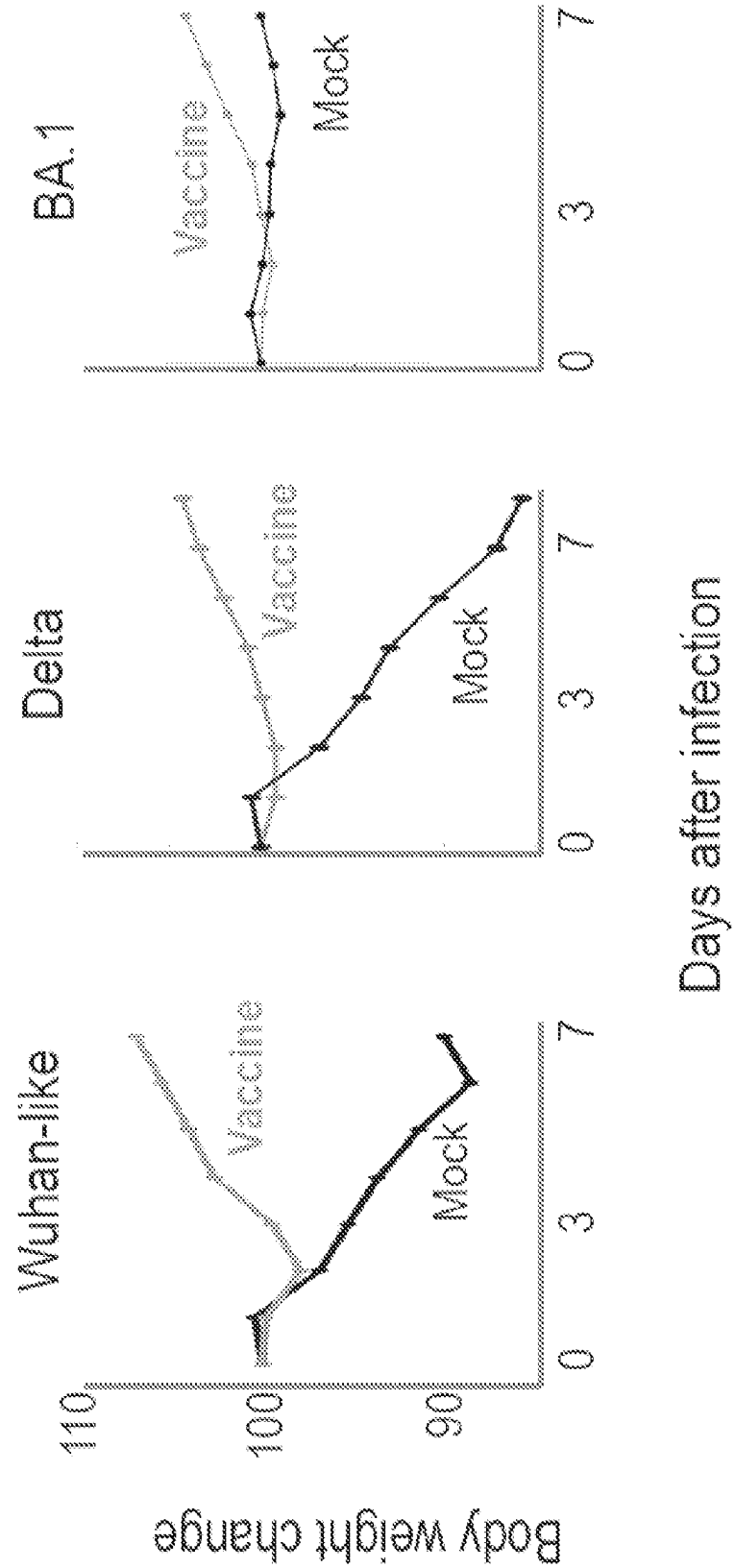
Wuhan-like
Delta
BA.1
Vaccine
Mock
110
100
90
0
3
7
Body weight change
Days after infection

Fig. 48

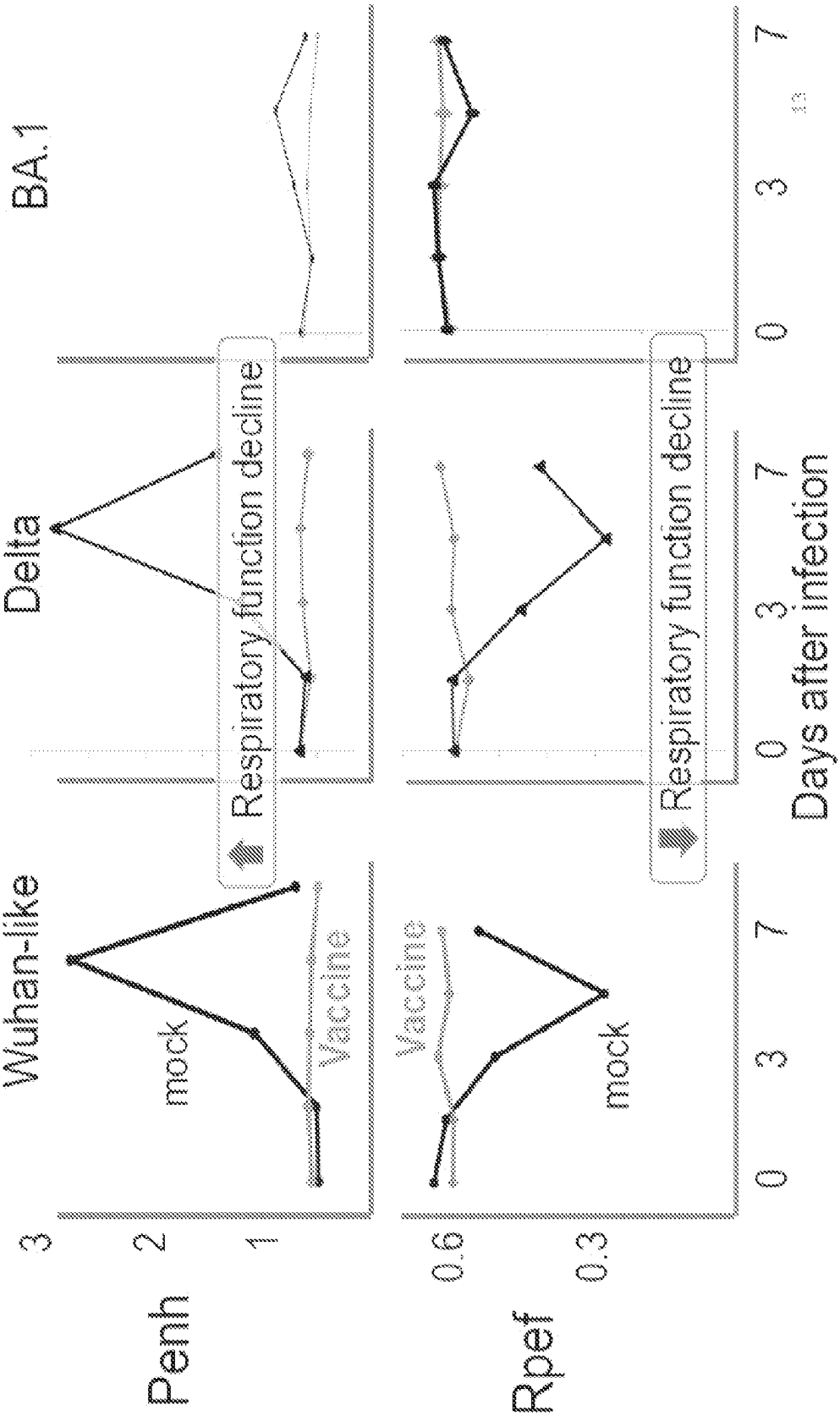
Wuhan-like
Delta
BA.1
Penh
Rpef
mock
Vaccine
Respiratory function decline
Days after infection

Fig. 49

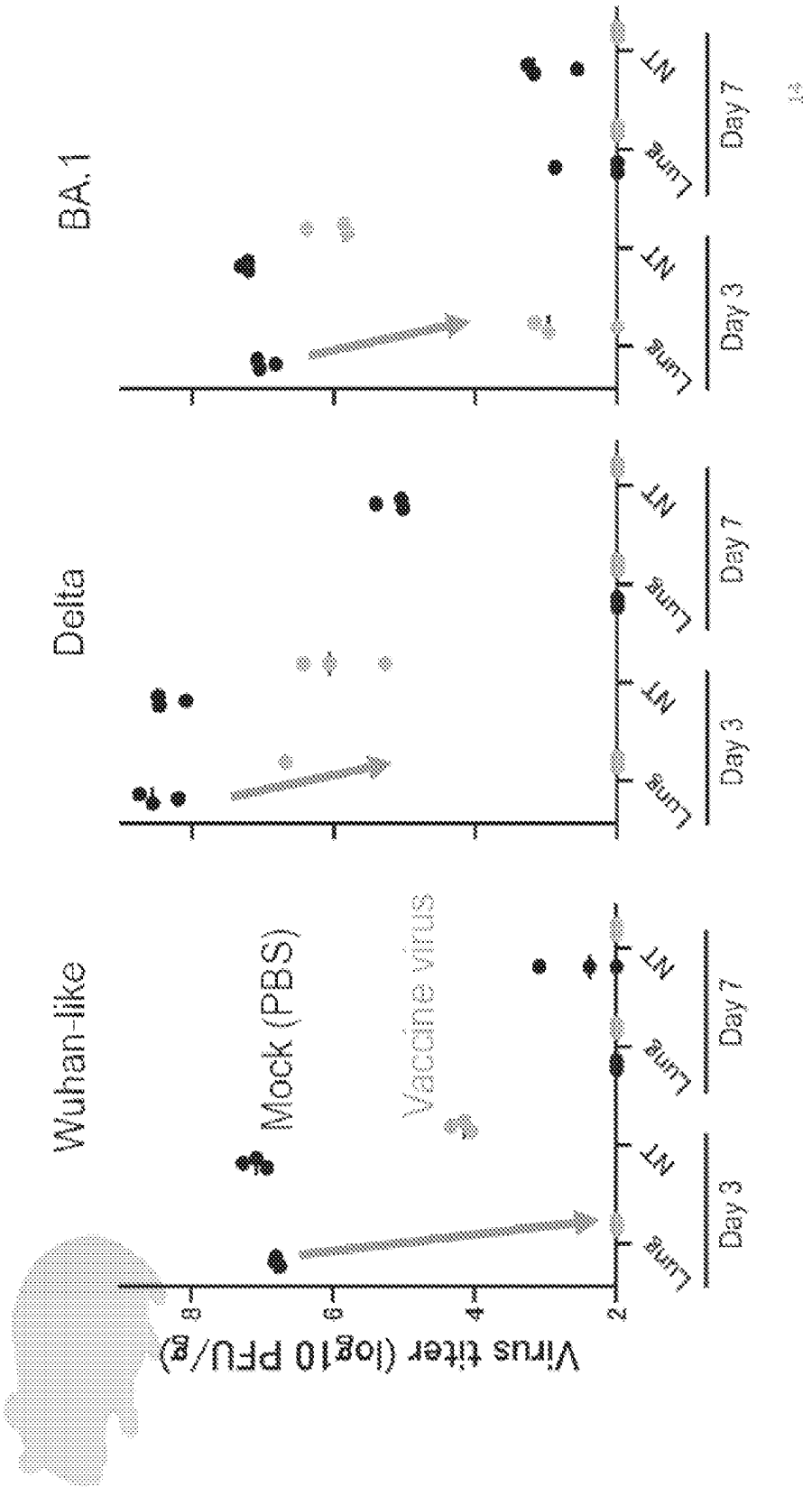
Wuhan-like
Delta
BA.1
Mock (PBS)
Vaccine virus
Virus titer (log10 PFU/g)
8
6
4
2
Lung
NT
Day 3
Day 7

Fig. 50

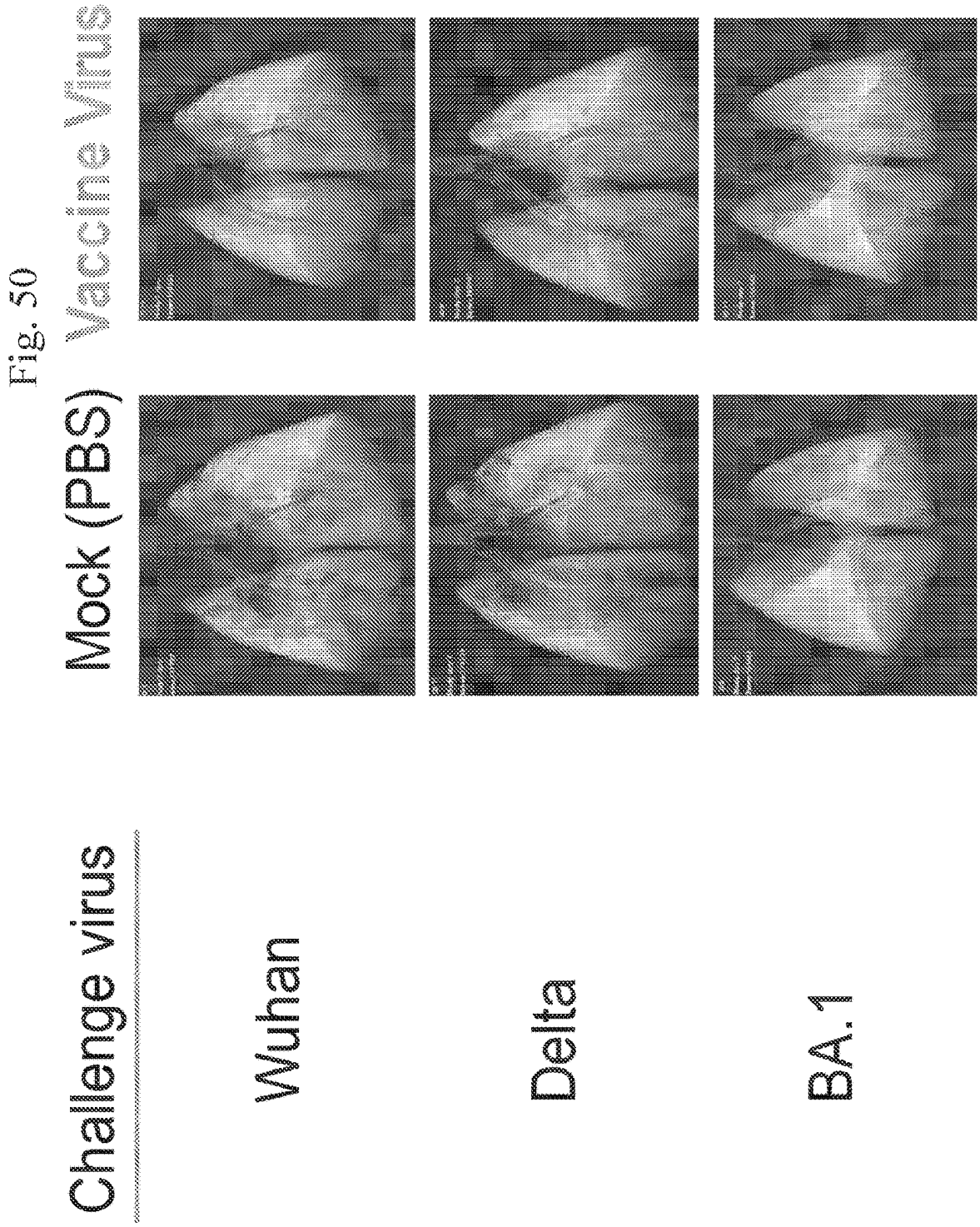

Fig. 51

Virus	Amino acid substitutions			
	Nsp1 85	Nsp3 272	Nsp6 260	S 1094
Vaccine candidate	Del	N	F	F
nsp1-M85ins	M	N	F	F
nsp3-N272Y	Del	Y	F	F
nsp6-F260L	Del	N	L	F
[illegible]	Del	N	F	V
Wild-type	M	Y	L	V

Fig. 52

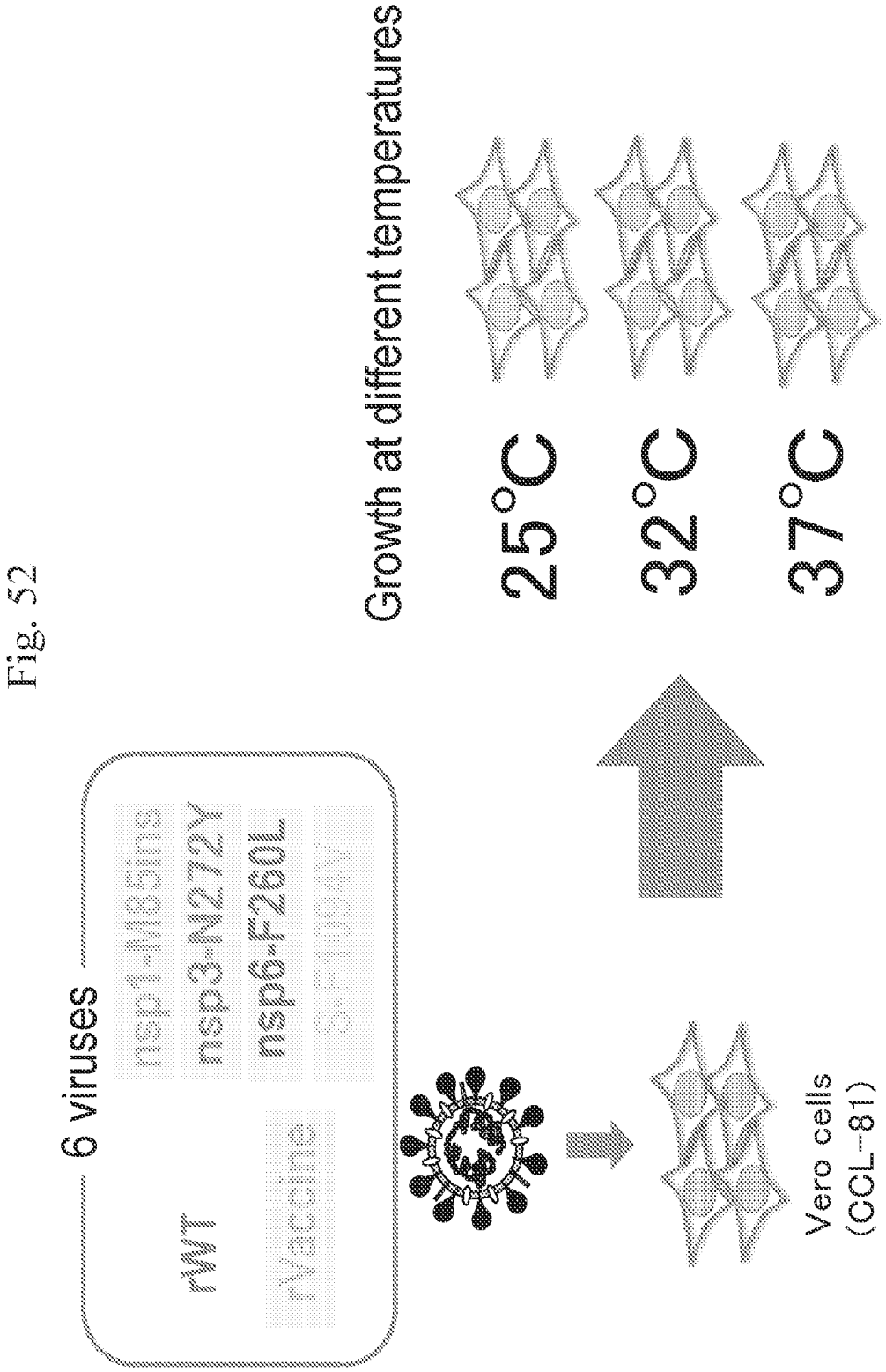
6 viruses
rWT
nsp1-M85ins
nsp3-N272Y
nsp6-F260L
Vaccine
Vero cells
(CCL-81)
Growth at different temperatures
25°C
32°C
37°C

Fig. 53

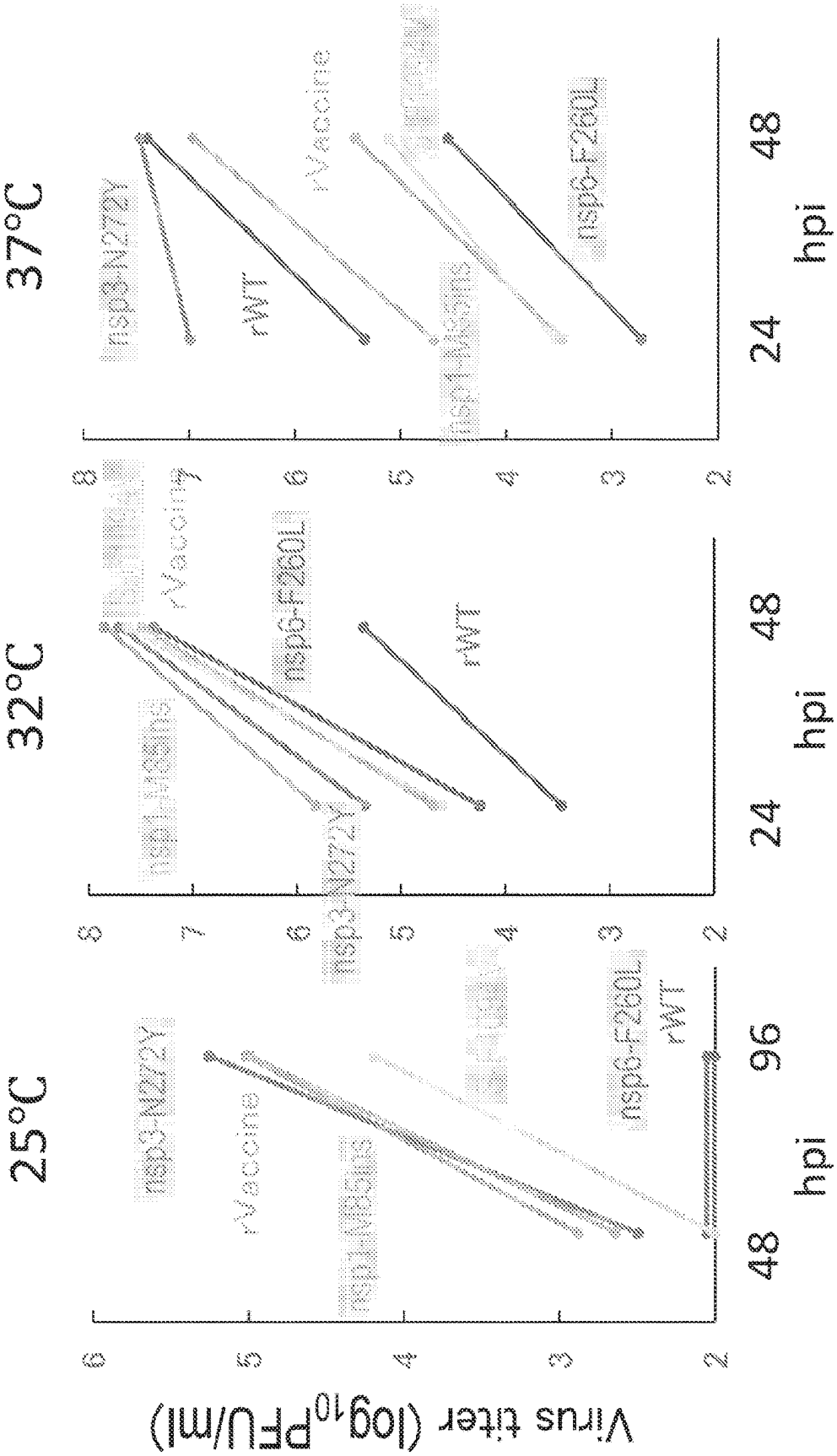
25°C
32°C
37°C
Virus titer (log10PFU/ml)
hpi
48
96
24
nsp3-N272Y
rVaccine
nsp6-F260L
rWT

Fig. 54

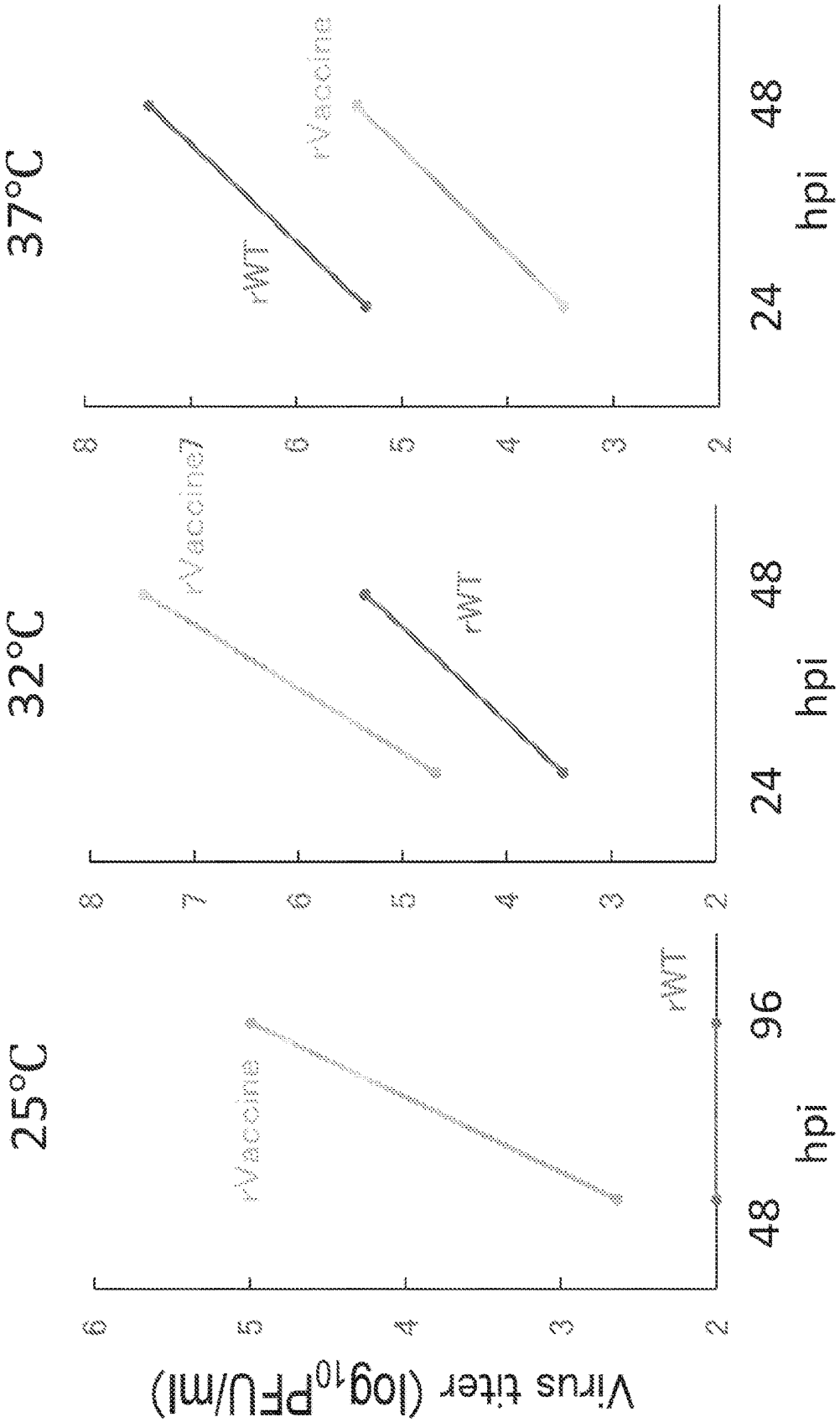
Virus titer (log_{10}PFU/ml)
25°C
32°C
37°C
rVaccine
rWT
rVaccine7
48
96
24
hpi

Fig. 55

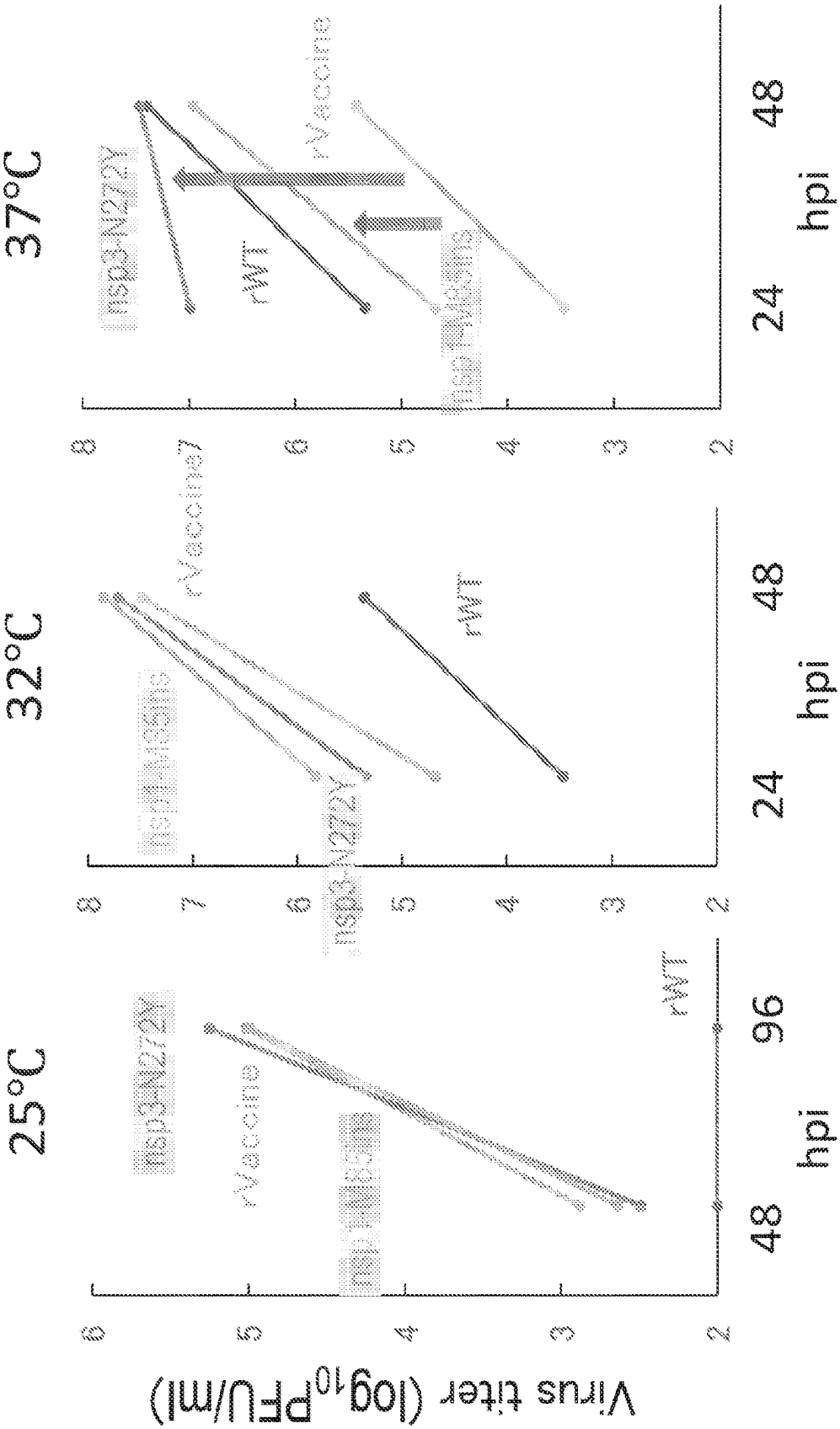
25°C
32°C
37°C
Virus titer (log10PFU/ml)
hpi
48
96
24
48
rVaccine
rWT
nsp3-N272Y

Fig.56

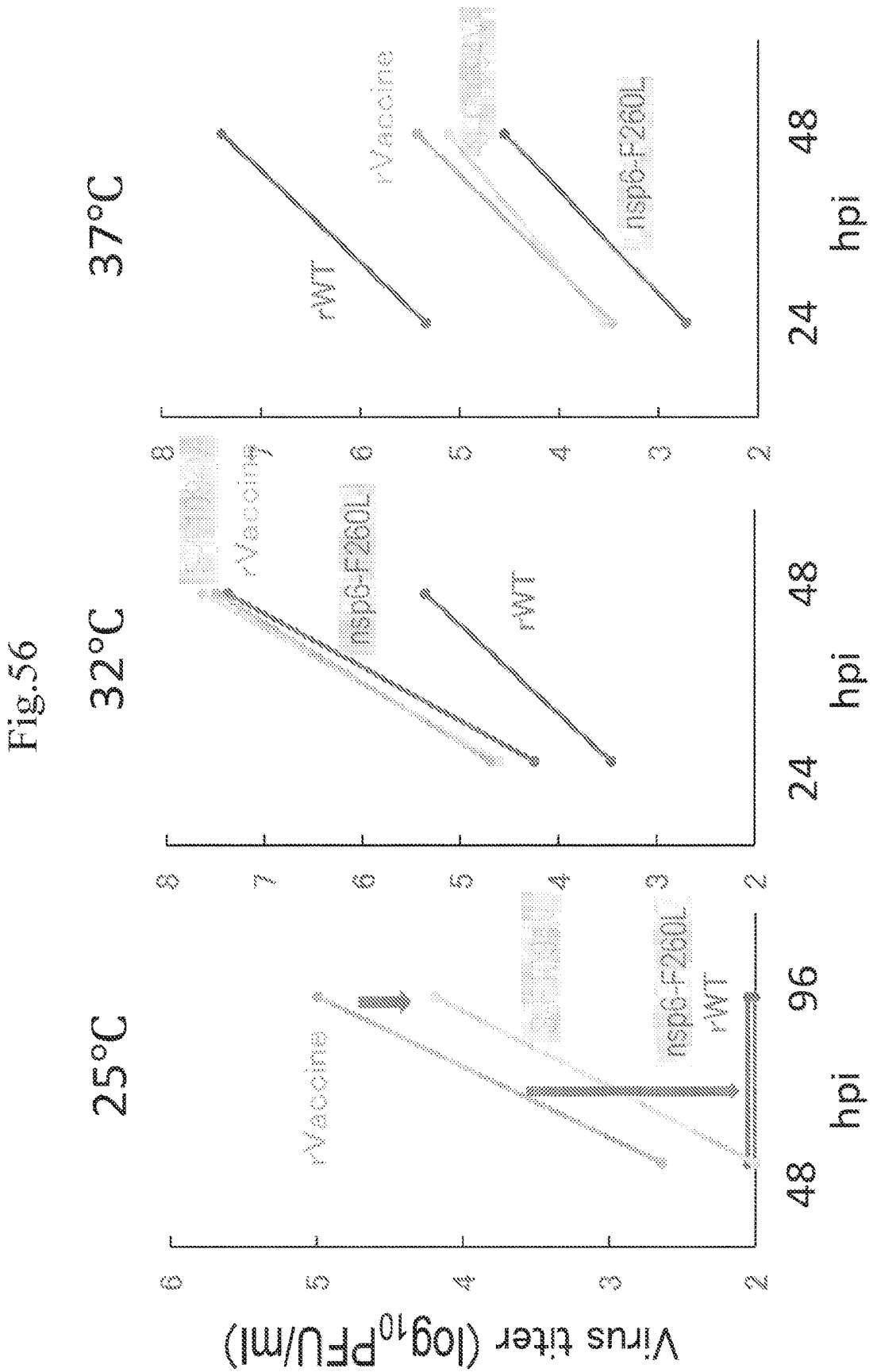
25°C
32°C
37°C
Virus titer (log_{10}PFU/ml)
hpi
48
96
24
rVaccine
nsp6-F260L
rWT

Fig. 57

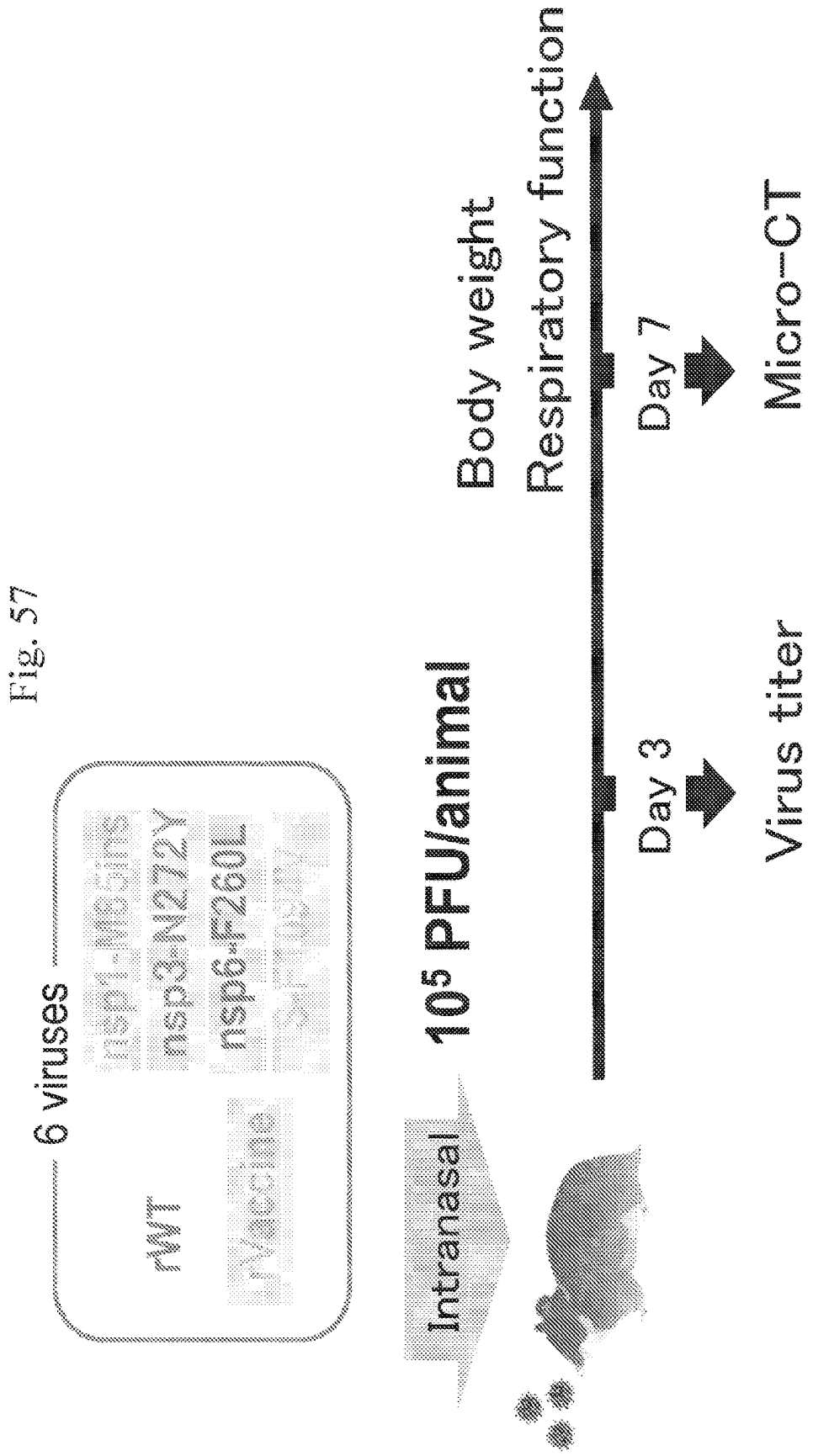
6 viruses
rWT
nsp3-N272Y
nsp6-F260L
Intranasal
10^5 PFU/animal
Body weight
Respiratory function
Day 3
Virus titer
Day 7
Micro-CT

Fig. 58

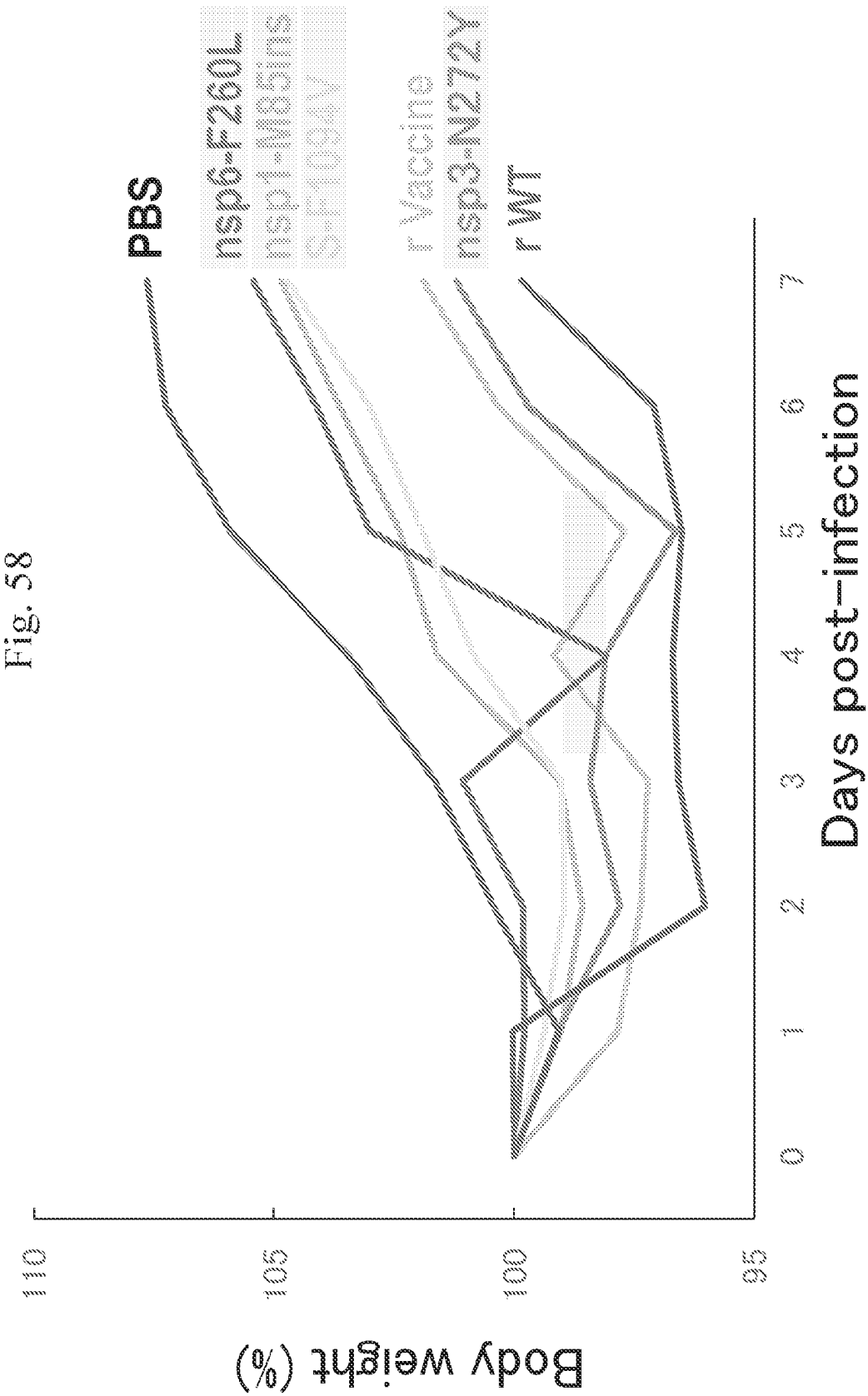
PBS
nsp6-F260L
nsp1-M85ins
S-F1094V
r Vaccine
nsp3-N272Y
r WT
Body weight (%)
110
105
100
95
0
1
2
3
4
5
6
7
Days post-infection

Fig. 59

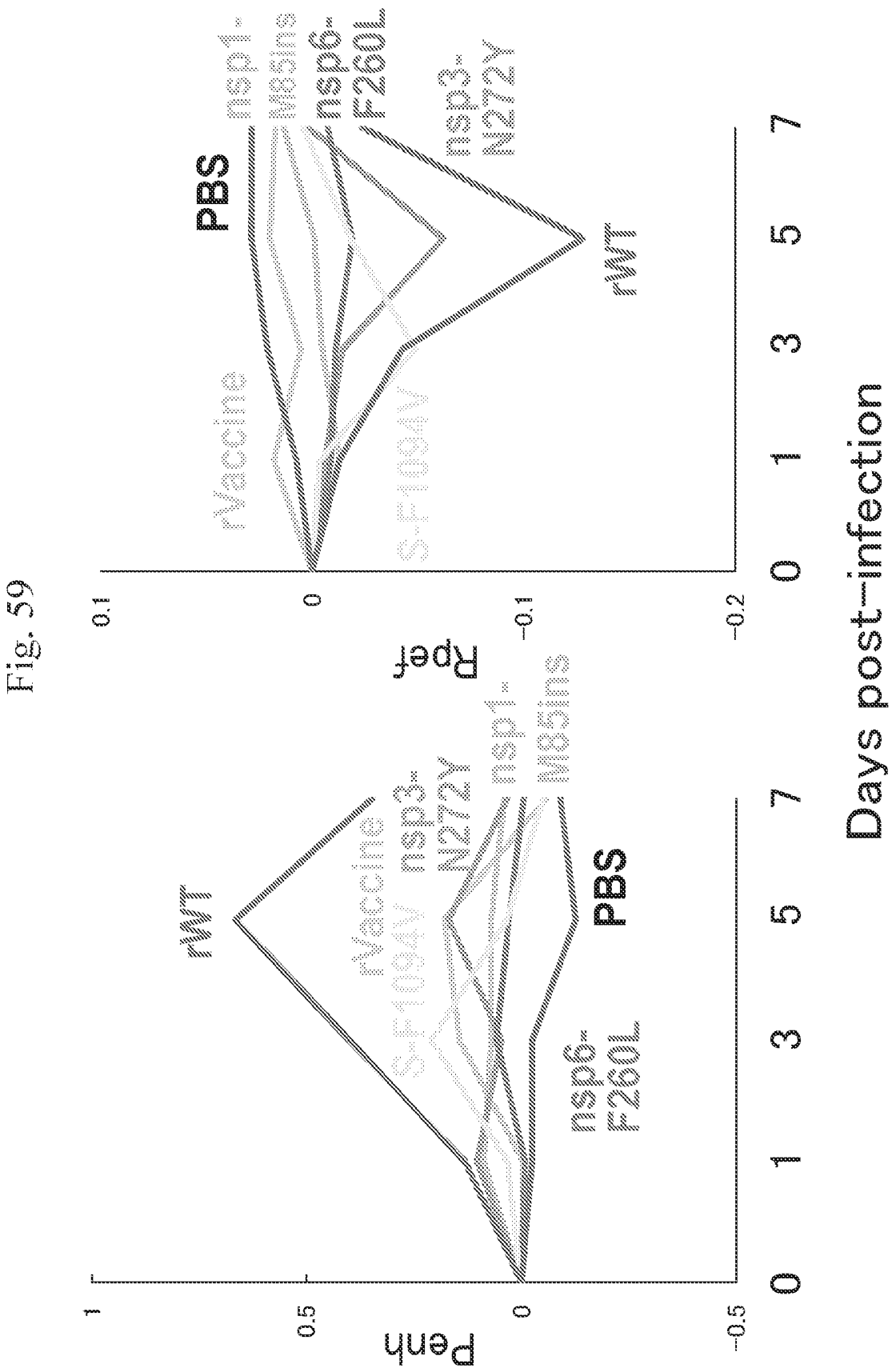
Penh
1
0.5
0
−0.5
rWT
rVaccine
S-F1094V
nsp3-
N272Y
nsp1-
M85ins
PBS
nsp6-
F260L
Rpef
0.1
0
−0.1
−0.2
PBS
nsp1-
M85ins
nsp6-
F260L
nsp3-
N272Y
rVaccine
S-F1094V
rWT
0
1
3
5
7
Days post−infection

Fig. 60

Nsp3-N272Y and nsp1-M85ins are correlated with attenuation in hamsters

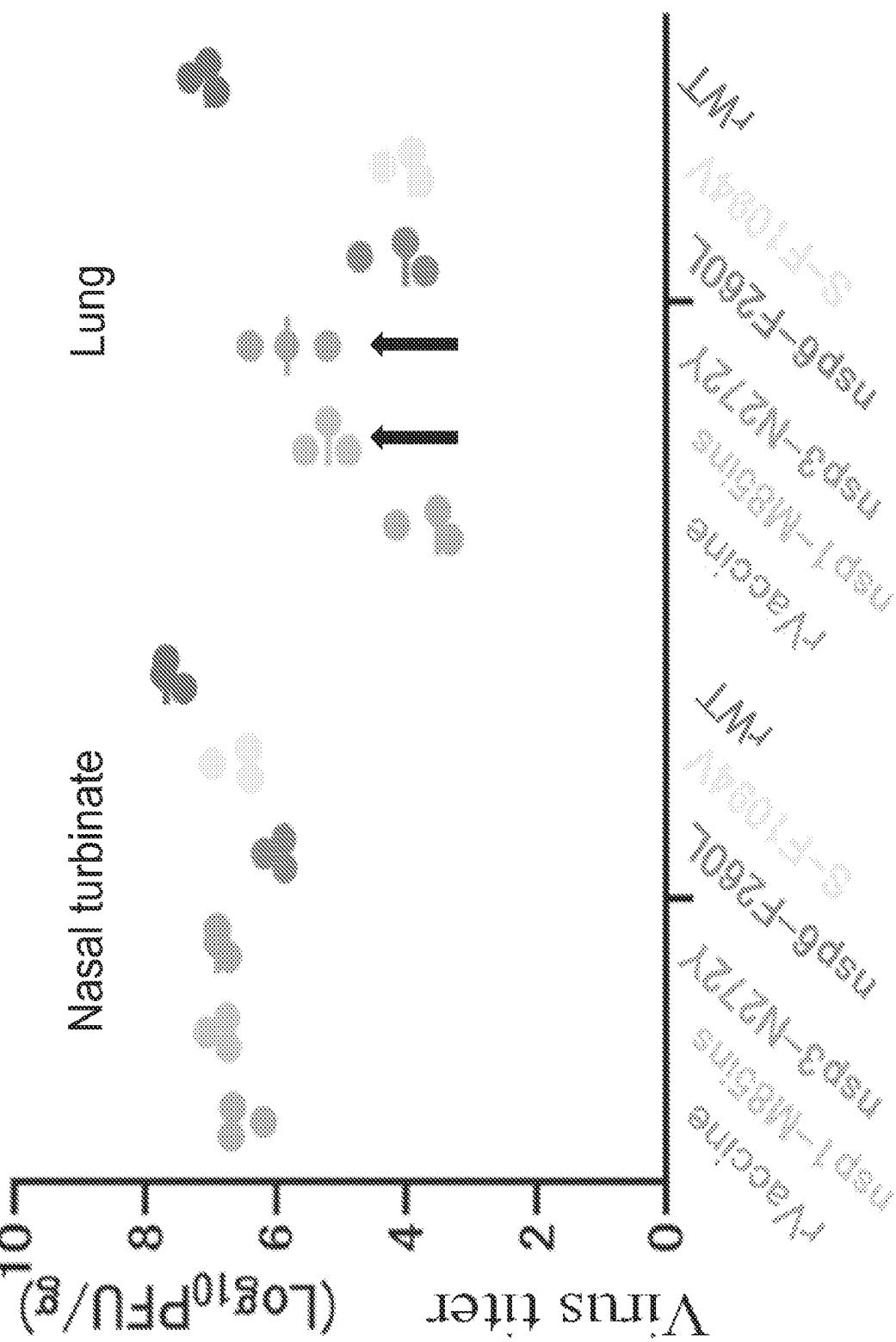

Fig. 61A

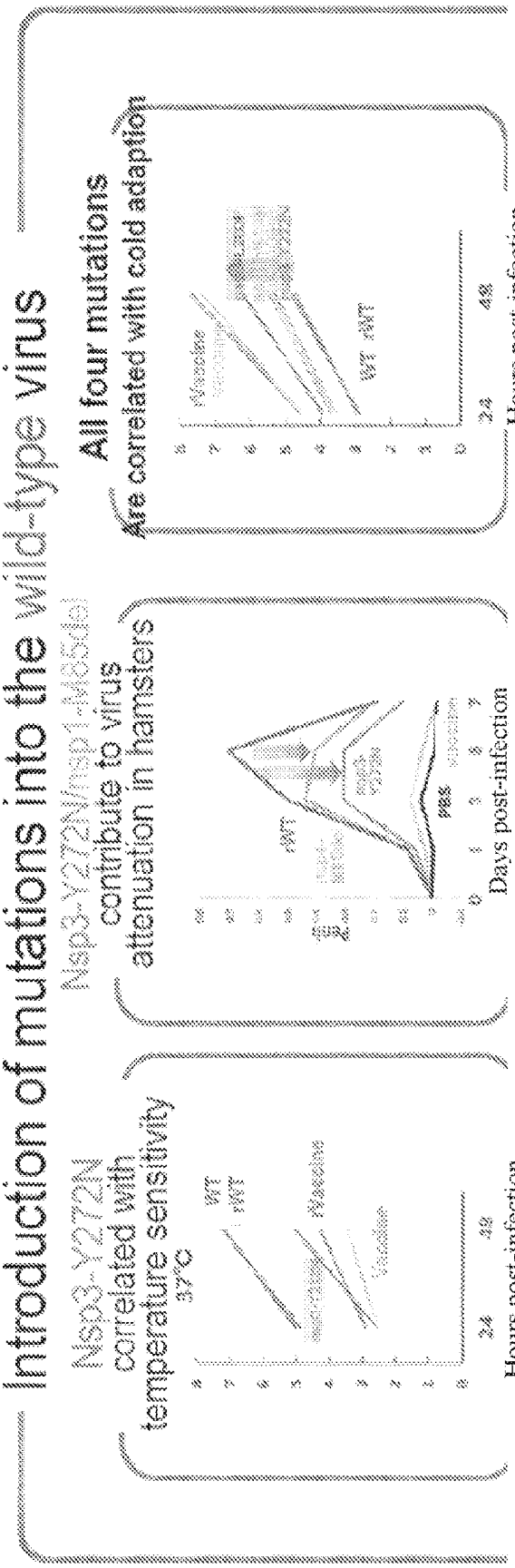
Introduction of mutations into the wild-type virus
Nsp3-Y272N
correlated with
temperature sensitivity
37°C
WT
rWT
Vaccine
Hours post-infection
Nsp3-Y272N/nsp1-Δ85del
contribute to virus
attenuation in hamsters
rWT
Days post-infection
All four mutations
are correlated with cold adaption
Vaccine
WT rWT
Hours post-infection

Fig. 61B

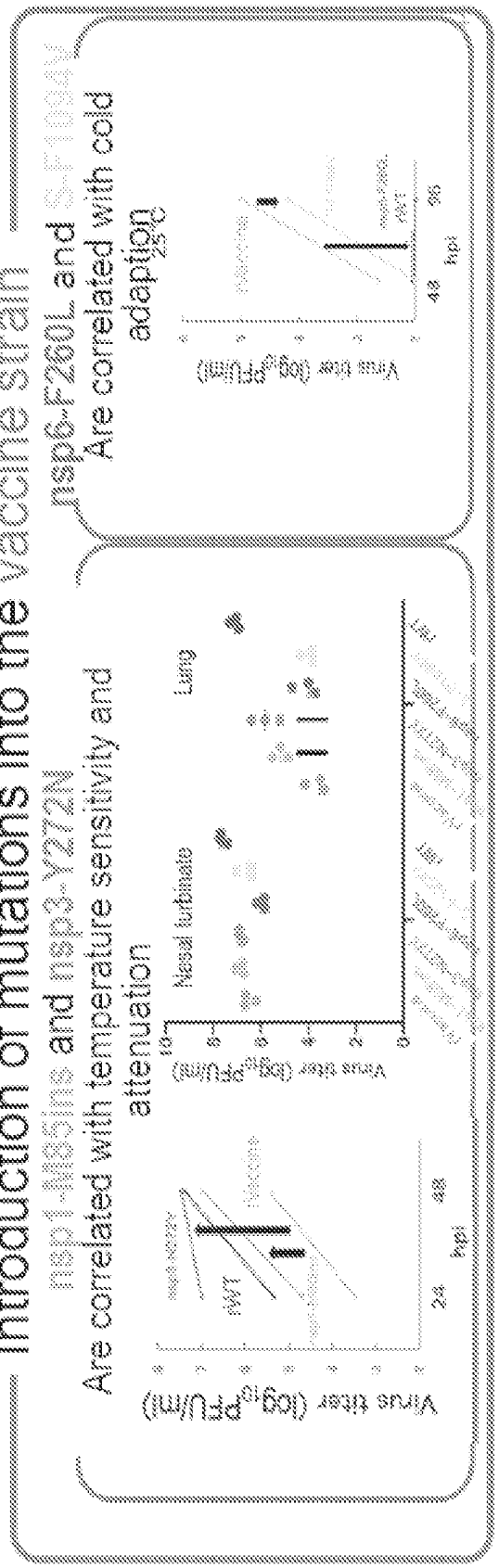
Introduction of mutations into the vaccine strain
nsp1-M85ins and nsp3-Y272N
Are correlated with temperature sensitivity and attenuation
Virus titer (log10PFU/ml)
rWT
Nasal turbinate
Lung
hpi
nsp6-F260L and S-F1094V
Are correlated with cold adaption
25°C
Virus titer (log10PFU/ml)
rWT
hpi

Fig. 62A

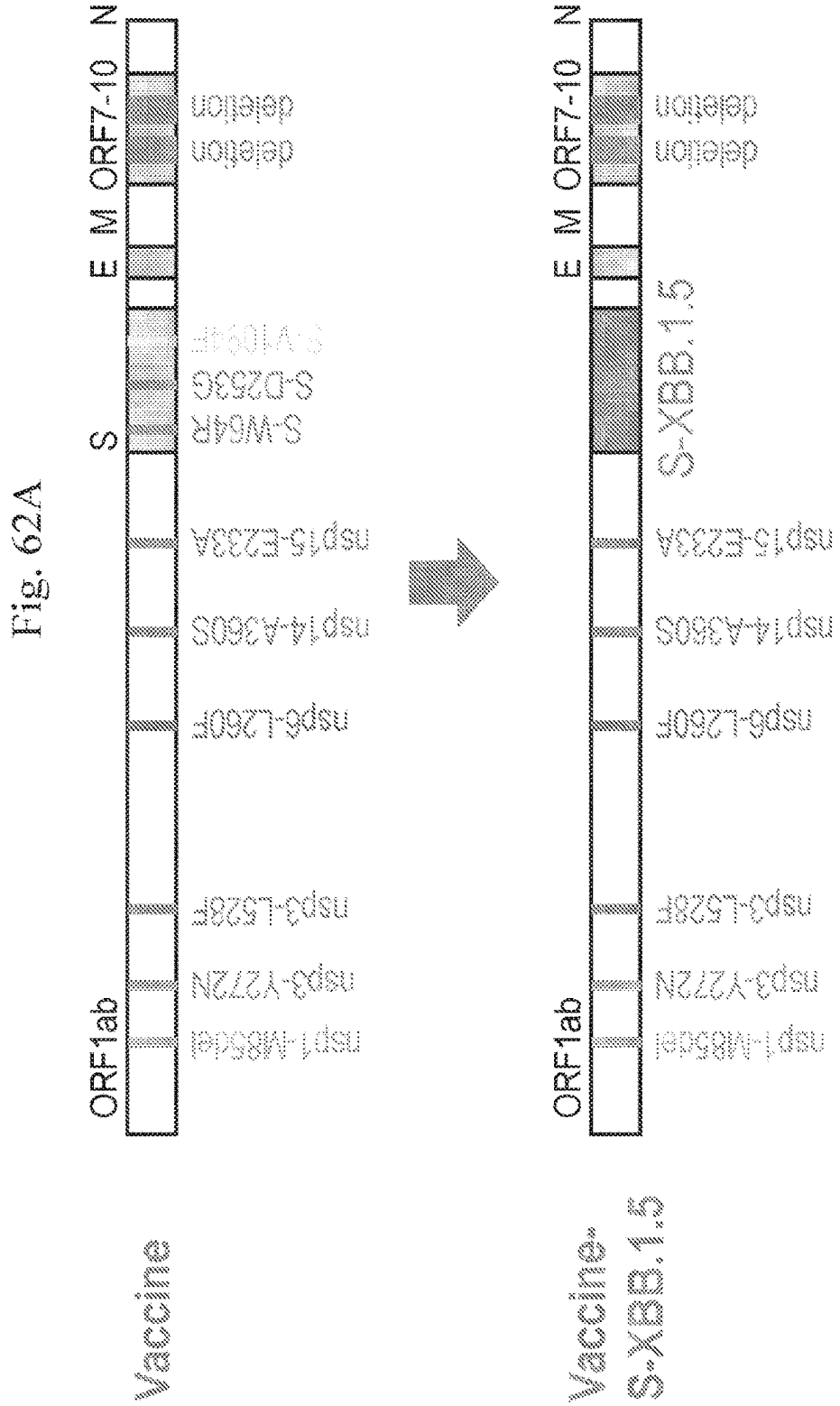
Vaccine
ORF1ab
S
E M ORF7-10 N
nsp1-M85del
nsp3-Y27N
nsp3-L528F
nsp6-L260F
nsp14-A360S
nsp15-E233A
S-W64R
S-D253G
deletion
deletion
Vaccine*
S-XBB.1.5
ORF1ab
S-XBB.1.5
E M ORF7-10 N
nsp1-M85del
nsp3-Y27N
nsp3-L528F
nsp6-L260F
nsp14-A360S
nsp15-E233A
deletion
deletion

FIG. 62B

Full genome sequence of EG.5.1

AGATCTGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATA
ATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCG
TGTTGCAGCCGATCATCAGCACATCTAGGTTTTGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCT
GGTTTCAACGAGAAAACACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTACGTGGCTTTG
GAGACTCCGTGGAGGAGGTCTTATCAGAGGCACGTCAACATCTTAGAGATGGCACTTGTGGCTTAGTAGAAGTTG
AAAAAGGCGTTTTGCCTCAACTTGAACAGCCCTATGTGTTCATCAAACGTTCGGATGCTCGAACTGCACCTCATGG
TCATGTTATGGTTGAGCTGGTAGCAGAACTCGAAGGCATTCAGTACGGTCGTAGTGGTGAGACACTTGGTGTCCTT
GTCCCTCATGTGGGCGAAATACCAGTGGCTTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAAGGAGCTGGT
GGCCATAGGTACGGCGCCGATCTAAAGTCATTTGACTTAGGCGACGAGCTTGGCACTGATCCTTATGAAGATTTTC
AAGAAAACTGGAACACTAAACATAGCAGTGGTGTTACCCGTGAACTCATGCGTGAGCTTAACGGAGGGGCATACA
CTCGCTATGTCGATAACAACTTCTGTGGCCCTGATGGCTACCCTCTTGAGTGCATTAAAGACCTTCTAGCACGTGCT
GGTAAAGCTTCATGCACTTTGTCCGAACAACTGGACTTTATTGACACTAAGAGGGGTGTATACTGCTGCCGTGAAC
ATGAGCATGAAATTGCTTGGTACACGGAACGTTCTGAAAAGAGCTATGAATTGCAGACACCTTTTGAAATTAAATT
GGCAAAGAAATTTGACACCTTCAATGGGGAATGTCCAAATTTTGTATTTCCCTTAAATTCCATAATCAAGACTATTC
AACCAAGGGTTGAAAAGAAAAAGCTTGATGGCTTTATGGGTAGAATTCGATCTGTCTATCCAGTTGCGTCACCAAA
TGAATGCAACCAAATGTGCCTTTCAACTCTCATGAAGTGTGATCATTGTGGTGAAACTTCATGGCAGACGGGCGAT
TTTGTTAAAGCCACTTGCGAATTTTGTGGCACTGAGAATTTGACTAAAGAAGGTGCCACTACTTGTGGTTACTTACC
CCAAAATGCTGTTGTTAAAATTTATTGTCCAGCATGTCACAATTCAGAAGTAGGACCTGAGCATAGTCTTGCCGAA
TACCATAATGAATCTGGCTTGAAAACCATTCTTCGTAAGGGTGGTCGCACTATTGCCTTTGGAGGCTGTGTGTTCTC
TTATGTTGGTTGCCATAACAAGTGTGCCTATTGGGTTCCACGTGCTAGCGCTAACATAGGTTGTAACCATACAGGT
GTTGTTGGAGAAGGTTCCGAAGGTCTTAATGACAACCTTCTTGAAATACTCCAAAAAGAGAAAGTCAACATCAATA
TTGTTGGTGACTTTAAACTTAATGAAGAGATCGCCATTATTTTGGCATCTTTTTCTGCTTCCACAAGTGCTTTTGTGG
AAACTGTGAAAGGTTTGGATTATAAAGCATTCAAACAAATTGTTGAATCCTGTGGTAATTTTAAAGTTACAAAAGG
AAAAGCTAAAAAAGGTGCCTGGAATATTGGTGAACAGAAATCAATACTGAGTCCTCTTTATGCATTTGCATCAGAG
GCTGCTCGTGTTGTACGATCAATTTTCTCCCGCACTCTTGAAACTGCTCAAAATTCTGTGCGTGTTTTACAGAAGGC
CGCTATAACAATACTAGATGGAATTTCACAGTATTCACTGAGACTCATTGATGCTATGATGTTCACATCTGATTTGG
CTACTAACAATCTAGTTGTAATGGCCTACATTACAGGTGGTGTTGTTCAGTTGACTTCGCAGTGGCTAACTAACATC
TTTGGCACTGTTTATGAAAAACTCAAACCCGTCCTTGATTGGCTTGAAGAGAAGTTTAAGGAAGGTGTAGAGTTTC
TTAGAGACGGTTGGGAAATTGTTAAATTTATCTCAACCTGTGCTTGTGAAATTGTCGGTGGACAAATTGTCACCTG
TGCAAAGGAAATTAAGGAGAGTGTTCAGACATTCTTTAAGCTTGTAAATAAATTTTTGGCTTTGTGTGTTGACTCTA
TCATTATTGGTGGAGCTAAACTTAAAGCCTTGAATTTAGGTGAAACATTTGTCACGCACTCAAAGGGATTGTACAG
AAAGTGTGTTAAATCCAGAGAAGAAACTGGCCTACTCATGCCTCTAAAAGCCCCAAAAGAAATTATCTTCTTAGAG
GGAGAAACACTTCCCACAGAAGTGTTAACAGAGGAAGTTGTCTTGAAAACTGGTGATTTACAACCATTAGAACAA
CCTACTAGTGAAGCTGTTGAAGCTCCATTGGTTGGTACACCAGTTTGTATTAACGGGCTTATGTTGCTCGAAATCAA
AGACACAGAAAAGTACTGTGCCCTTGCACCTAATATGATGGTAACAAACAATACCTTCACACTCAAAGGCGGTGCA
CCAACAAAGGTTACTTTTGGTGATGACACTGTGATAGAAGTGCAAGGTTACAAGAGTGTGAATATCATTTTTGAAC
TTGATGAAAGGATTGATAAAGTACTTAATGAGAAGTGCTCTGCCTATACAGTTGAACTCGGTACAGAAGTAAATG
AGTTCGCCTGTGTTGTGGCAGATGCTGTCATAAAAACTTTGCAACCAGTATCTGAATTACTTACACCACTGGGCATT
GATTTAGATGAGTGGAGTATGGCTACATACTACTTATTTGATGAGTCTGGTGAGTTTAAATTGGCTTCACATATGTA
TTGTTCTTTTTACCCTCCAGATGAGGATGAAGAAGAAGGTGATTGTGAAGAAGAAGAGTTTGAGCCATCAACTCA
ATATGAGTATGGTACTGAAGATGATTACCAAGGTAAACCTTTGGAATTTGGTGCCACTTCTGCTGCTCTTCAACCTG
AAGAAGAGCAAGAAGAAGATTGGTTAGATGATGATAGTCAACAAACTGTTGGTCAACAAGACGGCAGTGAGGAC
AATCAGACAACTACTATTCAAACAATTGTTGAGGTTCAACCTCAATTAGAGATGGAACTTACACCAGTTGTTCAGAC
TATTGAAGTGAATAGTTTTAGTGGTTATTTAAAACTTACTGACAATGTATACATTAAAAATGCAGACATTGTGGAA
GAAGCTAAAAAGGTAAAACCAACAGTGGTTGTTAATGCAGCCAATGTTTACCTTAAACATGGAGGAGGTGTTGCA
GGAGCCTTAAATAAGGCTACTAACAATGCCATGCAAGTTGAATCTGATGATTACATAGCTACTAATGGACCACTTA

FIG. 62B continued

AAGTGGGTGGTAGTTGTGTTTTAAGCGGACACAATCTTGCTAAACACTGTCTTCATGTTGTCGGCCCAAATGTTAA
CAAAGGTGAAGACATTCAACTTCTTAAGAGTGCTTATGAAAATTTTAATCAGCACGAAGTTCTACTTGCACCATTAT
TATCAGCTGGTATTTTTGGTGCTGACCCTATACATTCTTTAAGAGTTTGTGTAGATACTGTTCGCACAAATGTCTACT
TAGCTGTCTTTGATAAAAATCTCTATGACAAACTTGTTTCAAGCTTTTTGGAAATGAAGAGTGAAAAGCAAGTTGA
ACAAAAGATCGCTGAGATTCCTAAAGAGGAAGTTAAGCCATTTATAACTGAAAGTAAACCTTCAGTTGAACAGAG
AAAACAAGATGATAAGAAAATCAAAGCTTGTGTTGAAGAAGTTACAACAACTCTGGAAGAAACTAAGTTCCTCAC
AGAAAACTTGTTACTTTATATTGACATTAATGGCAATCTTCATCCAGATTCTGCCACTCTTGTTAGTGACATTGACAT
CACTTTCTTAAAGAAAGATGCTCCATATATAGTGGGTGATGTTGTTCAAGAGGGTGTTTTAACTGCTGTGGTTATAC
CTACTAAAAAGGCTAGTGGCACTACTGAAATGCTAGCGAAAGCTTTGAGAAAAGTGCCAACAGACAATTATATAA
CCACTTACCCGGGTCAGGGTTTAAATGGTTACACTGTAGAGGAGGCAAAGACAGTGCTTAAAAAGTGTAAAAGTG
CTTTTTACATTCTACCATCTATTATCTCTAATGAGAAGCAAGAAATTCTTGGAACTGTTTCTTGGAATTTGCGAGAAA
TGCTTGCACATGCAGAAGAAACACGCAAATTAATGCCTGTCTGTGTGGAAACTAAAGCCATAGTTTCAACTATACA
GCGTAAATATAAGGGTATTAAAATACAAGAGGGTGTGGTTGATTATGGTGCTAGATTTTACTTTTACACCAGTAAA
ACAACTGTAGCGTCACTTATCAACACACTTAACGATCTAAATGAAACTCTTGTTACAATGCCACTTGGCTATGTAAC
ACATGGCTTAAATTTGGAAGAAGCTGCTCGGTATATGAGATCTCTCAAAGTGCCAGCTACAGTTTCTGTTTCTTCAC
CTGATGCTGTTACAGCGTATAATGGTTATCTTACTTCTTCTTCTAAAACACCTGAAGAACATTTTATTGAAACCATCT
CACTTGCTGGTTCCTATAAAGATTGGTCCTATTCTGGACAATCTACACAACTAGGTATAGAATTTCTTAAGAGAGGT
GATAAAAGTGTATATTACACTAGTAATCCTACCACATTCCACCTAGATGGTGAAGTTATCACCTTTGACAATCTTAA
GACACTTCTTTCTTTGAGAGAAGTGAGGACTATTAAGGTGTTTACAACAGTAGACAACATTAACCTCCACACGCAA
GTTGTGGACATGTCAATGACATATGGACAACAGTTTGGTCCAACTTATTTGGATGGAGCTGATGTTACTAAAATAA
AACCTCATAATTCACATGAAGGTAAAACATTTTATGTTTTACCTAATGATGACACTCTACGTGTTGAGGCTTTTGAG
TACTACCACACAACTGATCCTAGTTTTCTGGGTAGGTACATGTCAGCATTAAATCACACTAAAAAGTGGAAATACCC
ACAAGTTAATGGTTTAACTTCTATTAAATGGGCAGATAACAACTGTTATCTTGCCACTGCATTGTTAACACTCCAAC
AAATAGAGTTGAAGTTTAATCCACCTGCTCTACAAGATGCTTATTACAGAGCAAGGGCTGGTGAAGCTGCTAACTT
TTGTGCACTTATCTTAGCCTACTGTAATAAGACAGTAGGTGAGTTAGGTGATGTTAGAGAAACAATGAGTTACTTG
TTTCAACATGCCAATTTAGATTCTTGCAAAAGAGTCTTGAACGTGGTGTGTAAAACTTGTGGACAACAGCAGACAA
CCCTTAAGGGTGTAGAAGCTGTTATGTACATGGGCACACTTTCTTATGAACAATTTAAGAAAGGTGTTCAGATACC
TTGTACGTGTGGTAAACAAGCTACAAAATATCTAGTACAACAGGAGTCACCTTTTGTTATGATGTCAGCACCACCT
GCTCAGTATGAACTTAAGCATAGTACATTTACTTGTGCTAGTGAGTACACTGGTAATTACCAGTGTGGTCACTATAA
ACATATAACTTCTAAAGAAACTTTGTATTGCATAGACGGTGCTTTACTTACAAAGTCCTCAGAATACAAAGGTCCTA
TTACGGATGTTTTCTACAAAGAAAACAGTTACACAACAACCATAAAACCAGTTACTTATAAATTGGATGGTGTTGTT
TGTACAGAAATTGACCCTAAGTTGGACAATTATTATAAGAAAGACAATTCTTATTTCACAGAGCAACCAATTGATCT
TGTACCAAACCAACCATATCCAAACGCAAGCTTCGATAATTTTAAGTTTGTATGTGATAATATCAAATTTGCTGATG
ATTTAAACCAGTTAACTGGTTATAAGAAACCTGCTTCAAGAGAGCTTAAAGTTACATTTTTCCCTGACTTAAATGGT
GATGTGGTGGCTATTGATTATAAACACTACACACCCTCTTTTAAGAAAGGAGCTAAATTGTTACATAAACCTATTGT
TTGGCATGTTAACAATGCAACTAATAAAGCCACGTATAAACCAAATACCTGGTGTATACGTTGTCTTTGGAGCACA
AAACCAGTTGAAACATCAAATTCGTTTGATGTACTGAAGTCAGAGGACGCGCAGGGAATGGATAATCTTGCCTGC
GAAGATCTAAAACCAGTCTCTGAAGAAGTAGTGGAAAATCCTACCATACAGAAAGACGTTCTTGAGTGTAATGTG
AAAACTACCGAAGTTGTAGGAGACATTATACTTAAACCAGCAAATAATAGTTTAAAAATTACAGAAGAGGTTGGC
CACACAGATCTAATGGCTGCTTATGTAGACAATTCTAGTCTTACTATTAAGAAACCTAATGAATTATCTAGAGTATT
AGGTTTGAAAACCCTTGCTACTCATGGTTTAGCTGCTGTTAATAGTGTCCCTTGGGATACTATAGCTAATTATGCTA
AGCCTTTTCTTAACAAAGTTGTTAGTACAACTACTAACATAGTTACACGGTGTTTAAACCGTGTTTGTACTAATTATA
TGCCTTATTTCTTTACTTTATTGCTACAATTGTGTACTTTTACTAGAAGTACAAATTCTAGAATTAAAGCATCTATGCC
GACTACTATAGCAAAGAATACTGTTAAGAGTGTCGGTAAATTTTGTCTAGAGGCTTCATTTAATTATTTGAAGTCAC
CTAATTTTTCTAAACTGATAAATATTATAATTTGGTTTTTACTATTAAGTGTTTGCCTAGGTTCTTTAATCTACTCAAC
CGCTGCTTTAGGTGTTTTAATGTCTAATTTAGGCATGCCTTCTTACTGTACTGGTTACAGAGAAGGCTATTTGAACT
CTACTAATGTCACTATTGCAACCTACTGTACTGGTTCTATACCTTGTAGTGTTTGTCTTAGTGGTTTAGATTCTTTAG
ACACCTATCCTTCTTTAGAAACTATACAAATTACCATTTCATCTTTTAAATGGGATTTAACTGCTTTTGGCTTAGTTGC

FIG. 62B continued

AGAGTGGTTTTTTGGCATATATTCTTTTCACTAGGTTTTTTCTATGTACTTGGATTGGCTGCAATCATGCAATTGTTTTTT
CAGCTATTTTGCAGTACATTTTATTAGTAATTCTTGGCTTATGTGGTTAATAATTAATCTTGTACAAATGGCCCCGAT
TTCAGCTATGGTTAGAATGTACATCTTCTTTGCATCATTTTATTATGTATGGAAAAGTTATGTGCATGTTGTAGACG
GTTGTAATTCATCAACTTGTATGATGTGTTACAAACGTAATAGAGCAACAAGAGTCGAATGTACAACTATTGTTAA
TGGTGTTAGAAGGTCCTTTTATGTCTATGCTAATGGAGGTAAAGGCTTTTGCAAACTACACAATTGGAATTGTGTT
AATTGTGATACATTCTGTGCTGGTAGTACATTTATTAGTGATGAAGTTGCGAGAGACTTGTCACTACAGTTTAAAA
GACCAATAAATCCTACTGACCAGTCTTCTTACATCGTTGATAGTGTTACAGTGAAGAATGGTTCCATCCATCTTTAC
TTTGATAAAGCTGGTCAAAAGACTTATGAAAGACATTCTCTCTCTCATTTTGTTAACTTAGACAACCTGAGAGCTAA
TAACACTAAAGGTTCATTGCCTATTAATGTTATAGTTTTTGATGGTAAATCAAAATGTGAAGAATCATCTGCAAAAT
CAGCGTCTGTTTACTACAGTCAGCTTATGTGTCAACCTATACTGTTACTAGATCAGGCATTAGTGTCTGATGTTGGT
GATAGTGCGGAAGTTGCAGTTAAAATGTTTGATGCTTACGTTAATACGTTTTCATCAACTTTTAACGTACCAATGGA
AAAACTCAAAACACTAGTTGCAACTGCAGAAGCTGAACTTGCAAAGAATGTGTCCTTAGACAATGTTTTATCTACTT
TTATTTCAGCAGCTCGGCAAGGGTTTGTTGATTCAGATGTAGAAACTAAAGATGTTGTTGAATGTCTTAAATTGTCA
CATCAATCTGACATAGAAGTTACTGGCGATAGTTGTAATAACTATATGCTCACCTATAACAAAGTTGAAAACATGA
CACCCCGTGACCTTGGTGCTTGTATTGACTGTAGTGCGCGTCATATTAATGCGCAGGTAGCAAAAAGTCACAACAT
TGCTTTGATATGGAACGTTAAAGATTTCATGTCATTGTCTGAACAACTACGAAAACAAATACGTAGTGCTGCTAAA
AAGAATAACTTACCTTTTAAGTTGACATGTGCAACTACTAGACAAGTTGTTAATGTTGTAACAACAAAGATAGCACT
TAAGGGTGGTAAAATTGTTAATAATTGGTTGAAGCAGTTAATTAAAGTTACACTTGTGTTCCTTTTTGTTGCTGCTA
TTTTCTATTTAATAACACCTGTTCATGTCATGTCTAAACATACTGACTTTTCAAGTGAAATCATAGGATACAAGGCTA
TTGATGGTGGTGTCACTCGTGACATAGCATCTACAGATACTTGTTTTGCTAACAAACATGCTGATTTTGACACATGG
TTTAGCCAGCGTGGTGGTAGTTATACTAATGACAAAGCTTGCCCATTGATTGCTGCAGTCATAACAAGAGAAGTGG
GTTTTGTCGTGCCTGGTTTGCCTGGCACGATATTACGCACAACTAATGGTGACTTTTTGCATTTCTTACCTAGAGTTT
TTAGTGCAGTTGGTAACATCTGTTACACACCATCAAAACTTATAGAGTACACTGACTTTGCAACATCAGCTTGTGTT
TTGGCTGCTGAATGTACAATTTTTAAAGATGCTTCTGGTAAGCCAGTACCATATTGTTATGATACCAATGTACTAGA
AGGTTCTGTTGCTTATGAAAGTTTACGCCCTGACACACGTTATGTGCTCATGGATGGCTCTATTATTCAATTTCCTAA
CACCTACCTTGAAGGTTCTGTTAGAGTGGTAACAACTTTTGATTCTGAGTACTGTAGGCACGGCACTTGTGAAAGA
TCAGAAGCTGGTGTTTGTGTATCTACTAGTGGTAGATGGGTACTTAACAATGATTATTACAGATCTTTACCAGGAG
TTTTCTGTGGTGTAGATGCTGTAAATTTATTTACTAATATGTTTACACCACTAATTCAACCTATTGGTGCTTTGGACA
TATCAGCATCTATAGTAGCTGGTGGTATTGTGGCTATCGTAGTAACATGCCTTGCCTACTATTTTATGAGGTTTAGA
AGAGCTTTTGGTGAATACAGTCATGTAGTTGCCTTTAATACTTTACTATTCCTTATGTCATTCATTGTACTCTGTTTA
ACACCAGTTTACTCATTCTTACCTGGTGTTTATTCTGTTATTTACTTGTACTTGACATTTTATCTTACTAATGATGTTTC
TTTTTTAGCACATATTCAGTGGATGGTTATGTTCACACCTTTAGTACCTTTCTGGATAACAATTGTTTATATCATTTGT
ATTTCCACAAAGCATTTCTATTGGTTCTTTAGTAATTACCTAAAGAGACGTGTAGTCTTTAATGGTGTTTCCTTTAGT
ACTTTTGAAGAAGCTGCGCTGTGCACCTTTTTGTTAAATAAAGAAATGTATCTAAAGTTGCGTAGTGATGTGCTATT
ACCTTTTACGCAATATAATAGATACTTAGCTCTTTATAATAAGTACAAGTATTTTAGTGGAGCAATGGATACAACTA
GCTACAGAGAAGCTGCTTGTTGTCATCTCGCAAAGGCTCTCAATGACTTCAGTAACTCAGGTTCTGATGTTCTTTAC
CAACCACCACAAATCTCTATCACCTCAGCTGTTTTGCAGAGTGGTTTTAGAAAAATGGCATTCCCATCTGGTAAAGT
TGAGGGTTGTATGGTACAAGTAACTTGTGGTACAACTACACTTAACGGTCTTTGGCTTGATGACGTAGTTTACTGT
CCAAGACATGTGATCTGCACCTCTGAAGATATGCTTAACCCTAATTATGAAGATTTACTCATTCGTAAGTCTAATCA
TAATTTCTTGGTACAGGCTGGTAATGTTCAACTCAGGGTTATTGGACATTCTATGCAAAATTGTGTACTTAAGCTTA
AGGTTGATACAGCCAATCCTAAGACACCTAAGTATAAGTTTGTTCGCATTCAACCAGGACAGACTTTTTCAGTGTTA
GCTTGTTACAATGGTTCACCATCTGGTGTTTACCAATGTGCTATGAGACACAATTTCACTATTAAGGGTTCATTCCTT
AATGGTTCATGTGGTAGTGTTGGTTTTAACATAGATTATGACTGTGTCTCTTTTTGTTACATGCACCATATGGAATT
ACCAACTGGAGTTCATGCTGGCACAGACTTAGAAGGTAACTTTTATGGACCTTTTGTTGACAGGCAAACAGCACAA
GCAGCTGGTACGGACACAACTATTACAGTTAATGTTTTAGCTTGGTTGTACGCTGCTGTTATAAATGGAGACAGGT
GGTTTCTCAATCGATTTACCACAACTCTTAATGACTTTAACCTTGTGGCTATGAAGTACAATTATGAACCTCTAACAC
AAGACCATGTTGACATACTAGGACCTCTTTCTGCTCAAACTGGAATTGCCGTTTTAGATATGTGTGCTTCATTAAAA
GAATTACTGCAAAATGGTATGAATGGACGTACCATATTGGGTAGTGCTTTATTAGAAGATGAATTTACACCTTTTG

FIG. 62B continued

ATGTTGTTAGACAATGCTCAGGTGTTACTTTCCAAAGTGCAGTGAAAAGAACAATCAAGGGTACACACCACTGGTT
GTTACTCACAATTTTGACTTCACTTTTAGTTTTAGTCCAGAGTACTCAATGGTCTTTGTTCTTTTTTTTGTATGAAAAT
GCCTTTTTACCTTTTGCTATGGGTATTATTGCTATGTCTGCTTTTGCAATGATGTTTGTCAAACATAAGCATGCATTT
CTCTGTTTGTTTTTGTTACCTTCTCTTGCCACTGTAGCTTATTTTAATATGGTCTATATGCCTGCTAGTTGGGTGATGC
GTATTATGACATGGTTGGATATGGTTGATACTAGTTTGAAGCTAAAAGACTGTGTTATGTATGCATCAGCTGTAGT
GTTACTAATCCTTATGACAGCAAGAACTGTGTATGATGATGGTGCTAGGAGAGTGTGGACACTTATGAATGTCTTG
ACACTCGTTTATAAAGTTTATTATGGTAATGCTTTAGATCAAGCCATTTCCATGTGGGCTCTTATAATCTCTGTTACT
TCTAACTACTCAGGTGTAGTTACAACTGTCATGTTTTTGGCCAGAGGTATTGTTTTTATGTGTGTTGAGTATTGCCCT
ATTTTCTTCATAACTGGTAATACACTTCAGTGTATAATGCTAGTTTATTGTTTCTTAGGCTATTTTTGTACTTGTTACT
TTGGCCTCTTTTGTTTACTCAACCGCTACTTTAGACTGACTCTTGGTGTTTATGATTACTTAGTTTCTACACAGGAGT
TTAGATATATGAATTCACAGGGACTACTCCCACCCAAGAATAGCATAGATGCCTTCAAACTCAACATTAAATTGTTG
GGTGTTGGTGGCAAACCTTGTATCAAAGTAGCCACTGTACAGTCTAAAATGTCAGATGTAAAGTGCACATCAGTA
GTCTTACTCTCAGTTTTGCAACAACTCAGAGTAGAATCATCATCTAAATTGTGGGCTCAATGTGTCCAGTTACACAA
TGACATTCTCTTAGCTAAAGATACTACTGAAGCCTTTGAAAAAATGGTTTCACTACTTTCTGTTTTGCTTTCCATGCA
GGGTGCTGTAGACATAAACAAGCTTTGTGAAGAAATGCTGGACAACAGGGCAACCTTACAAGCTATAGCCTCAGA
GTTTAGTTCCCTTCCATCATATGCAGCTTTTGCTACTGCTCAAGAAGCTTATGAGCAGGCTGTTGCTAATGGTGATT
CTGAAGTTGTTCTTAAAAAGTTGAAGAAGTCTTTGAATGTGGCTAAATCTGAATTTGACCGTGATGCAGCCATGCA
ACGTAAGTTGGAAAAGATGGCTGATCAAGCTATGACCCAAATGTATAAACAGGCTAGATCTGAGGACAAGAGGG
CAAAAGTTACTAGTGCTATGCAGACAATGCTTTTCACTATGCTTAGAAAGTTGGATAATGATGCACTCAACAACATT
ATCAACAATGCAAGAGATGGTTGTGTTCCCTTGAACATAATACCTCTTACAACAGCAGCCAAACTAATGGTTGTCAT
ACCAGACTATAACACATATAAAAATACGTGTGATGGTACAACATTTACTTATGCATCAGCATTGTGGGAAATCCAA
CAGGTTGTAGATGCAGATAGTAAAATTGTTCAACTTAGTGAAATTAGTATGGACAATTCACCTAATTTAGCATGGC
CTCTTATTGTAACAGCTTTAAGGGCCAATTCTGCTGTCAAATTACAGAATAATGAGCTTAGTCCTGTTGCACTACGA
CAGATGTCTTGTGCTGCCGGTACTACACAAACTGCTTGCACTGATGACAATGCGTTAGCTTACTACAACACAATAA
AGGGAGGTAGGTTTGTACTTGCACTGTTATCCGATTTACAGGATTTGAAATGGGCTAGATTCCCTAAGAGTGATGG
AACTGGTACTATTTATACAGAACTGGAACCACCTTGTAGGTTTGTTACAGACACACCTAAAGGTCCTAAAGTGAAG
TATTTATACTTTATTAAAGGATTAAACAACCTAAATAGAGGTATGGTACTTGGTAGTTTAGCTGCCACAGTACGTCT
ACAAGCTGGTAATGCAACAGAAGTGCCTGCCAATTCAACTGTATTATCTTTCTGTGCTTTTGCTGTAGATGCTGCTA
AAGCTTACAAAGATTATCTAGCTAGTGGGGGACAACCAATCACTAATTGTGTTAAGATGTTGTGTACACACACTGG
TACTGGTCAGGCAATAACAGTTACACCGGAAGCCAATATGGATCAAGAATCCTTTGGTGGTGCATCGTGTTGTCTG
TACTGCCGTTGCCACATAGATCATCCAAATCCTAAAGGATTTTGTGACTTAAAAGGTAAGTATGTACAAATACCTAC
AACTTGTGCTAATGACCCTGTGGGTTTTACACTTAAAAACACAGTCTGTACCGTCTGCGGTATGTGGAAAGGTTAT
GGCTGTAGTTGTGATCAACTCCGCGAACCCATGCTTCAGTCAGCTGATGCACAATCGTTTTTAAACGGGTTTGCGG
TGTAAGTGCAGCCCGTCTTACACCGTGCGGCACAGGCACTAGTACTGATGTCGTATACAGGGCTTTTGACATCTAC
AATGATAAAGTAGCTGGTTTTGCTAAATTCCTAAAAACTAATTGTTGTCGCTTCCAAGAAAAGGACGAAGATGACA
ATTTAATTGATTCTTACTTTGTAGTTAAGAGACACACTTTCTCTAACTACCAACATGAAGAAACAATTTATAATTTAC
TTAAGGATTGTCCAGCTGTTGCTAAACATGACTTCTTTAAGTTTAGAATAGACGGTGACATGGTACCACATATATCA
CGTCAACGTCTTACTAAATACACAATGGCAGACCTCGTCTATGCTTTAAGGCATTTTGATGAAGGTAATTGTGACAC
ATTAAAAGAAATACTTGTCACATACAATTGTTGTGATGATGATTATTTCAATAAAAAGGACTGGTATGATTTTGTAG
AAAACCCAGATATATTACGCGTATACGCCAACTTAGGTGAACGTGTACGCCAAGCTTTGTTAAAAACAGTACAATT
CTGTGATGCCATGCGAAATGCTGGTATTGTTGGTGTACTGACATTAGATAATCAAGATCTCAATGGTAACTGGTAT
GATTTCGGTGATTTCATACAAACCACGCCAGGTAGTGGAGTTCCTGTTGTAGATTCTTATTATTCATTGTTAATGCC
TATATTAACCTTGACCAGGGCTTTAACTGCAGAGTCACATGTTGACACTGACTTAACAAAGCCTTACATTAAGTGG
GATTTGTTAAAATATGACTTCACGGAAGAGAGGTTAAAACTCTTTGACCGTTATTTTAAATATTGGGATCAGACAT
ACCACCCAAATTGTGTTAACTGTTTGGATGACAGATGCATTCTGCATTGTGCAAACTTTAATGTTTTATTCTCTACAG
TGTTCCCACTTACAAGTTTTGGACCACTAGTGAGAAAAATATTTGTTGATGGTGTTCCATTTGTAGTTTCAACTGGA
TACCACTTCAGAGAGCTAGGTGTTGTACATAATCAGGATGTAAACTTACATAGCTCTAGACTTAGTTTTAAGGAATT
ACTTGTGTATGCTGCTGACCCTGCTATGCACGCTGCTTCTGGTAATCTATTACTAGATAAACGCACTACGTGCTTTTC

FIG. 62B continued

AGTAGCTGCACTTACTAACAATGTTGCTTTTCAAACTGTCAAACCCGGTAATTTTAACAAAGACTTCTATGACTTTG
CTGTGTCTAAGGGTTTCTTTAAGGAAGGAAGTTCTGTTGAATTAAAACACTTCTTCTTTGCTCAGGATGGTAATGCT
GCTATCAGCGATTATGACTACTATCGTTATAATCTACCAACAATGTGTGATATCAGACAACTACTATTTGTAGTTGA
AGTTGTTGATAAGTACTTTGATTGTTACGATGGTGGCTGTATTAATGCTAACCAAGTCATCGTCAACAACCTAGACA
AATCAGCTGGTTTTCCATTTAATAAATGGGGTAAGGCTAGACTTTATTATGATTCAATGAGTTATGAGGATCAAGA
TGCACTTTTCGCATATACAAAACGTAATGTCATCCCTACTATAACTCAAATGAATCTTAAGTATGCCATTAGTGCAA
AGAATAGAGCTCGCACCGTAGCTGGTGTCTCTATCTGTAGTACTATGACCAATAGACAGTTTCATCAAAAATTATT
GAAATCAATAGCCGCCACTAGAGGAGCTACTGTAGTAATTGGAACAAGCAAATTCTATGGTGGTTGGCACAACAT
GTTAAAAACTGTTTATAGTGATGTAGAAAACCCTCACCTTATGGGTTGGGATTATCCTAAATGTGATAGAGCCATG
CCTAACATGCTTAGAATTATGGCCTCACTTGTTCTTGCTCGCAAACATACAACGTGTTGTAGCTTGTCACACCGTTTC
TATAGATTAGCTAATGAGTGTGCTCAAGTATTGAGTGAAATGGTCATGTGTGGCAGTTCACTATATGTTAAACCAG
GTGGAACCTCATCAGGAGATGCCACAACTGCTTATGCTAATAGTGTTTTTAACATTTGTCAAGCTGTCACGGCCAAT
GTTAATGCACTTTTATCTACTGATGGTAACAAAATTGCCGATAAGTATGTCCGCAATTTACAACACAGACTTTATGA
GTGTCTCTATAGAAATAGAGATGTTGACACAGACTTTGTGAATGAGTTTTACGCATATTTGCGTAAACATTTCTCAA
TGATGATACTTTCTGACGATGCTGTTGTGTGTTTTAATAGCACTTATGCATCTCAAGGTCTAGTGGCTAGCATAAAG
AACTTTAAGTCAGTTCTTTATTATCAAAACAATGTTTTTATGTCTGAAGCAAAATGTTGGACTGAGACTGACCTTAC
TAAAGGACCTCATGAATTTTGCTCTCAACATACAATGCTAGTTAAACAGGGTGATGATTATGTGTACCTTCCTTACC
CAGACCCATCAAGAATCCTAGGGGCCGGCTGTTTTGTAGATGATATCGTAAAAACAGATGGTACACTTATGATTGA
ACGGTTCGTGTCTTTAGCTATAGATGCTTACCCACTTACTAAACATCCTAATCAGGAGTATGCTGATGTCTTTCATTT
GTACTTACAATACATAAGAAAGCTACATGATGAGTTAACAGGACACATGTTAGACATGTATTCTGTTATGCTTACTA
ATGATAACACTTCAAGGTATTGGGAACCTGAGTTTTATGAGGCTATGTACACACCGCATACAGTCTTACAGGCTGT
TGGGGCTTGTGTTCTTTGCAATTCACAGACTTCATTAAGATGTGGTGCTTGCATACGTAGACCATTCTTATGTTGTA
AATGCTGTTACGACCATGTCATACCAACATCACATAAATTAGTCTTGTCTGTTAATCCGTATGTTTGCAATGCTCCA
GGTTGTGATGTCACAGATGTGACTCAACTTTACTTAGGAGGTATGAGCTATTATTGTAAATCACATAAACCACCCAT
TAGTTTTCCATTGTGTGCTAATGGACAAGTTTTTGGTTTATATAAAAATACATGTGTTGGTAGCGATAATGTTACTG
ACTTTAATGCAATTGCAACATGTGACTGGACAAATGCTGGTGATTACATTTTAGCTAACACCTGTACTGAAAGACTC
AAGCTTTTTGCAGCAGAAACGCTCAAAGCTACTGAGGAGACATTTAAACTGTCTTATGGTATTGCTACTGTACGTG
AAGTGCTGTCTGACAGAGAATTACATCTTTCATGGGAAGTTGGTAAACCTAGACCACCACTTAACCGAAATTATGT
CTTTACTGGTTATCGTGTAACTAAAAACAGTAAAGTACAAATAGGAGAGTACACCTTTGAAAAAGGTGACTATGGT
GATGCTGTTGTTTACCGAGGTACTACAACTTACAAATTAAATGTTGGTGATTATTTTGTGCTGACATCACATACAGT
AATGCCATTAAGTGCACCTACACTAGTGCCACAAGAGCACTATGTTAGAATTACTGGCTTATACCCAACACTCAATA
TCTCAGATGAGTTTTCTAGCAATGTTGCAAATTATCAAAAGGTTGGTATGCAAAAGTATTCTACACTCCAGGGACC
ACCTGGTACTGGTAAGAGTCATTTTGCTATTGGCCTAGCTCTCTACTACCCTTCTGCTCGCATAGTGTATACAGCTT
GCTCTCATGCCGCTGTTGATGCACTATGTGAGAAGGCATTAAAATATTTGCCTATAGATAAATGTAGTAGAATTAT
ACCTGCACGTGCTCGTGTAGAGTGTTTTGATAAATTCAAAGTGAATTCAACATTAGAACAGTATGTCTTTTGTACTG
TAAATGCATTGCCTGAGACGACAGCAGATATAGTTGTCTTTGATGAAATTTCAATGGCCACAAATTATGATTTGAG
TGTTGTCAATGCCAGATTATGTGCTAAGCACTATGTGTACATTGGCGACCCTGCTCAATTACCTGCACCACGCACAT
TGCTAACTAAGGGCACACTAGAACCAGAATATTTCAATTCAGTGTGTAGACTTATGAAAACTATAGGTCCAGACAT
GTTCCTCGGAACTTGTCGGCGTTGTCCTGCTGAAATTGTTGACACTGTGAGTGCTTTGGTTTATGATAATAAGCTTA
AAGCACATAAAGACAAATCAGCTCAATGCTTTAAAATGTTTTATAAGGGTGTTATCACGCATGATGTTTCATCTGCA
ATTAACAGGCCACAAATAGGCGTGGTAAGAGAATTCCTTACACGTAACCCTGCTTGGAGAAAAGCTGTCTTTATTT
CACCTTATAATTCACAGAATGCTGTAGCCTCAAAGATTTTGGGACTACCAACTCAAACTGTTGATTCATCACAGGGC
TCAGAATACGACTATGTCATATTCACTCAAACCACTGAAACAGCTCACTCTTGTAATGTAAACAGATTTAATGTTGC
TATTACCAGAGCAAAAGTAGGCATACTTTGCATAATGTCTGATAGAGACCTTTATGACAAGTTGCAATTTACAAGT
CTTGAAATTCCACGTAGGAATGTGGCAACTTTACAAGCTGAAAATGTAACAGGACTCTTTAAAGATTGTAGTAAGG
TAATCACTGGGTTACATCCTACACAGGCACCTACACACCTCAGTGTTGACACTAAATTCAAAACTGAAGGTTTATGT
GTTGACGTACCTGGCATACCTAAGGACATGACCTATAGAAGACTCATCTCTATGATGGGTTTTAAAATGAATTATC
AAGTTAATGGTTACCCTAACATGTTTATCACCCGCGAAGAAGCTATAAGACATGTACGTGCATGGATTGGCTTCGA

FIG. 62B continued

TGTCGAGGGGTGTCATGCTACTAGAGAAGCTGTTGGTACCAATTTACCTTTACAGCTAGGTTTTTTCTACAGGTGTTA
ACCTAGTTGCTATACCTACAGGTTATGTTGATACACCTAATAATACAGATTTTTCCAGAGTTAGTGCTAAACCACCG
CCTGGAGATCAATTTAAACACCTCATACCGCTTATGTACAAAGGACTTCCTTGGAATGTAGTGCGTATAAAGATTG
TACAAATGTTAAGTGACACACTTAAAAATCTCTCTGACAGAGTCGTATTTGTCTTATGGGCACATGGCTTTGAGTTG
ACATCTATGAAGTATTTTGTGAAAATAGGACCTGAGCGCACCTGTTGTCTATGTGATAGACGTGCCACATGCTTTTC
CACTGCTTCAGACACTTATGCCTGTTGGCATCATTCTATTGGATTTGATTACGTCTATAATCCGTTTATGATTGATGT
TCAACAATGGGGTTTTACAGGTAACCTACAAAGCAACCATGATCTGTATTGTCAAGTCCATGGTAATGCACATGTA
GCTAGTTGTGATGCAATCATGACTAGGTGTCTAGCTGTCCACGAGTGCTTTGTTAAGCGTGTTGACTGGACTATTG
AATATCCTATAATTGGTGATGAACTGAAGATTAATGCGGCTTGTAGAAAGGTTCAACACATGGTTGTTAAAGCTGC
ATTATTAGCAGACAAATTCCCAGTTCTTCACGACATTGGTAACCCTAAAGCTATTAAGTGTGTACCTCAAGCTGATG
TAGAATGGAAGTTCTATGATGCACAGCCTTGTAGTGACAAAGCTTATAAAATAGAAGAATTATTCTATTCTTATGCC
ACACATTCTGACAAATTCACAGATGGTGTATGCCTATTTTGGAATTGCAATGTCGATAGATATCCTGCTAATTCCAT
TGTTTGTAGATTTGACACTAGAGTGCTATCTAACCTTAACTTGCCTGGTTGTGATGGTGGCAGTTTGTATGTAAATA
AACATGCATTCCACACACCGGCTTTTGATAAAAGTGCTTTTGTTAATTTAAAACAATTACCATTTTTCTATTACTCTG
ACAGTCCATGTGAGTCTCATGGAAAACAAGTAGTGTCAGATATAGATTATGTACCACTAAAGTCTGCTACGTGTAT
AACACGTTGCAATTTAGGTGGTGCTGTCTGTAGACATCATGCTAATGAGTACAGATTGTATCTCGATGCTTATAAC
ATGATGATCTCAGCTGGCTTTAGCTTGTGGGTTTACAAACAATTTGATACTTATAACCTCTGGAACACTTTTACAAG
ACTTCAGAGTTTAGAAAATGTGGCTTTTAATGTTGTAAATAAGGGACACTTTGATGGACAACAGGGTGAAGTACC
AGTTTCTATCATTAATAACACTGTTTACACAAAAGTTGATGGTGTTGATGTAGAATTGTTTGAAAATAAAACAACAT
TACCTGTTAATGTAGCATTTGAGCTTTGGGCTAAGCGCAACATTAAACCAGTACCAGAGGTGAAAATACTCAATAA
TTTGGGTGTGGACATTGCTGCTAATACTGTGATCTGGGACTACAAAAGAGATGCTCCAGCACATATATCTACTATT
GGTGTTTGTTCTATGACTGACATAGCCAAGAAACCAATTGAAACGATTTGTGCACCACTCACTGTCTTTTTTGATGG
TAGAGTTGATGGTCAAGTAGACTTATTTAGAAATGCCCGTAATGGTGTTCTTATTACAGAGGGTAGTGTTAAAGGT
TTACAACCATCTGTAGGTCCCAAACAAGCTAGTCTTAATGGAGTCACATTAATTGGAGAAGCCGTAAAAACACAGT
TCAATTATTATAAGAAAGTTGATGGTGTTGTCCAACAATTACCTGAAACTTACTTTACTCAGAGTAGAAATTTACAA
GAATTTAAACCCAGGAGTCAAATGGAAATTGATTTCTTAGAATTAGCTATGGATGAATTCATTGAACGGTATAAAT
TAGAAGGCTATGCCTTCGAACATATCGTTTATGGAGATTTTAGTCATAGTCAGTTAGGTGGTTTACATCTACTGATT
GGACTAGCTAAACGTTTTAAGGAATCACCTTTTGAATTAGAAGATTTTATTCCTATGGACAGTACAGTTAAAAACTA
TTTCATAACAGATGCGCAAACAGGTTCATCTAAGTGTGTGTGTTCTGTTATTGATTTATTACTTGATGATTTTGTTGA
AATAATAAAATCCCAAGATTTATCTGTAGTTTCTAAGGTTGTCAAAGTGACTATTGACTATACAGAAATTTCATTTA
TGCTTTGGTGTAAAGATGGCCACGTAGAAACATTTTACCCAAAATTACAATCTAGTCAAGCGTGGCAACCGGGTGT
TGCTATGCCTAATCTTTACAAAATGCAAAGAATGCTATTAGAAAAGTGTGACCTTCAAAATTATGGTGATAGTGCA
ACATTACCTAAAGGCATAATGATGAATGTCGCAAAATATACTCAACTGTGTCAATATTTAAACACATTAACATTAGC
TGTACCCTATAATATGAGAGTTATACATTTTGGTGCTGGTTCTGATAAAGGAGTTGCACCAGGTACAGCTGTTTTAA
GACAGTGGTTGCCTACGGGTACGCTGCTTGTCGATTCAGATCTTAATGACTTTGTCTCTGATGCAGATTCAACTTTG
ATTGGTGATTGTGCAACTGTACATACAGCTAATAAATGGGATCTCATTATTAGTGATATGTACGACCCTAAGACTA
AAAATGTTACAAAAGAAAATGACTCTAAAGAGGGTTTTTTCACTTACATTTGTGGGTTTATACAACAAAAGCTAGC
TCTTGGAGGTTCCGTGGCTATAAAGATAACAGAACATTCTTGGAATGCTGATCTTTATAAGCTCATGGGACACTTC
GCATGGTGGACAGCCTTTGTTACTAATGTGAATGCGTCATCATCTGAAGCATTTTTAATTGGATGTAATTATCTTGG
CAAACCACGCGAACAAATAGATGGTTATGTCATGCATGCAAATTACATATTTTGGAGGAATACAAATCCAATTCAG
TTGTCTTCCTATTCTTTATTTGACATGAGTAAATTTCCCCTTAAATTAAGGGGTACTGCTGTTATGTCTTTAAAAGAA
GGTCAAATCAATGATATGATTTTATCTCTTCTTAGTAAAGGTAGACTTATAATTAGAGAAAACAACAGAGTTGTTAT
TTCTAGTGATGTTCTTGTTAACAACTAAACGAACAATGTTTGTTTTTCTTGTTTTATTGCCACTAGTCTCTAGTCAGT
GTGTTAATCTTATAACCAGAACTCAATCATACACTAATTCTTTCACACGTGGTGTTTATTACCCTGACAAAGTTTTCA
GATCCTCAGTTTTACATTCAACTCATGACTTGTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGT
CTCTGGGACCAATGGTACTAAGAGGTTTGATAACCCTGCCCTACCATTTAATGATGGTGTTTATTTTGCTTCCACTG
AGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCCAGTCCCTACTTATTGTTAAT
AACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTTGTAATGATCCATTTTTGGATGTTTACCAAAAAAAC

FIG. 62B continued

AACAAAAGTTGGATGGAAAGTGAGTTCGGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGC
CTTTTCTTATGGACCTTGAAGGAAAAGAGGGTAATTTCAAAAATCTTAGGGAATTTGTGTTTAAGAATATTGATGG
TTATTTTAAAATATATTCTAAGCACACGCCTATTAATTTAGAGCGTGATCTCCCTCAGGGTTTTTCGGCTTTAGAACC
ATTGGTAGATTTGCCAATAGGTATTAACATCACTAGGTTTCAAACTTTACTTGCTTTACATAGAAGTTATTTGACTCC
TGTTGATTCTTCTTCAGGTTGGACAGCTGGTGCTGCAGCTTATTATGTGGGTTATCTTCAACCTAGGACTTTTCTATT
AAAATATAATGAAAATGGAACCATTACAGATGCTGTAGACTGTGCACTTGACCCTCTCTCAGAAACAAAGTGTACG
TTGAAATCCTTTACTGTAGAAAAAGGAATCTATCAAACTTCTAACTTTAGAGTCCAACCAACAGAATCTATTGTTAG
ATTTCCTAATATTACAAACTTGTGCCCTTTTCATGAAGTTTTTAACGCCACCACATTTGCATCTGTTTATGCTTGGAA
CAGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGTCATATATAATTTCGCACCATTTTTCGCTTTTAAGTGTTA
TGGAGTGTCTCCTACTAAATTAAATGATCTCTGCTTTACTAATGTCTATGCAGATTCATTTGTAATTAGAGGTAATG
AAGTCAGCCAAATCGCTCCAGGGCAAACTGGAAATATTGCTGATTATAATTATAAATTACCAGATGATTTTACAGG
CTGCGTTATAGCTTGGAATTCTAACAAGCTTGATTCTAAGCCTAGTGGTAATTATAATTACCTGTATAGATTGTTAA
GGAAGTCTAAGCTCAAACCTTTTGAGAGAGATATTTCAACTGAAATCTATCAGGCCGGTAACAAACCTTGTAATGG
TGTTGCAGGTCCTAATTGTTACTCTCCTTTACAATCATATGGTTTCCGACCCACTTATGGTGTTGGTCACCAACCATA
CAGAGTAGTAGTACTTTCTTTTGAACTTCTACATGCACCAGCAACTGTTTGTGGACCTAAAAAGTCTACTAATTTGG
TTAAAAACAAATGTGTCAATTTCAACTTCAATGGTTTAACAGGCACAGGTGTTCTTACTGAGTCTAACAAAAAGTTT
CTGCCTTTCCAACAATTTGGCAGAGACATTGCTGACACTACTGATGCTGTCCGTGATCCACAGACACTTGAGATTCT
TGACATTACACCATGTTCTTTTGGTGGTGTCAGTGTTATAACACCAGGAACAAATACTTCTAACCAGGTTGCTGTTC
TTTATCAGGGTGTTAACTGCACAGAAGTCCCTGTTGCTATTCATGCAGATCAACTTACTCCTACTTGGCGTGTTTATT
CTACAGGTTCTAATGTTTTTCAAACACGTGCAGGCTGTTTAATAGGGGCTGAATATGTCAACAACTCATATGAGTG
TGACATACCCATTGGTGCAGGTATATGCGCTAGTTATCAGACTCAGACTAAGTCTCATCGGCGGGCACGTAGTGTA
GCTAGTCAATCCATCATTGCCTACACTATGTCACTTGGTGCAGAAAATTCAGTTGCTTACTCTAATAACTCTATTGCC
ATACCCACAAATTTTACTATTAGTGTTACCACAGAAATTCTACCAGTGTCTATGACCAAGACATCAGTAGATTGTAC
AATGTACATTTGTGGTGATTCAACTGAATGCAGCAATCTTTTGTTGCAATATGGCAGTTTTTGTACACAATTAAAAC
GTGCTTTAACTGGAATAGCTGTTGAACAAGACAAAAACACCCAAGAAGTTTTTGCACAAGTCAAACAAATTTACAA
AACACCACCAATTAAATATTTTGGTGGTTTTAATTTTTCACAAATATTACCAGATCCATCAAAACCAAGCAAGAGGT
CATTTATTGAAGATCTACTTTTCAACAAAGTGACACTTGCAGATGCTGGCTTCATCAAACAATATGGTGATTGCCTT
GGTGATATTGCTGCTAGAGACCTCATTTGTGCACAAAAGTTTAACGGCCTTACTGTTTTGCCACCTTTGCTCACAGA
TGAAATGATTGCTCAATACACTTCTGCACTGTTAGCGGGTACAATCACTTCTGGTTGGACCTTTGGTGCAGGTGCT
GCATTACAAATACCATTTGCTATGCAAATGGCTTATAGGTTTAATGGTATTGGAGTTACACAGAATGTTCTCTATGA
GAACCAAAAATTGATTGCCAACCAATTTAATAGTGCTATTGGCAAAATTCAAGACTCACTTTCTTCCACAGCAAGTG
CACTTGGAAAACTTCAAGATGTGGTCAACCATAATGCACAAGCTTTAAACACGCTTGTTAAACAACTTAGCTCCAA
ATTTGGTGCAATTTCAAGTGTTTTAAATGATATCCTTTCACGTCTTGACAAAGTTGAGGCTGAAGTGCAAATTGATA
GGTTGATCACAGGCAGACTTCAAAGTTTGCAGACATATGTGACTCAACAATTAATTAGAGCTGCAGAAATCAGAG
CTTCTGCTAATCTTGCTGCTACTAAAATGTCAGAGTGTGTACTTGGACAATCAAAAAGAGTTGATTTTTGTGGAAA
GGGCTATCATCTTATGTCCTTCCCTCAGTCAGCACCTCATGGTGTAGTCTTCTTGCATGTGACTTATGTCCCTGCACA
AGAAAAGAACTTCACAACTGCTCCTGCCATTTGTCATGATGGAAAAGCACACTTTCCTCGTGAAGGTGTCTTTGTTT
CAAATGGCACACACTGGTTTGTAACACAAAGGAATTTTTATGAACCACAAATCATTACTACAGACAACACATTTGT
GTCTGGTAACTGTGATGTTGTAATAGGAATTGTCAACAACACAGTTTATGATCCTTTGCAACCTGAATTAGATTCAT
TCAAGGAGGAGTTAGATAAATATTTTAAGAATCATACATCACCAGATGTTGATTTAGGTGACATCTCTGGCATTAA
TGCTTCAGTTGTAAACATTCAAAAAGAAATTGACCGCCTCAATGAGGTTGCCAAGAATTTAAATGAATCTCTCATCG
ATCTCCAAGAACTTGGAAAGTATGAGCAGTATATAAAATGGCCATGGTACATTTGGCTAGGTTTTATAGCTGGCTT
GATTGCCATAGTAATGGTGACAATTATGCTTTGCTGTATGACCAGTTGCTGTAGTTGTCTCAAGGGCTGTTGTTCTT
GTGGATCCTGCTGCAAATTTGATGAAGACGACTCTGAGCCAGTGCTCAAAGGAGTCAAATTACATTACACATAAAC
GAACTTATGGATTTGTTTATGAGAATCTTTACAATTGGAACTGTAACTTTGAAGCAAGGTGAAATCAAGGATGCTA
CTCCTTCAGATTTTGTTCGCGCTACTGCAACGATACCGATACAAGCCTCACTCCCTTTCGGATGGCTTATTGTTGGC
GTTGCACTTCTTGCTGTTTTTCAGAGCGCTTCCAAAATCATAACTCTCAAAAAGAGATGGCAACTAGCACTCTCCAA
GGGTGTTCACTTTGTTTGCAACTTGCTGTTGTTGTTTGTAACAGTTTACTCACACCTTTTGCTCGTTGCTGCTGGCCT

FIG. 62B continued

TGAAGCCCCTTTTCTCTATCTTTATGCTTTAGTCTACTTCTTGCAGAGTATAAACTTTGTAAGAATAATAATGAGGCT
TTGGCTTTGCTGGAAATGCCGTTCCAAAAACCCATTACTTTATGATGCCAACTATTTTCTTTGCTGGCATACTAATTG
TTACGACTATTGTATACCTTACAATAGTGTAACTTCTTCAATTGTCATTACTTCAGGTGATGGCACAACAAGTCCTAT
TTCTGAACATGACTACCAGATTGGTGGTTATACTGAAAAATGGGAATCTGGAGTAAAAGACTGTGTTGTATTACAC
AGTTACTTCACTTCAGACTATTACCAGCTGTACTCAACTCAATTGAGTACAGACATTGGTGTTGAACATGTTACCTT
CTTCATCTACAATAAAATTGTTGATGAGCCTGAAGAACATGTCCAAATTCACACAATCGACGGTTCATCCGGAGTT
GTTAATCCAGTAATGGAACCAATTTATGATGAACCGACGACGACTACTAGCGTGCCTTTGTAAGCACAAGCTGATG
AGTACGAACTTATGTACTCATTCGTTTCGGAAGAGATAGGTGCGTTAATAGTTAATAGCGTACTTCTTTTTCTTGCT
TTCGTGGTATTCTTGCTAGTTACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATATTGTTAAC
GTGAGTCTTGTAAAACCTTCTTTTTACGTTTACTCTCGTGTTAAAAATCTGAATTCTTCTAGAGTTCCTGATCTTCTG
GTCTAAACGAACTAAATATTATATTAGTTTTTCTGTTTGGAACTTTAATTTTAGCCATGGCAGATTCCAACGGTACTA
TTACCGTTGAAGAGCTTAAAAAGCTCCTTGAAGAATGGAACCTAGTAATAGGTTTCCTATTCCTTACATGGATTTGT
CTTCTACAATTTGCCTATGCCAACAGGAATAGGTTTTTGTATATAATTAAGTTAATTTTCCTCTGGCTGTTATGGCCA
GTAACTTTAACTTGTTTTGTGCTTGCTGCTGTTTACAGAATAAATTGGATCACCGGTGGAATTGCTATCGCAATGGC
TTGTCTTGTAGGCTTGATGTGGCTCAGCTACTTCATTGCTTCTTTCAGACTGTTTGCGCGTACGCGTTCCATGTGGTC
ATTTAATCCAGAAACTAACATTCTTCTCAACGTGCCACTCCATGGCACTATTCTGACCAGACCGCTTCTAGAAAGTG
AACTCGTAATCGGAGCTGTGATCCTTCGTGGACATCTTCGTATTGCTGGACACCATCTAGGACGCTGTGACATCAA
GGACCTGCCTAAAGAAATCACTGTTGCTACATCACGAACGCTTTCTTATTACAAATTGGGAGCTTCGCAGCGTGTA
GCAGGTGACTCAGGTTTTGCTGCATACAGTCGCTACAGGATTGGCAACTATAAATTAAACACAGACCATTCCAGTA
GCAGTGACAATATTGCTTTGCTTGTACAGTAAGTGACAACAGATGTTTCATCTCGTTGACTTTCAGGTTACTATAGC
AGAGATATTACTAATTATTATGCGGACTTTTAAAGTTTCCATTTGGAATCTTGATTACATCATAAACCTCATAATTAA
AAATTTATCTAAGTCACTAACTGAGAATAAATATTCTCAATTAGATGAAGAGCAACCAATGGAGATTCTCTAAACG
AACATGAAAATTATTCTTTTCTTGGCACTGATAACACTCGCTACTTGTGAGCTTTATCACTACCAAGAGTGTGTTAG
AGGTACAACAGTACTTTTAAAAGAACCTTGCTCTTCTGGCACATACGAGGGCAATTCACCATTTCATCCTCTAGCTG
ATAACAAATTTGCACTGACTTGCTTTAGCACTCAATTTGCTTTTGCTTGTCCTGACGGCGTAAAACACGTCTATCAGT
TACGTGCCAGATCAGTTTCACCTAAACTGTTCATCAGACAAGAGGAAGTTCAAGAACTTTACTCTCCAATTTTTCTT
ATTGTTGCGGCAATAGTGTTTATAACACTTTGCTTCACACTCAAAAGAAAGACAGAATGATTGAACTTTCATTAATT
GACTTCTATTTGTGCTTTTTAGCCTTTCTGTTATTCCTTGTTTTAATTATGCTTATTATCTTTTGGTTCTCACTTGAACT
GCAAGATCATAATGAAACTTGTCACGCCTAAACGAACATGAAATTTCTTGTTTTCTTATGAATCATCACAACTGTAG
CTGCATTTCACCAAGAATGTAGTTTACAGTCATGTACTCAACATCAACCATATGTAGTTGATGACCCGTGTCCTATT
CACTTCTATTCTAAATGGTATATTAGAGTAGGAGCTAGAAAATCAGCACCTTTAATTGAATTGTGCGTGGATGAGG
CTGGTTCTAAATCACCCATTCAGTACATCGATATCGGTAATTATACAGTTTCCTGTTTACCTTTTACAATTAATTGCC
AGGAACCTAAATTGGGTAGTCTTGTAGTGCGTTGTTCGTTCTATGAAGACTTTTTAGAGTATCATGACGTTCGTGTT
GTTTTAGATTTCATCTAAACGAACAAACTTAAATGTCTGATAATGGACCCCAAAACCAGCGAAATGCACTCCGCATT
ACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGTGGGGCGCGATCAAAACAACGTCGGCCCCAA
GGTTTACCCAATAATACTGCGTCTTGGTTCACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATTCCCTCGAGG
ACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATGACCAAATTGGCTACTACCGAAGAGCTACCAGACGAATT
CGTGGTGGTGACGGTAAAATGAAAGATCTCAGTCCAAGATGGTATTTCTACTACCTAGGAACTGGGCCAGAAGCT
GGACTTCCCTATGGTGCTAACAAAGACGGCATCATATGGGTTGCAACTGAGGGAGCCTTGAATACACCAAAAGAT
CACATTGGCACCCGCAATCCTGCTAACAATGCTGCAATCGTGCTACAACTTCCTCAAGGAACAACATTGCCAAAAG
GCTTCTACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCATCACGTAGTCGCAACAGTTCAA
GAAATTCAACTCCAGGCAGCAGTAAACGAACTTCTCCTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCTTGC
TTTGCTGCTGCTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTGGTAAAGGCCAACAACAACAAGGCCAAAC
TGTCACTAAGAAATCTGCTGCTGAGGCTTCTAAGAAGCCTCGGCAAAAACGTACTGCCACTAAAGCATACAATGTA
ACACAAGCTTTCGGCAGACGTGGTCCAGAACAAACCCAAGGAAATTTTGGGGACCAGGAACTAATCAGACAAGG
AACTGATTACAAACATTGGCCGCAAATTGCACAATTTGCCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGCATT
GGCATGGAAGTCACACCTTCGGGAACGTGGTTGACCTACACAGGTGCCATCAAATTGGATGACAAAGATCCAAAT
TTCAAAGATCAAGTCATTTTGCTGAATAAGCATATTGACGCATACAAAACATTCCCACCAACAGAGCCTAAAAAGG

FIG. 62B continued

ACAAAAAGAAGAAGGCTGATGAAACTCAAGCCTTACCGCAGAGACAGAAGAAACAGCAAACTGTGACTCTTCTTC
CTGCTGCAGATTTGGATGATTTCTCCAAACAATTGCAACAATCCATGAGCCGTGCTGACTCAACTCAGGCCTAAACT
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ATGGAAGAGCCCTAATGTGTAAAATTAATTTTAGTAGTGCTATCCC

FIG. 63

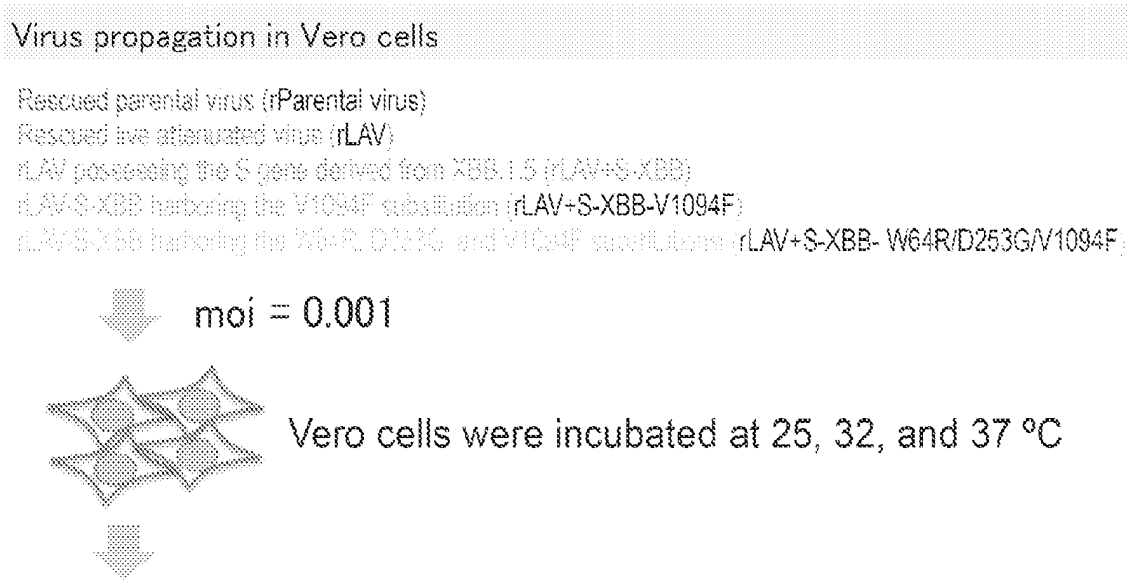
Virus propagation in Vero cells
Rescued parental virus (rParental virus)
Rescued live attenuated virus (rLAV)
rLAV possessing the S gene derived from XBB.1.5 (rLAV+S-XBB)
rLAV-S-XBB harboring the V1094F substitution (rLAV+S-XBB-V1094F)
rLAV-S-XBB harboring the W64R, D253G, and V1094F substitutions (rLAV+S-XBB- W64R/D253G/V1094F)
moi = 0.001
Vero cells were incubated at 25, 32, and 37 °C
Virus titers were measured at 24, 48, or 96 hpi by plaque assay.

FIG. 64

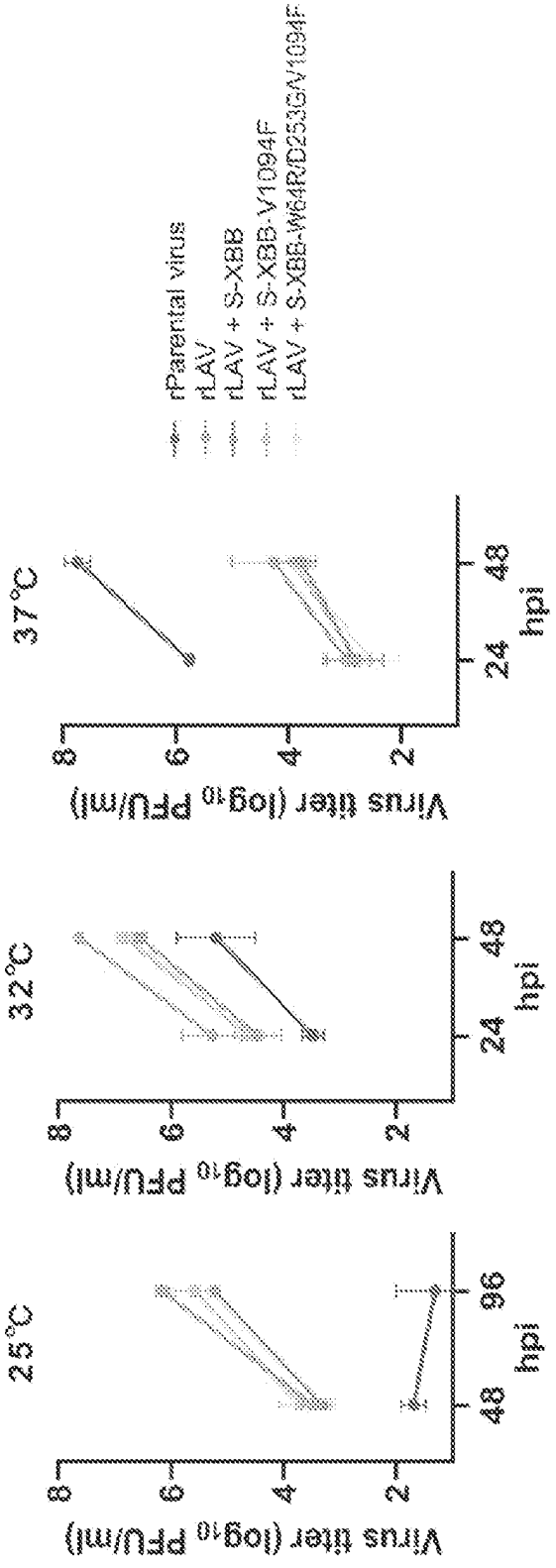
rParental virus
rLAV
rLAV + S-XBB
rLAV + S-XBB-V1094F
rLAV + S-XBB-W64R/D253G/V1094F
25°C
32°C
37°C
Virus titer (log10 PFU/ml)
hpi
48
96
24

FIG. 65

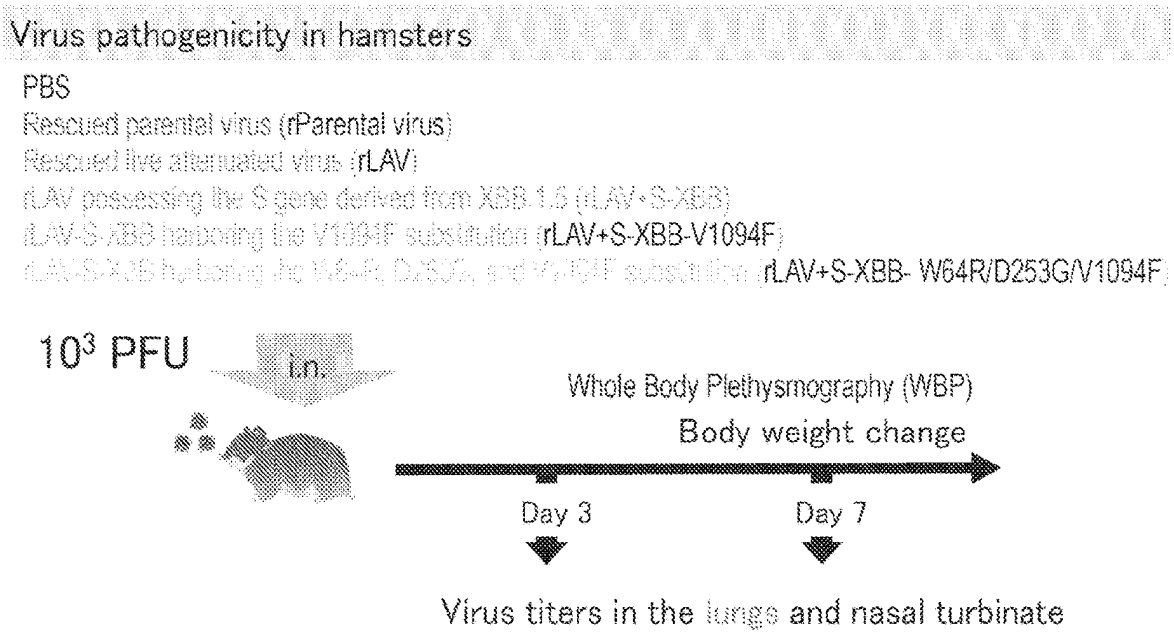
Virus pathogenicity in hamsters
PBS
Rescued parental virus (rParental virus)
Rescued live attenuated virus (rLAV)
rLAV possessing the S gene derived from XBB.1.5 (rLAV+S-XBB)
rLAV-S-XBB harboring the V1094F substitution (rLAV+S-XBB-V1094F)
rLAV-S-XBB harboring the W64R, D253G, and V1094F substitution (rLAV+S-XBB- W64R/D253G/V1094F)
10^3 PFU
i.n.
Whole Body Plethysmography (WBP)
Body weight change
Day 3
Day 7
Virus titers in the lungs and nasal turbinate

FIG. 66

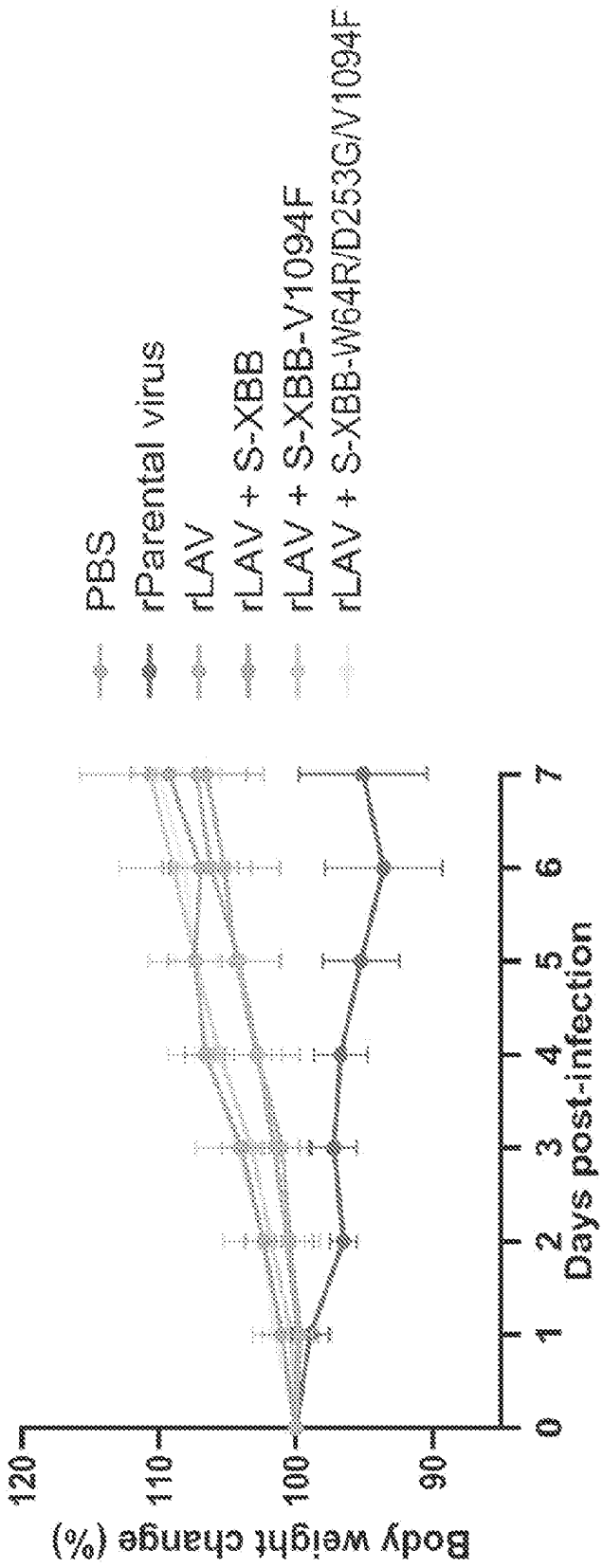
PBS
rParental virus
rLAV
rLAV + S-XBB
rLAV + S-XBB-V1094F
rLAV + S-XBB-W64R/D253C/V1094F
Body weight change (%)
120
110
100
90
0
1
2
3
4
5
6
7
Days post-infection

FIG. 67

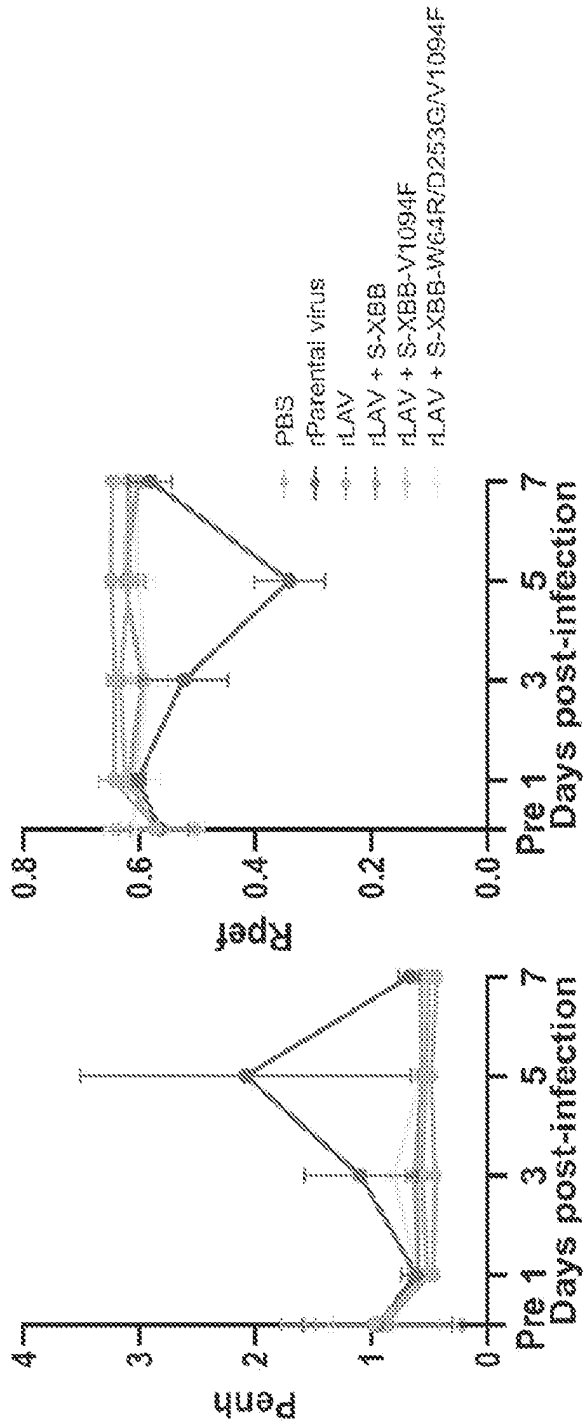
Penh
Rpef
Pre 1 3 5 7
Days post-infection
PBS
rParental virus
rLAV
rLAV + S-XBB
rLAV + S-XBB-V1094F
rLAV + S-XBB-W64R/D253G/V1094F

FIG. 68

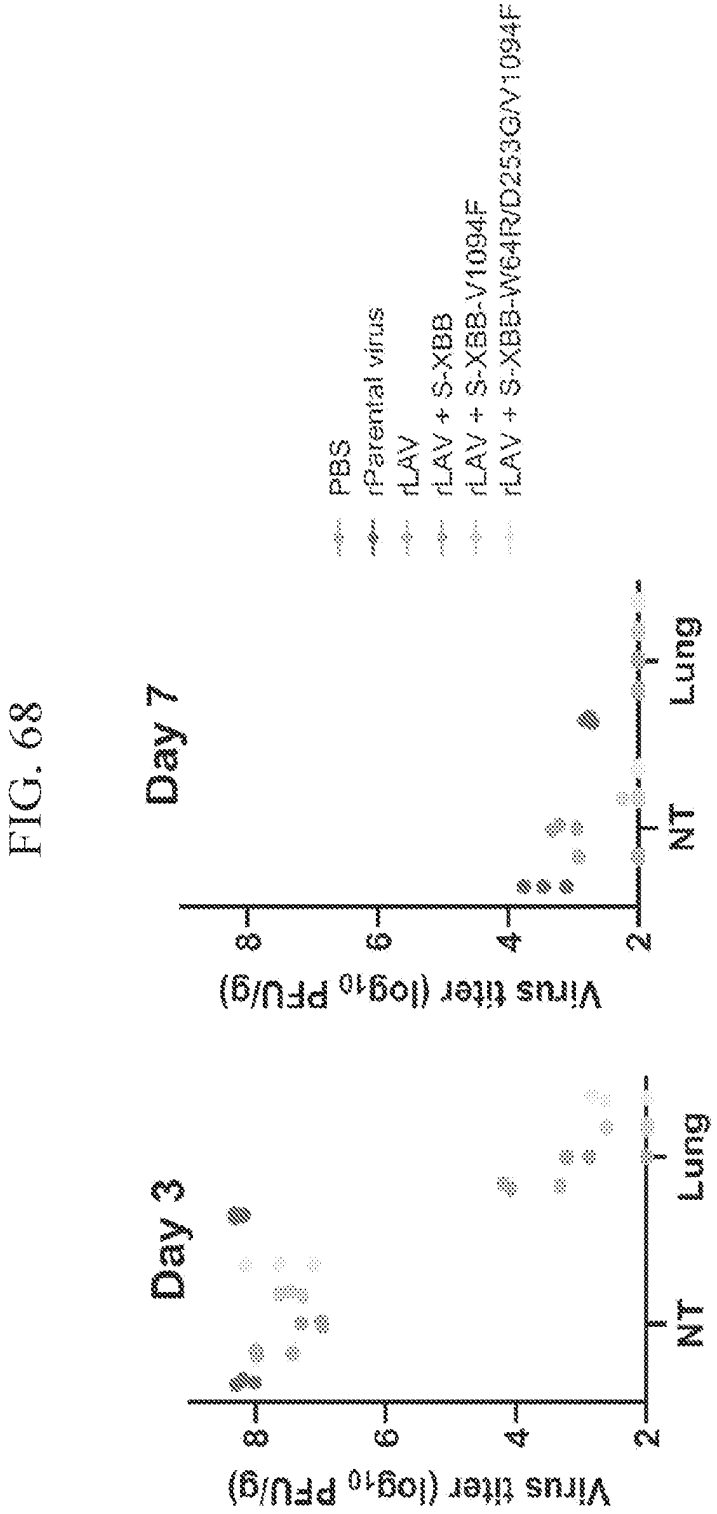
PBS
rParental virus
rLAV
rLAV + S-XBB
rLAV + S-XBB-V1094F
rLAV + S-XBB-W64R/D253G/V1094F
Day 7
Day 3
Virus titer (log10 PFU/g)
8
6
4
2
NT
Lung

Fig. 69A

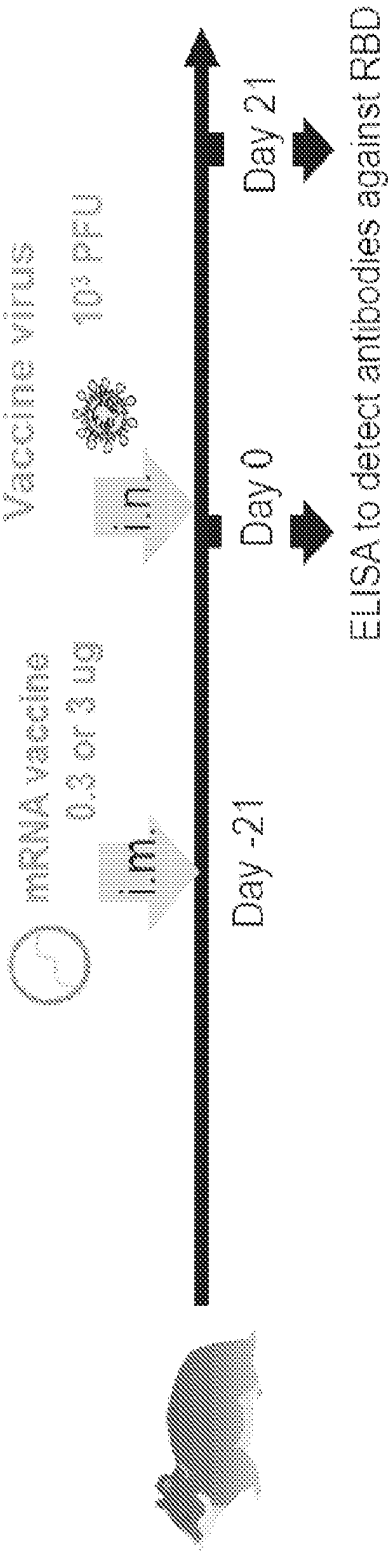
mRNA vaccine
0.3 or 3 ug
i.m.
Vaccine virus
10⁵ PFU
i.n.
Day -21
Day 0
Day 21
ELISA to detect antibodies against RBD

Fig. 69B

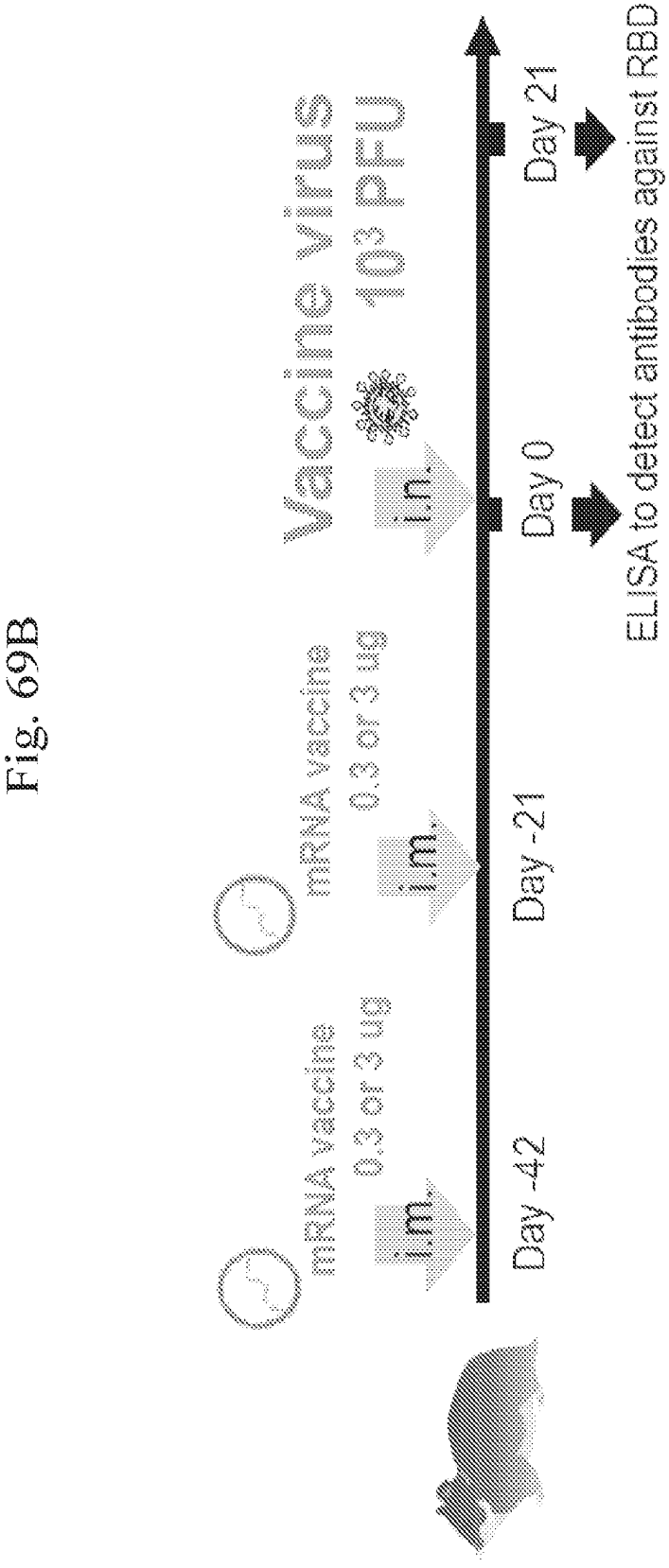
mRNA vaccine
0.3 or 3 ug
i.m.
Day -42
mRNA vaccine
0.3 or 3 ug
i.m.
Day -21
Vaccine virus
10^3 PFU
i.n.
Day 0
Day 21
ELISA to detect antibodies against RBD

FIG. 70

Boost immunization with vaccine virus in hamsters that received mRNA vaccination

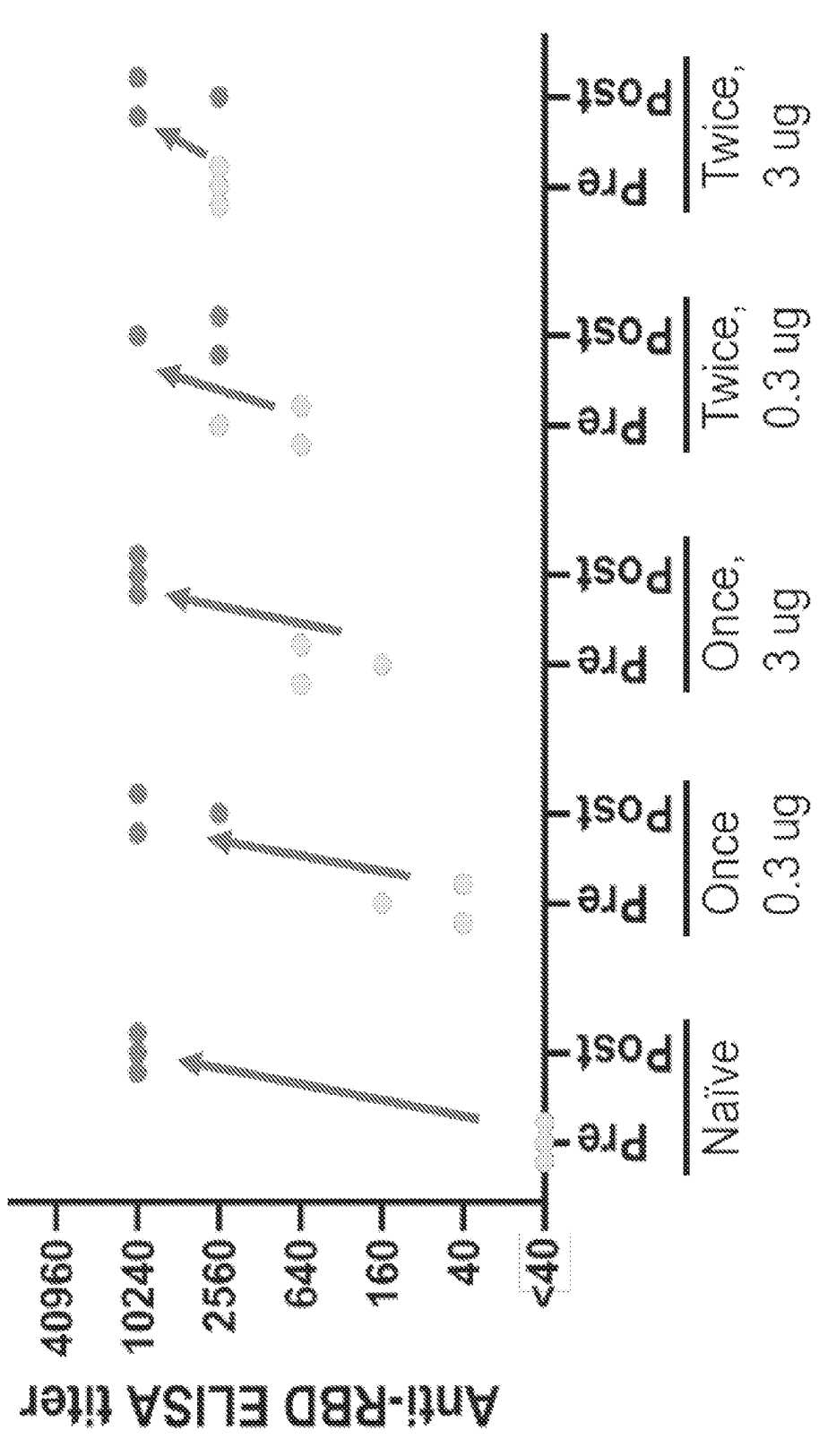

FIG. 71

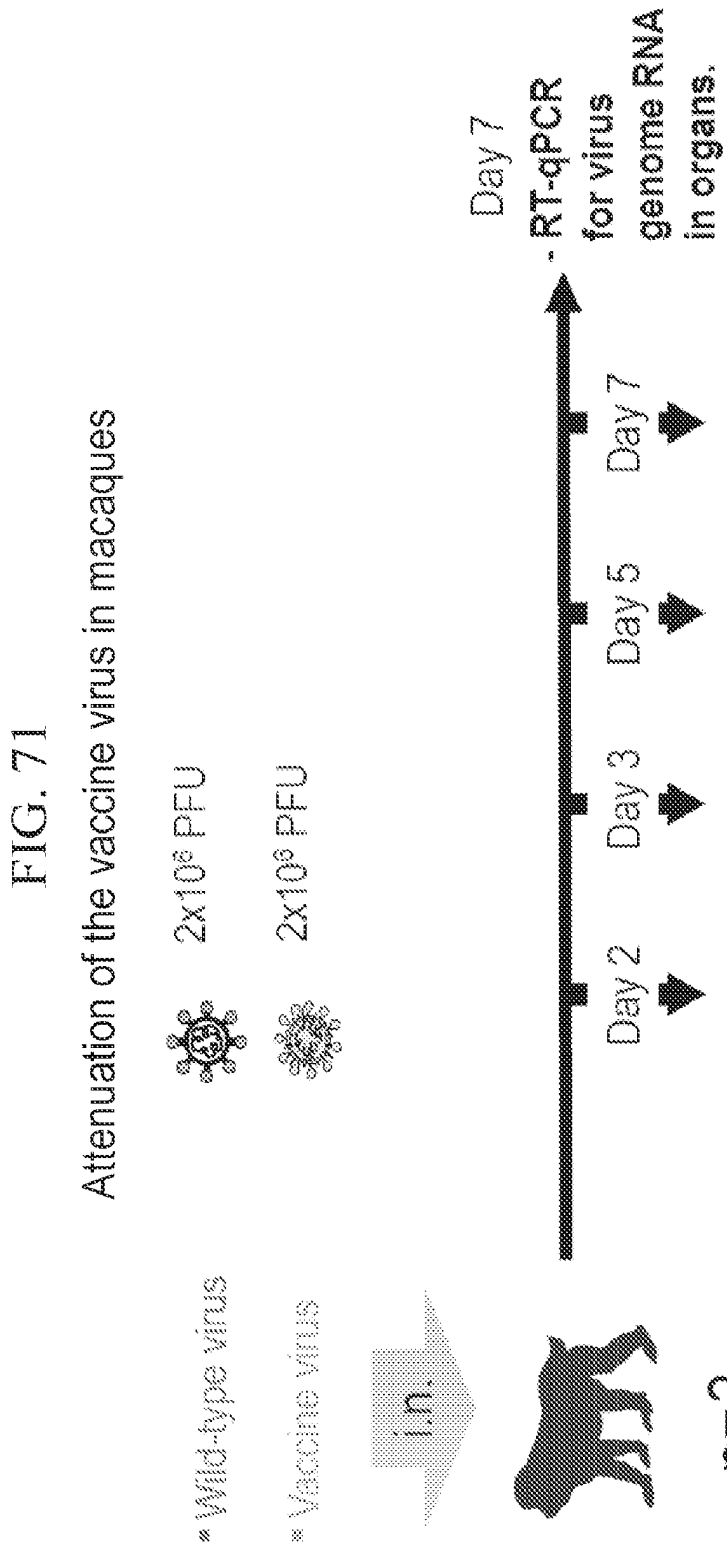
Attenuation of the vaccine virus in macaques
* Wild-type virus
2x10⁶ PFU
* Vaccine virus
2x10⁶ PFU
i.n.
n=3
Day 2
Day 3
Day 5
Day 7
Day 7
- RT-qPCR for virus genome RNA in organs.
- Virus loads in nasal, oral, and rectal swabs
- Body weight

FIG. 72

Body weight change of infected macaques

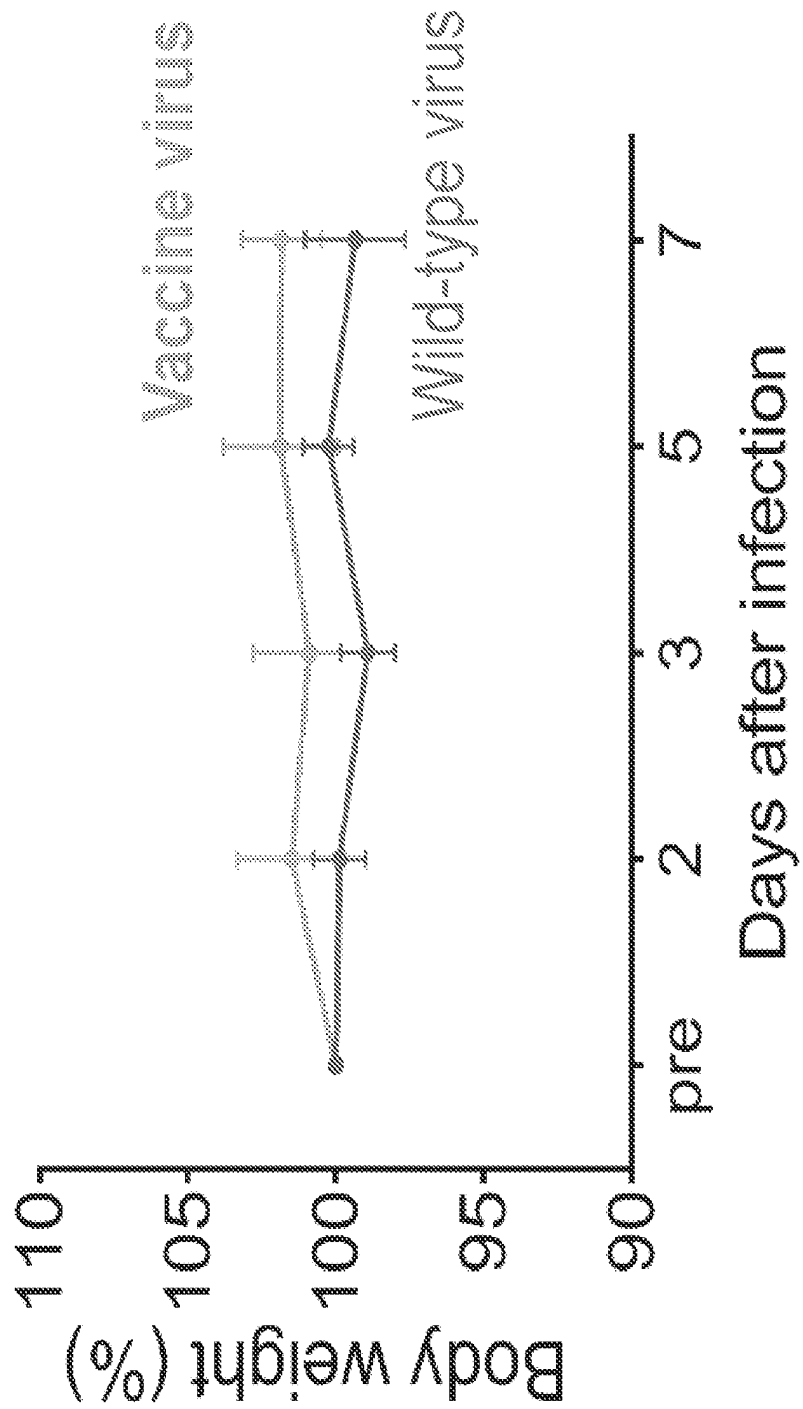

FIG. 73

Virus loads in the nasal, oral, and rectal swabs of infected macaques

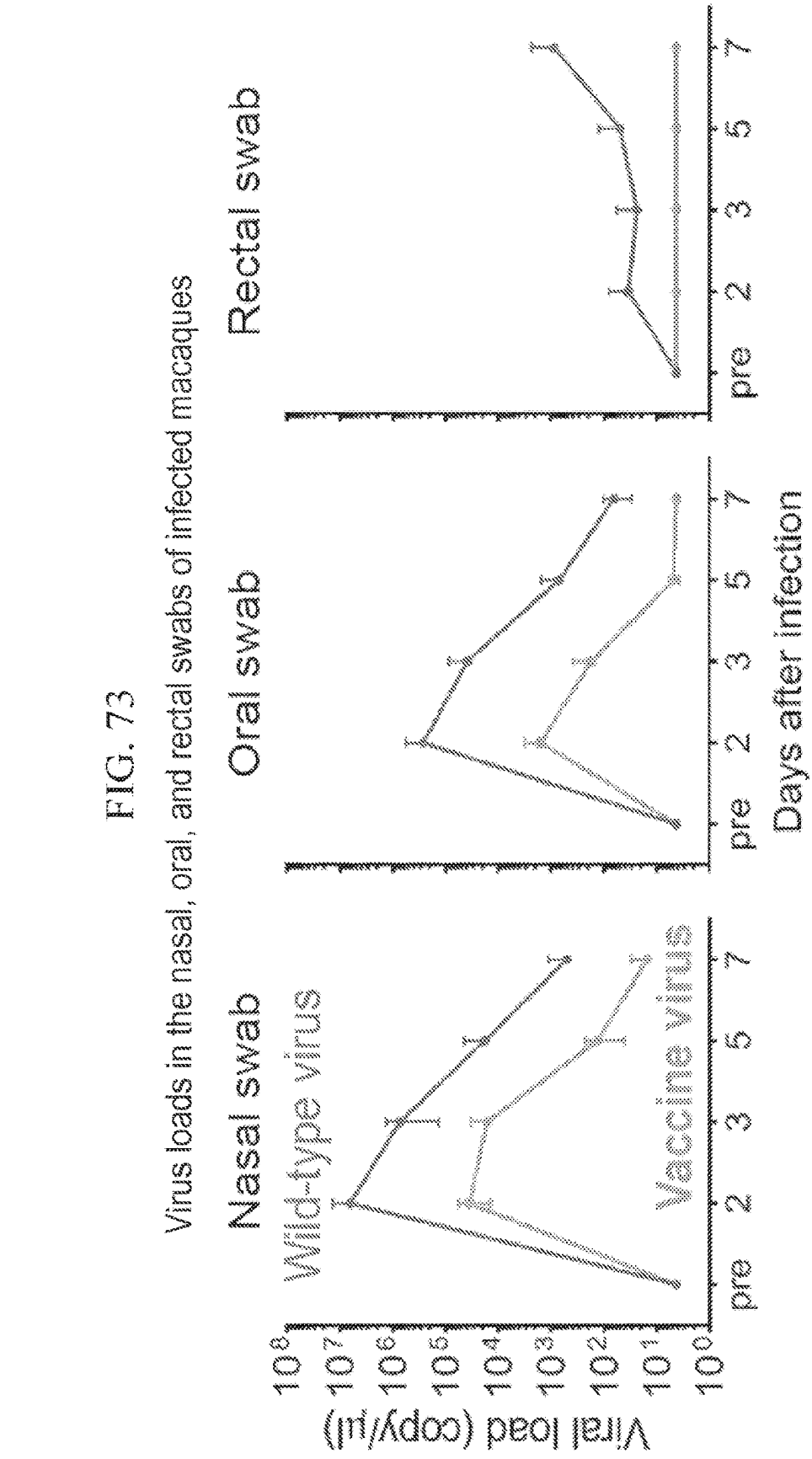

FIG. 74

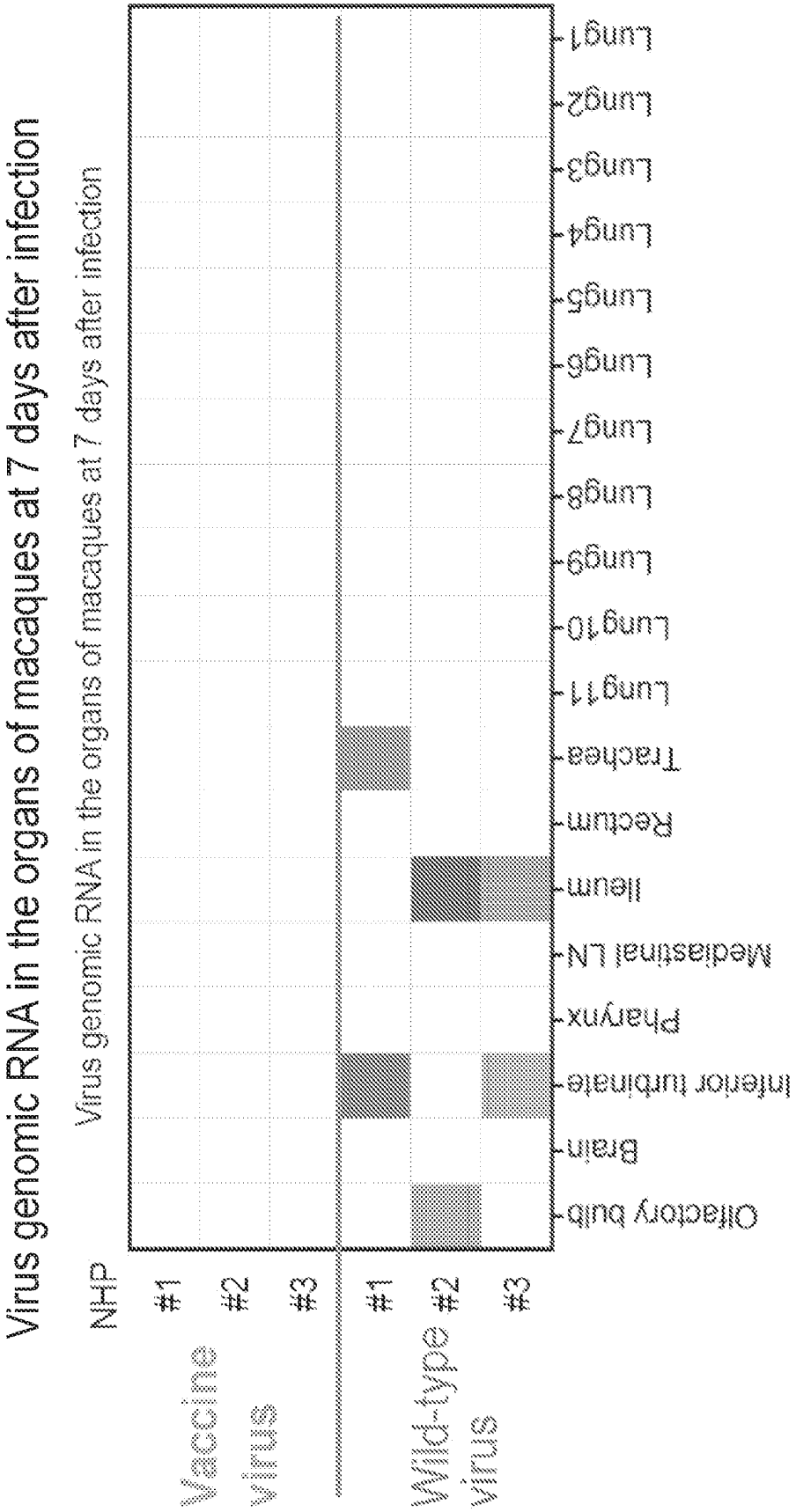
Virus genomic RNA in the organs of macaques at 7 days after infection
Virus genomic RNA in the organs of macaques at 7 days after infection
NHP
Vaccine virus
Wild-type virus
#1
#2
#3
#1
#2
#3
Olfactory bulb
Brain
Inferior turbinate
Pharynx
Mediastinal LN
Ileum
Rectum
Trachea
Lung11
Lung10
Lung9
Lung8
Lung7
Lung6
Lung5
Lung4
Lung3
Lung2
Lung1

FIG. 75

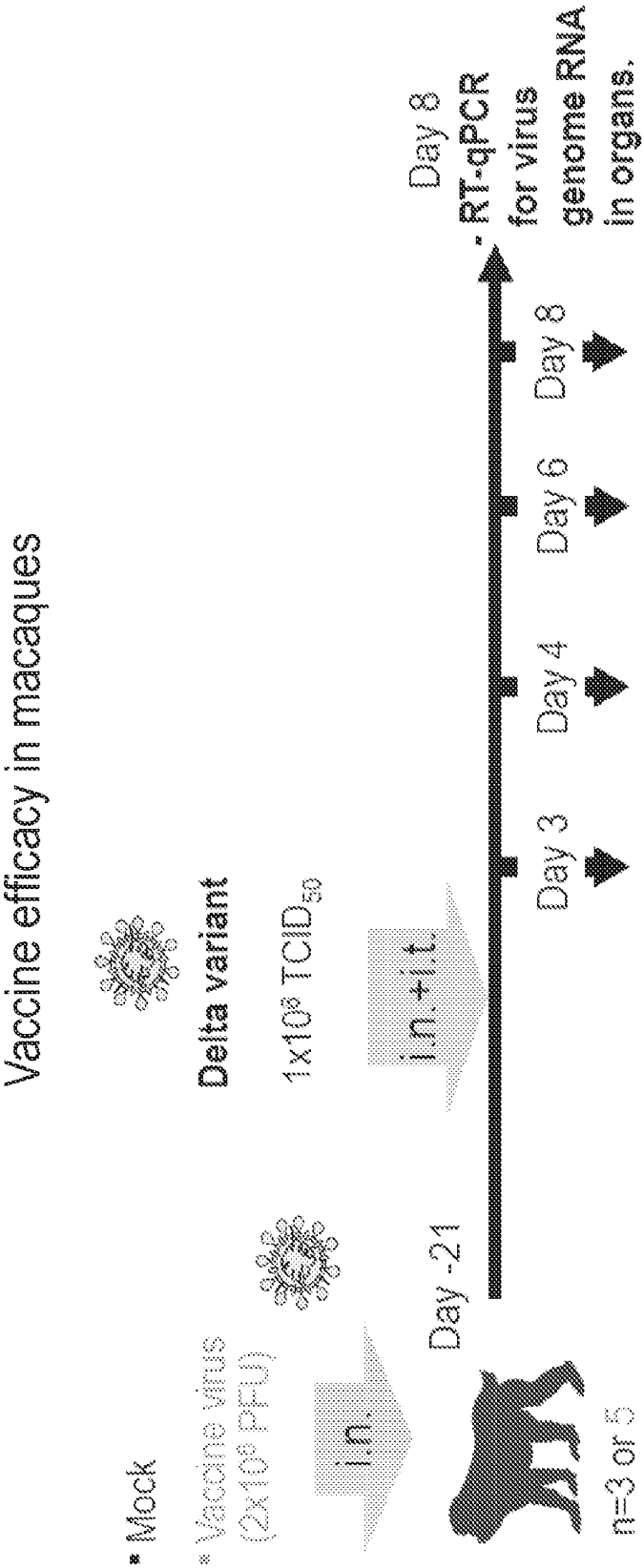
Vaccine efficacy in macaques
Mock
Vaccine virus (2x10⁶ PFU)
i.n.
Delta variant
1x10⁶ TCID₅₀
i.n.+i.t.
n=3 or 5
Day -21
Day 3
Day 4
Day 6
Day 8
Day 8
- RT-qPCR for virus genome RNA in organs.
- Virus load in nasal, oral, and rectal swabs
- Body weight

FIG. 76

Body weight change of immunized macaques after challenge infection

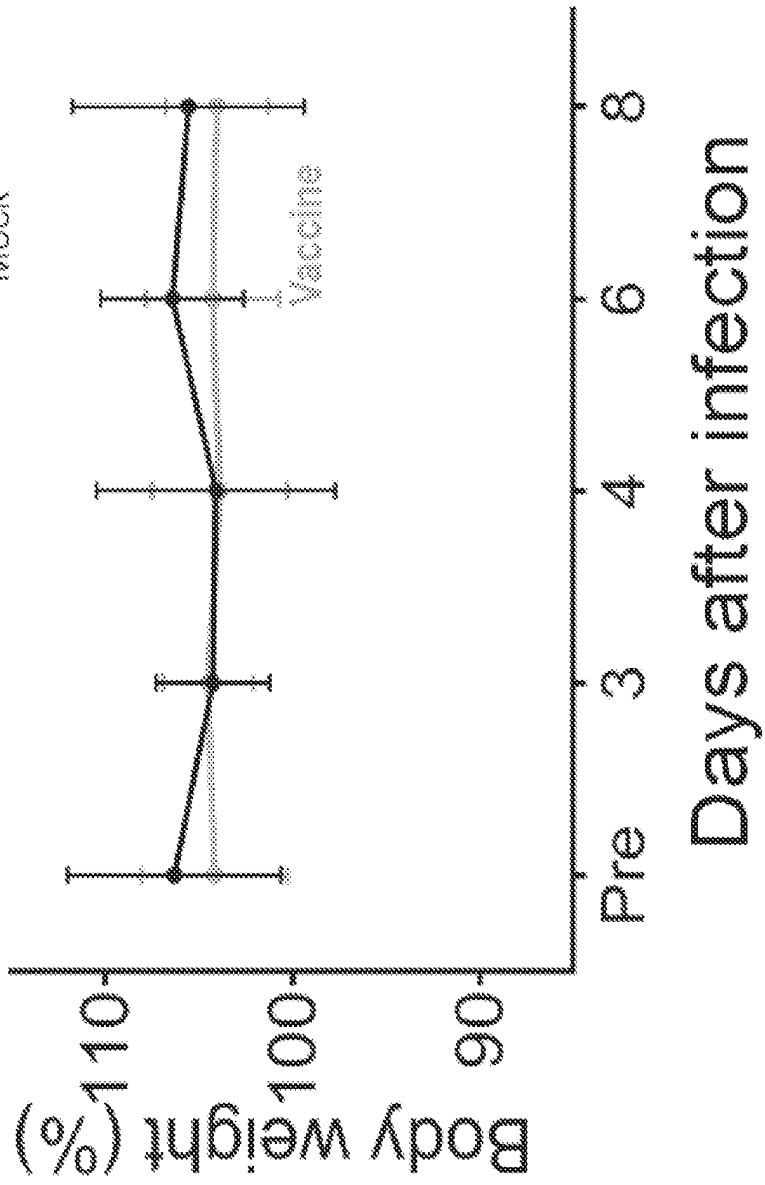

FIG. 77

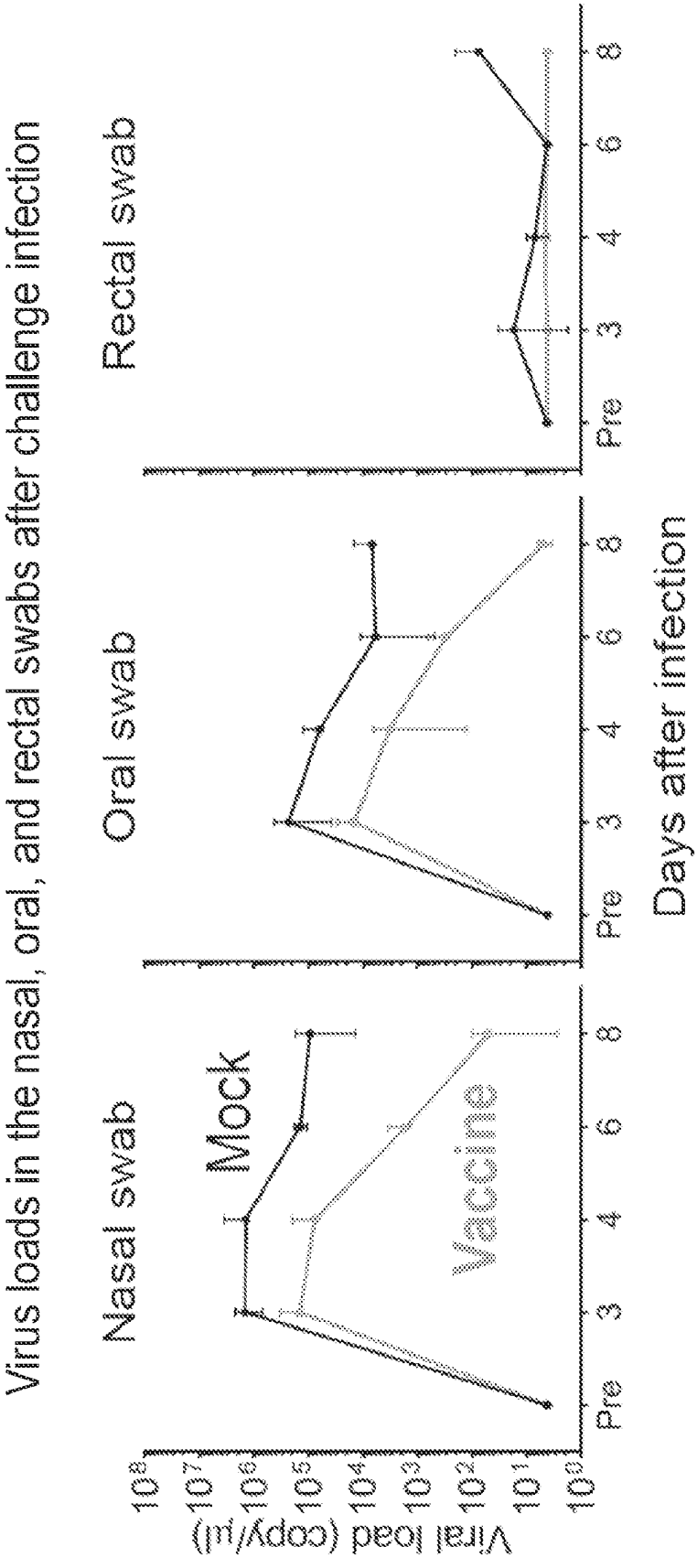
Virus loads in the nasal, oral, and rectal swabs after challenge infection
Nasal swab
Oral swab
Rectal swab
Mock
Vaccine
Viral load (copy/μl)
Days after infection
Pre
3
4
6
8

FIG. 78

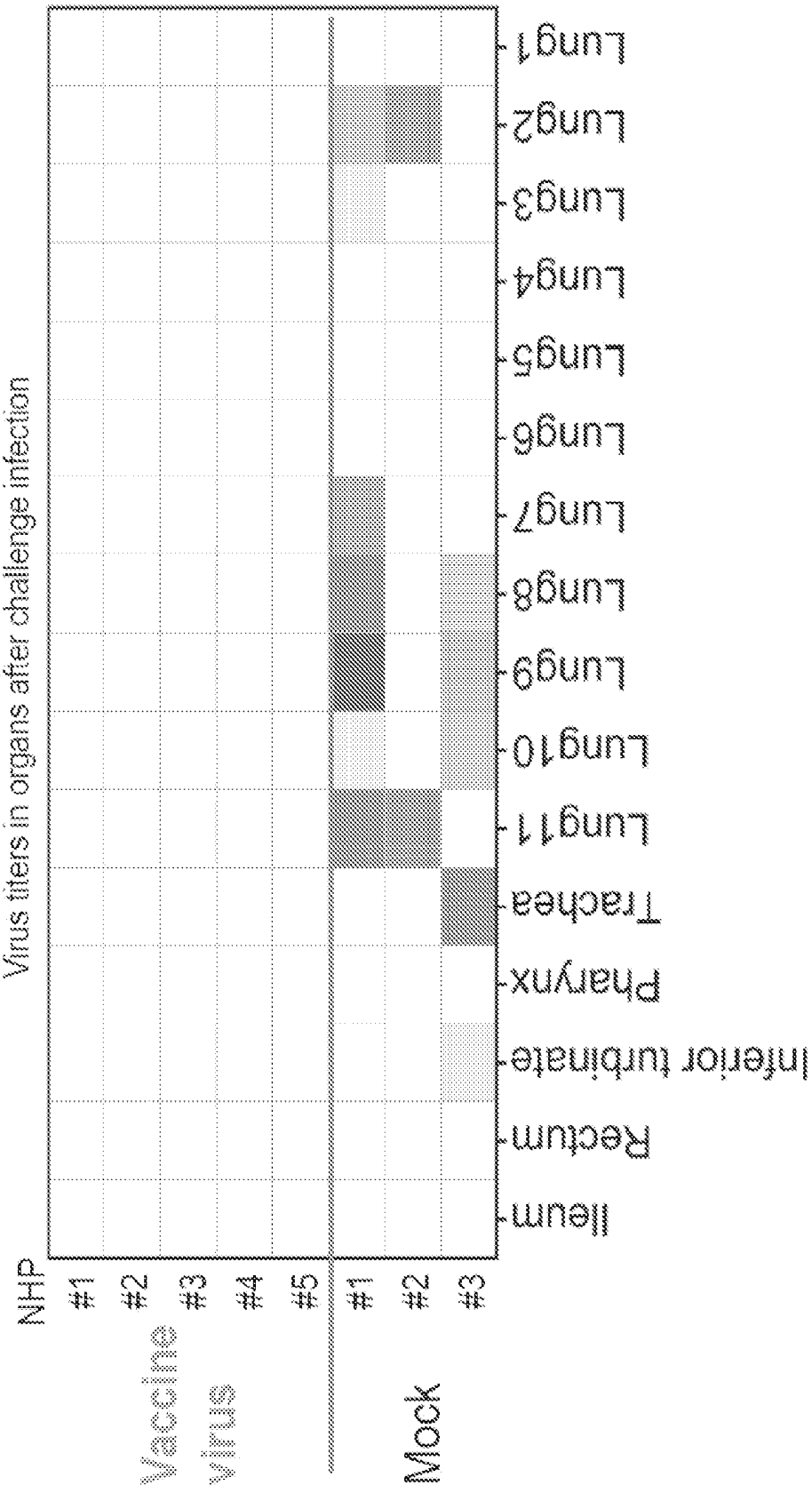
Virus titers in organs after challenge infection
NHP
#1
#2
#3
#4
#5
#1
#2
#3
Vaccine virus
Mock
Ileum
Rectum
Inferior turbinate
Pharynx
Trachea
Lung11
Lung10
Lung9
Lung8
Lung7
Lung6
Lung5
Lung4
Lung3
Lung2
Lung1

Fig. 79 Wuhan-Hu-1 (NCBI Reference Sequence: NC_045512.2) (SEQ ID NO:5)

```
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20341 agtcatagtc agttaggtgg tttacatcta ctgattggac tagctaaacg ttttaaggaa
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20461 acagatgcgc aaacaggttc atctaagtgt gtgtgttctg ttattgattt attacttgat
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20521 gattttgttg aaataataaa atcccaagat ttatctgtag tttctaaggt tgtcaaagtg
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20641 ttttacccaa aattacaatc tagtcaagcg tggcaaccgg gtgttgctat gcctaatctt
20701 tacaaaatgc aaagaatgct attagaaaag tgtgaccttc aaaattatgg tgatagtgca
20761 acattaccta aaggcataat gatgaatgtc gcaaaatata ctcaactgtg tcaatattta
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21301 ccacgcgaac aaatagatgg ttatgtcatg catgcaaatt acatattttg gaggaataca
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21541 cttgttaaca actaaacgaa caatgtttgt ttttcttgtt ttattgccac tagtctctag
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21961 tcaattttgt aatgatccat ttttgggtgt ttattaccac aaaaacaaca aaagttggat
22021 ggaaagtgag ttcagagttt attctagtgc gaataattgc acttttgaat atgtctctca
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27601 ggcgtaaaac acgtctatca gttacgtgcc agatcagttt cacctaaact gttcatcaga
27661 caagaggaag ttcaagaact ttactctcca atttttctta ttgttgcggc aatagtgttt
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28501 caatagcagt ccagatgacc aaattggcta ctaccgaaga gctaccagac gaattcgtgg
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29761 acagtgaaca atgctaggga gagctgccta tatggaagag ccctaatgtg taaaattaat
29821 tttagtagtg ctatccccat gtgattttaa tagcttctta ggagaatgac aaaaaaaaaa
29881 aaaaaaaaaa aaaaaaaaaa aaa
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Fig. 80

SARS-Cov-2/UT-HPCo-038/human/2020/Tokyo (SEQ ID NO:78):

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GCAGCCGATCATCAGCACATCTAGGTTTTGTCCGGGTGTGACCGAAAGGTAA
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CTGGTAGCAGAACTCGAAGGCATTCAGTACGGTCGTAGTGGTGAGACACTTG
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Fig. 80 continued

TGTTAAATCCAGAGAAGAAACTGGCCTACTCATGCCTCTAAAAGCCCCAAAA
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GATTTTACTTTTACACCAGTAAAACAACTGTAGCGTCACTTATCAACACACTT
AACGATCTAAATGAAACTCTTGTTACAATGCCACTTGGCTATGTAACACATGG
CTTAAATTTGGAAGAAGCTGCTCGGTATATGAGATCTCTCAAAGTGCCAGCTA
CAGTTTCTGTTTCTTCACCTGATGCTGTTACAGCGTATAATGGTTATCTTACTT
CTTCTTCTAAAACACCTGAAGAACATTTTATTGAAACCATCTCACTTGCTGGT
TCCTATAAAGATTGGTCCTATTCTGGACAATCTACACAACTAGGTATAGAATTT
CTTAAGAGAGGTGATAAAAGTGTATATTACACTAGTAATCCTACCACATTCCA

Fig. 80 continued

CCTAGATGGTGAAGTTATCACCTTTGACAATCTTAAGACACTTCTTTCTTTGA
GAGAAGTGAGGACTATTAAGGTGTTTACAACAGTAGACAACATTAACCTCCA
CACGCAAGTTGTGGACATGTCAATGACATATGGACAACAGTTTGGTCCAACT
TATTTGGATGGAGCTGATGTTACTAAAATAAAACCTCATAATTCACATGAAGG
TAAAACATTTTATGTTTTACCTAATGATGACACTCTACGTGTTGAGGCTTTTGA
GTACTACCACACAACTGATCCTAGTTTTCTGGGTAGGTACATGTCAGCATTAA
ATCACACTAAAAAAGTGGAAATACCCACAAGTTAATGGTTTAACTTCTATTAAA
TGGGCAGATAACAACTGTTATCTTGCCACTGCATTGTTAACACTCCAACAAAT
AGAGTTGAAGTTTAATCCACCTGCTCTACAAGATGCTTATTACAGAGCAAGG
GCTGGTGAAGCTGCTAACTTTTGTGCACTTATCTTAGCCTACTGTAATAAGAC
AGTAGGTGAGTTAGGTGATGTTAGAGAAACAATGAGTTACTTGTTTCAACAT
GCCAATTTAGATTCTTGCAAAAGAGTCTTGAACGTGGTGTGTAAAACTTGTG
GACAACAGCAGACAACCCTTAAGGGTGTAGAAGCTGTTATGTACATGGGCAC
ACTTTCTTATGAACAATTTAAGAAAGGTGTTCAGATACCTTGTACGTGTGGTA
AACAAGCTACAAAATATCTAGTACAACAGGAGTCACCTTTTGTTATGATGTCA
GCACCACCTGCTCAGTATGAACTTAAGCATGGTACATTTACTTGTGCTAGTGA
GTACACTGGTAATTACCAGTGTGGTCACTATAAACATATAACTTCTAAAGAAA
CTTTGTATTGCATAGACGGTGCTTTACTTACAAAGTCCTCAGAATACAAAGGT
CCTATTACGGATGTTTTCTACAAAGAAAACAGTTACACAACAACCATAAAAC
CAGTTACTTATAAATTGGATGGTGTTGTTTGTACAGAAATTGACCCTAAGTTG
GACAATTATTATAAGAAAGACAATTCTTATTTCACAGAGCAACCAATTGATCT
TGTACCAAACCAACCATATCCAAACGCAAGCTTCGATAATTTTAAGTTTGTAT
GTGATAATATCAAATTTGCTGATGATTTAAACCAGTTAACTGGTTATAAGAAAC
CTGCTTCAAGAGAGCTTAAAGTTACATTTTTCCCTGACTTAAATGGTGATGTG
GTGGCTATTGATTATAAACACTACACACCCTCTTTTAAGAAAGGAGCTAAATT
GTTACATAAACCTATTGTTTGGCATGTTAACAATGCAACTAATAAAGCCACGT
ATAAACCAAATACCTGGTGTATACGTTGTCTTTGGAGCACAAAACCAGTTGA
AACATCAAATTCGTTTGATGTACTGAAGTCAGAGGACGCGCAGGGAATGGAT
AATCTTGCCTGCGAAGATCTAAAACCAGTCTCTGAAGAAGTAGTGGAAAATC
CTACCATACAGAAAGACGTTCTTGAGTGTAATGTGAAAACTACCGAAGTTGT
AGGAGACATTATACTTAAACCAGCAAATAATAGTTTAAAAATTACAGAAGAG
GTTGGCCACACAGATCTAATGGCTGCTTATGTAGACAATTCTAGTCTTACTATT
AAGAAACCTAATGAATTATCTAGAGTATTAGGTTTGAAAACCCTTGCTACTCA
TGGTTTAGCTGCTGTTAATAGTGTCCCTTGGGATACTATAGCTAATTATGCTAA
GCCTTTTCTTAACAAAGTTGTTAGTACAACTACTAACATAGTTACACGGTGTT
TAAACCGTGTTTGTACTAATTATATGCCTTATTTCTTTACTTTATTGCTACAATT
GTGTACTTTTACTAGAAGTACAAATTCTAGAATTAAAGCATCTATGCCGACTA
CTATAGCAAAGAATACTGTTAAGAGTGTCGGTAAATTTTGTCTAGAGGCTTCA
TTTAATTATTTGAAGTCACCTAATTTTTCTAAACTGATAAATATTATAATTTGGT
TTTTACTATTAAGTGTTTGCCTAGGTTCTTTAATCTACTCAACCGCTGCTTTAG
GTGTTTTAATGTCTAATTTAGGCATGCCTTCTTACTGTACTGGTTACAGAGAAG
GCTATTTGAACTCTACTAATGTCACTATTGCAACCTACTGTACTGGTTCTATAC
CTTGTAGTGTTTGTCTTAGTGGTTTAGATTCTTTAGACACCTATCCTTCTTTAG
AAACTATACAAATTACCATTTCATCTTTTAAATGGGATTTAACTGCTTTTGGCT
TAGTTGCAGAGTGGTTTTTGGCATATATTCTTTTCACTAGGTTTTTCTATGTACT
TGGATTGGCTGCAATCATGCAATTGTTTTTCAGCTATTTTGCAGTACATTTTAT
TAGTAATTCTTGGCTTATGTGGTTAATAATTAATCTTGTACAAATGGCCCCGAT

Fig. 80 continued

TTCAGCTATGGTTAGAATGTACATCTTCTTTGCATCATTTTATTATGTATGGAAA
AGTTATGTGCATGTTGTAGACGGTTGTAATTCATCAACTTGTATGATGTGTTAC
AAACGTAATAGAGCAACAAGAGTCGAATGTACAACTATTGTTAATGGTGTTA
GAAGGTCCTTTTATGTCTATGCTAATGGAGGTAAAGGCTTTTGCAAACTACAC
AATTGGAATTGTGTTAATTGTGATACATTCTGTGCTGGTAGTACATTTATTAGT
GATGAAGTTGCGAGAGACTTGTCACTACAGTTTAAAAGACCAATAAATCCTA
CTGACCAGTCTTCTTACATCGTTGATAGTGTTACAGTGAAGAATGGTTCCATC
CATCTTTACTTTGATAAAGCTGGTCAAAAGACTTATGAAAGACATTCTCTCTC
TCATTTTGTTAACTTAGACAACCTGAGAGCTAATAACACTAAAGGTTCATTGC
CTATTAATGTTATAGTTTTTGATGGTAAATCAAAATGTGAAGAATCATCTGCAA
AATCAGCGTCTGTTTACTACAGTCAGCTTATGTGTCAACCTATACTGTTACTAG
ATCAGGCATTAGTGTCTGATGTTGGTGATAGTGCGGAAGTTGCAGTTAAAATG
TTTGATGCTTACGTTAATACGTTTTCATCAACTTTTAACGTACCAATGGAAAAA
CTCAAAACACTAGTTGCAACTGCAGAAGCTGAACTTGCAAAGAATGTGTCCT
TAGACAATGTCTTATCTACTTTTATTTCAGCAGCTCGGCAAGGGTTTGTTGATT
CAGATGTAGAAACTAAAGATGTTGTTGAATGTCTTAAATTGTCACATCAATCT
GACATAGAAGTTACTGGCGATAGTTGTAATAACTATATGCTCACCTATAACAA
AGTTGAAAACATGACACCCCGTGACCTTGGTGCTTGTATTGACTGTAGTGCG
CGTCATATTAATGCGCAGGTAGCAAAAAGTCACAACATTGCTTTGATATGGAA
CGTTAAAGATTTCATGTCATTGTCTGAACAACTACGAAAACAAATACGTAGTG
CTGCTAAAAAGAATAACTTACCTTTTAAGTTGACATGTGCAACTACTAGACAA
GTTGTTAATGTTGTAACAACAAAGATAGCACTTAAGGGTGGTAAAATTGTTAA
TAATTGGTTGAAGCAGTTAATTAAAGTTACACTTGTGTTCCTTTTTGTTGCTGC
TATTTTCTATTTAATAACACCTGTTCATGTCATGTCTAAACATACTGACTTTTCA
AGTGAAATCATAGGATACAAGGCTATTGATGGTGGTGTCACTCGTGACATAGC
ATCTACAGATACTTGTTTTGCTAACAAACATGCTGATTTTGACACATGGTTTAG
CCAGCGTGGTGGTAGTTATACTAATGACAAAGCTTGCCCATTGATTGCTGCAG
TCATAACAAGAGAAGTGGGTTTTGTCGTGCCTGGTTTGCCTGGCACGATATTA
CGCACAACTAATGGTGACTTTTTGCATTTCTTACCTAGAGTTTTTAGTGCAGT
TGGTAACATCTGTTACACACCATCAAAACTTATAGAGTACACTGACTTTGCAA
CATCAGCTTGTGTTTTGGCTGCTGAATGTACAATTTTTAAAGATGCTTCTGGTA
AGCCAGTACCATATTGTTATGATACCAATGTACTAGAAGGTTCTGTTGCTTATG
AAAGTTTACGCCCTGACACACGTTATGTGCTCATGGATGGCTCTATTATTCAAT
TTCCTAACACCTACCTTGAAGGTTCTGTTAGAGTGGTAACAACTTTTGATTCT
GAGTACTGTAGGCACGGCACTTGTGAAAGATCAGAAGCTGGTGTTTGTGTAT
CTACTAGTGGTAGATGGGTACTTAATAATGATTATTACAGATCTTTACCAGGAG
TTTTCTGTGGTGTAGATGCTGTAAATTTACTTACTAATATGTTTACACCACTAAT
TCAACCTATTGGTGCTTTGGACATATCAGCATCTATAGTAGCTGGTGGTATTGT
AGCTATCGTAGTAACATGCCTTGCCTACTATTTTATGAGGTTTAGAAGAGCTTT
TGGTGAATACAGTCATGTAGTTGCCTTTAATACTTTACTATTCCTTATGTCATTC
ACTGTACTCTGTTTAACACCAGTTTACTCATTCTTACCTGGTGTTTATTCTGTT
ATTTACTTGTACTTGACATTTTATCTTACTAATGATGTTTCTTTTTTTAGCACATA
TTCAGTGGATGGTTATGTTCACACCTTTAGTACCTTTCTGGATAACAATTGCTT
ATATCATTTGTATTTCCACAAAGCATTTCTATTGGTTCTTTAGTAATTACCTAAA
GAGACGTGTAGTCTTTAATGGTGTTTCCTTTAGTACTTTTGAAGAAGCTGCGC
TGTGCACCTTTTTGTTAAATAAAGAAATGTATCTAAAGTTGCGTAGTGATGTG
CTATTACCTCTTACGCAATATAATAGATACTTAGCTCTTTATAATAAGTACAAGT

Fig. 80 continued

ATTTTAGTGGAGCAATGGATACAACTAGCTACAGAGAAGCTGCTTGTTGTCAT
CTCGCAAAGGCTCTCAATGACTTCAGTAACTCAGGTTCTGATGTTCTTTACCA
ACCACCACAAACCTCTATCACCTCAGCTGTTTTGCAGAGTGGTTTTAGAAAA
ATGGCATTCCCATCTGGTAAAGTTGAGGGTTGTATGGTACAAGTAACTTGTGG
TACAACTACACTTAACGGTCTTTGGCTTGATGACGTAGTTTACTGTCCAAGAC
ATGTGATCTGCACCTCTGAAGACATGCTTAACCCTAATTATGAAGATTTACTC
ATTCGTAAGTCTAATCATAATTTCTTGGTACAGGCTGGTAATGTTCAACTCAGG
GTTATTGGACATTCTATGCAAAATTGTGTACTTAAGCTTAAGGTTGATACAGCC
AATCCTAAGACACCTAAGTATAAGTTTGTTCGCATTCAATCAGGACAGACTTT
TTCAGTGTTAGCTTGTTACAATGGTTCACCATCTGGTGTTTACCAATGTGCTAT
GAGGCCCAATTTCACTATTAAGGGTTCATTCCTTAATGGTTCATGTGGTAGTGT
TGGTTTTTAACATAGATTATGACTGTGTCTCTTTTTGTTACATGCACCATATGGA
ATTACCAACTGGAGTTCATGCTGGCACAGACTTAGAAGGTAACTTTTATGGAC
CTTTTGTTGACAGGCAAACAGCACAAGCAGCTGGTACGGACACAACTATTAC
AGTTAATGTTTTAGCTTGGTTGTACGCTGCTGTTATAAATGGAGACAGGTGGT
TTCTCAATCGATTTACCACAACTCTTAATGACTTTAACCTTGTGGCTATGAAGT
ACAATTATGAACCTCTAACACAAGACCATGTTGACATACTAGGACCTCTTTCT
GCTCAAACTGGAATTGCCGTTTTAGATATGTGTGCTTCATTAAAAGAATTACT
GCAAAATGGTATGAATGGACGTACCATATTGGGTAGTGCTTTATTAGAAGATG
AATTTACACCTTTTGATGTTGTTAGACAATGCTCAGGTGTTACTTTCCAAAGT
GCAGTGAAAAGAACAATCAAGGGTACACACCACTGGTTGTTACTCACAATTT
TGACTTCACTTTTAGTTTTAGTCCAGAGTACTCAATGGTCTTTGTTCTTTTTTT
TGTATGAAAATGCCTTTTTACCTTTTGCTATGGGTATTATTGCTATGTCTGCTTT
TGCAATGATGTTTGTCAAACATAAGCATGCATTTCTCTGTTTGTTTTTGTTACC
TTCTCTTGCCACTGTAGCTTATTTTAATATGGTCTATATGCCTGCTAGTTGGGTG
ATGCGTATTATGACATGGTTGGATATGGTTGATACTAGTTTGTCTGGTTTTAAG
CTAAAAGACTGTGTTATGTATGCATCAGCTGTAGTGTTACTAATCCTTATGACA
GCAAGAACTGTGTATGATGATGGTGCTAGGAGAGTGTGGACACTTATGAATG
TCTTGACACTCGTTTATAAAGTTTATTATGGTAATGCTTTAGATCAAGCCATTT
CCATGTGGGCTCTTATAATCTCTGTTACTTCTAACTACTCAGGTGTAGTTACAA
CTGTCATGTTTTTGGCCAGAGGTATTGTTTTTATGTGTGTTGAGTATTGCCCTA
TTTTCTTCATAACTGGTAATACACTTCAGTGTATAATGCTAGTTTATTGTTTCTT
AGGCTATTTTTGTACTTGTTACTTTGGCCTCTTTTGTTTACTCAACCGCTACTT
TAGACTGACTCTTGGTGTTTATGATTACTTAGTTTCTACACAGGAGTTTAGATA
TATGAATTCACAGGGACTACTCCCACCCAAGAATAGCATAGATGCCTTCAAAC
TCAACATTAAATTGTTGGGTGTTGGTGGCAAACCTTGTATCAAAGTAGCCACT
GTACAGTCTAAAATGTCAGATGTAAAGTGCACATCAGTAGTCTTACTCTCAGT
TTTGCAACAACTCAGAGTAGAATCATCATCTAAATTGTGGGCTCAATGTGTCC
AGTTACACAATGACATTCTCTTAGCTAAAGATACTACTGAAGCCTTTGAAAAA
ATGGTTTCACTACTTTCTGTTTTGCTTTCCATGCAGGGTGCTGTAGACATAAA
CAAGCTTTGTGAAGAAATGCTGGACAACAGGGCAACCTTACAAGCTATAGCC
TCAGAGTTTAGTTCCCTTCCATCATATGCAGCTTTTGCTACTGCTCAAGAAGC
TTATGAGCAGGCTGTTGCTAATGGTGATTCTGAAGTTGTTCTTAAAAAGTTGA
AGAAGTCTTTGAATGTGGCTAAATCTGAATTTGACCGTGATGCAGCCATGCA
ACGTAAGTTGGAAAAGATGGCTGATCAAGCTATGACCCAAATGTATAAACAG
GCTAGATCTGAGGACAAGAGGGCAAAAGTTACTAGTGCTATGCAGACAATGC
TTTTCACTATGCTTAGAAAGTTGGATAATGATGCACTCAACAACATTATCAAC

Fig. 80 continued

AATGCAAGAGATGGTTGTGTTCCCTTGAACATAATACCTCTTACAACAGCAGC
CAAACTAATGGTTGTCATACCAGACTATAACACATATAAAAATACGTGTGATG
GTACAACATTTACTTATGCATCAGCATTGTGGGAAATCCAACAGGTTGTAGAT
GCAGATAGTAAAATTGTTCAACTTAGTGAAATTAGTATGGACAATTCACCTAA
TTTAGCATGGCCTCTTATTGTAACAGCTTTAAGGGCCAATTCTGCTGTCAAATT
ACAGAATAATGAGCTTAGTCCTGTTGCACTACGACAGATGTCTTGTGCTGCCG
GTACTACACAAACTGCTTGCACTGATGACAATGCGTTAGCTTACTACAACACA
ACAAAGGGAGGTAGGTTTGTACTTGCACTGTTATCCGATTTACAGGATTTGAA
ATGGGCTAGATTCCCTAAGAGTGATGGAACTGGTACTATCTATACAGAACTGG
AACCACCTTGTAGGTTTGTTACAGACACACCTAAAGGTCCTAAAGTGAAGTA
TTTATACTTTATTAAAGGATTAAACAACCTAAATAGAGGTATGGTACTTGGTAG
TTTAGCTGCCACAGTACGTCTACAAGCTGGTAATGCAACAGAAGTGCCTGCC
AATTCAACTGTATTATCTTTCTGTGCTTTTGCTGTAGATGCTGCTAAAGCTTAC
AAAGATTATCTAGCTAGTGGGGGACAACCAATCACTAATTGTGTTAAGATGTT
GTGTACACACACTGGTACTGGTCAGGCAATAACAGTTACACCGGAAGCCAAT
ATGGATCAAGAATCCTTTGGTGGTGCATCGTGTTGTCTGTACTGCCGTTGCCA
CATAGATCATCCAAATCCTAAAGGATTTTGTGACTTAAAAGGTAAGTATGTAC
AAATACCTACAACTTGTGCTAATGACCCTGTGGGTTTTACACTTAAAAACACA
GTCTGTACCGTCTGCGGTATGTGGAAAGGTTATGGCTGTAGTTGTGATCAACT
CCGCGAACCCATGCTTCAGTCAGCTGATGCACAATCGTTTTTAAACGGGTTTG
CGGTGTAAGTGCAGCCCGTCTTACACCGTGCGGCACAGGCACTAGTACTGAT
GTCGTATACAGGGCTTTTGACATCTACAATGATAAAGTAGCTGGTTTTGCTAA
ATTCCTAAAAACTAATTGTTGTCGCTTCCAAGAAAAGGACGAAGATGACAAT
TTAATTGATTCTTACTTTGTAGTTAAGAGACACACTTTCTCTAACTACCAACAT
GAAGAAACAATTTATAATTTACTTAAGGATTGTCCAGCTGTTGCTAAACATGA
CTTCTTTAAGTTTAGAATAGACGGTGACATGGTACCACATATATCACGTCAAC
GTCTTACTAAATACACAATGGCAGACCTCGTCTATGCTTTAAGGCATTTTGATG
AAGGTAATTGTGACACATTAAAAGAAATACTTGTCACATACAATTGTTGTGAT
GATGATTATTTCAATAAAAAGGACTGGTATGATTTTGTAGAAAACCCAGATAT
ATTACGCGTATACGCCAACTTAGGTGAACGTGTACGCCAAGCTTTGTTAAAAA
CAGTACAATTCTGTGATGCCATGCGAAATGCTGGTATTGTTGGTGTACTGACA
TTAGATAATCAAGATCTCAATGGTAACTGGTATGATTTCGGTGATTTCATACAA
ACCACGCCAGGTAGTGGAGTTCCTGTTGTAGATTCTTATTATTCATTGTTAATG
CCTATATTAACCTTGACCAGGGCTTTAACTGCAGAGTCACATGTTGACACTGA
CTTAACAAAGCCTTACATTAAGTGGGATTTGTTAAAATATGACTTCACGGAAG
AGAGGTTAAAACTCTTTGACCGTTATTTTAAATATTGGGATCAGACATACCAC
CCAAATTGTGTTAACTGTTTGGATGACAGATGCATTCTGCATTGTGCAAACTT
TAATGTTTTATTCTCTACAGTGTTCCCACTTACAAGTTTTGGACCACTAGTGAG
AAAAATATTTGTTGATGGTGTTCCATTTGTAGTTTCAACTGGATACCACTTCAG
AGAGCTAGGTGTTGTACATAATCAGGATGTAAACTTACATAGCTCTAGACTTA
GTTTTAAGGAATTACTTGTGTATGCTGCTGACCCTGCTATGCACGCTGCTTCT
GGTAATCTATTACTAGATAAACGCACTACGTGCTTTTCAGTAGCTGCACTTACT
AACAATGTTGCTTTTCAAACTGTCAAACCCGGTAATTTTAACAAAGACTTCTA
TGACTTTGTTGTGTCTAAGGGTTTCTTTAAGGAAGGAAGTTCTGTTGAATTAA
AACACTTCTTCTTTGCTCAGGATGGTAATGCTGCTATCAGCGATTATGACTACT
ATCGTTATAATCTACCAACAATGTGTGATATCAGACAACTACTATTTGTAGTTG
AAGTTGTTGATAAGTACTTTGATTGTTACGATGGTGGCTGTATTAATGCTAACC

Fig. 80 continued

AAGTCATCGTCAACAACCTAGACAAATCAGCTGGTTTTCCATTTAATAAATGG
GGTAAGGCTAGACTTTATTATGATTCAATGAGTTATGAGGATCAAGATGCACT
TTTCGCATATACAAAACGTAATGTCATCCCTACTATAACTCAAATGAATCTTAA
GTATGCCATTAGTGCAAAGAATAGAGCTCGCACCGTAGCTGGTGTCTCTATCT
GTAGTACTATGACCAATAGACAGTTTCATCAAAAATTATTGAAATCAATAGCC
GCCACTAGAGGAGCTACTGTAGTAATTGGAACAAGCAAATTCTATGGTGGTT
GGCACAACATGTTAAAAACTGTTTATAGTGATGTAGAAAACCCTCACCTTATG
GGTTGGGATTATCCTAAATGTGATAGAGCCATGCCTAACATGCTTAGAATTATG
GCCTCACTTGTTCTTGCTCGCAAACATACAACGTGTTGTAGCTTGTCACACCG
TTTCTATAGATTAGCTAATGAGTGTGCTCAAGTATTGAGTGAAATGGTCATGTG
TGGCGGTTCACTATATGTTAAACCAGGTGGAACCTCATCAGGAGATGCCACA
ACTGCTTATGCTAATAGTGTTTTTAACATTTGTCAAGCTGTCACGGCCAATGTT
AATGCACTTTTATCTACTGATGGTAACAAAATTGCCGATAAGTATGTCCGCAAT
TTACAACACAGACTTTATGAGTGTCTCTATAGAAATAGAGATGTTGACACAGA
CTTTGTGAATGAGTTTTACGCATATTTGCGTAAACATTTCTCAATGATGATACT
CTCTGACGATGCTGTTGTGTGTTTCAATAGCACTTATGCATCTCAAGGTCTAG
TGGCTAGCATAAAGAACTTTAAGTCAGTTCTTTATTATCAAAACAATGTTTTTA
TGTCTGAAGCAAAATGTTGGACTGAGACTGACCTTACTAAAGGACCTCATGA
ATTTTGCTCTCAACATACAATGCTAGTTAAACAGGGTGATGATTATGTGTACCT
TCCTTACCCAGATCCATCAAGAATCCTAGGGGCCGGCTGTTTTGTAGATGATA
TCGTAAAAACAGATGGTACACTTATGATTGAACGGTTCGTGTCTTTAGCTATA
GATGCTTACCCACTTACTAAACATCCTAATCAGGAGTATGCTGATGTCTTTCAT
TTGTACTTACAATACATAAGAAAGCTACATGATGAGTTAACAGGACACATGTT
AGACATGTATTCTGTTATGCTTACTAATGATAACACTTCAAGGTATTGGGAACC
TGAGTTTTATGAGGCTATGTACACACCGCATACAGTCTTACAGGCTGTTGGGG
CTTGTGTTCTTTGCAATTCACAGACTTCATTAAGATGTGGTGCTTGCATACGTA
GACCATTCTTATGTTGTAAATGCTGTTACGACCATGTCATATCAACATCACATA
AATTAGTCTTGTCTGTTAATCCGTATGTTTGCAATGCTCCAGGTTGTGATGTCA
CAGATGTGACTCAACTTTACTTAGGAGGTATGAGCTATTATTGTAAATCACATA
AACCACCCATTAGTTTTCCATTGTGTGCTAATGGACAAGTTTTTGGTTTATATA
AAAATACATGTGTTGGTAGCGATAATGTTACTGACTTTAATGCAATTGCAACAT
GTGACTGGACAAATGCTGGTGATTACATTTTAGCTAACACCTGTACTGAAAG
ACTCAAGCTTTTTGCAGCAGAAACGCTCAAAGCTACTGAGGAGACATTTAAA
CTGTCTTATGGTATTGCTACTGTACGTGAAGTGCTGTCTGACAGAGAATTACA
TCTTTCATGGGAAGTTGGTAAACCTAGACCACCACTTAACCGAAATTATGTCT
TTACTGGTTATCGTGTAACTAAAAACAGTAAAGTACAAATAGGAGAGTACAC
CTTTGAAAAAGGTGACTATGGTGATGCTGTTGTTTACCGAGGTACAACAACT
TACAAATTAAATGTTGGTGATTATTTTGTGCTGACATCACATACAGTAATGCCA
TTAAGTGCACCTACACTAGTGCCACAAGAGCACTATGTTAGAATTACTGGCTT
ATACCCAACACTCAATATCTCAGATGAGTTTTCTAGCAATGTTGCAAATTATCA
AAAGGTTGGTATGCAAAAGTATTCTACACTCCAGGGACCACCTGGTACTGGT
AAGAGTCATTTTGCTATTGGCCTAGCTCTCTACTACCCTTCTGCTCGCATAGTG
TATACAGCTTGCTCTCATGCCGCTGTTGATGCACTATGTGAGAAGGCATTAAA
ATATTTGCCTATAGATAAATGTAGTAGAATTATACCTGCACGTGCTCGTGTAGA
GTGTTTTGATAAATTCAAAGTGAATTCAACATTAGAACAGTATGTCTTTTGTA
CTGTAAATGCATTGCCTGAGACGACAGCAGATATAGTTGTCTTTGATGAAATT
TCAATGGCCACAAATTATGATTTGAGTGTTGTCAATGCCAGATTACGTGCTAA

Fig. 80 continued

GCACTATGTGTACATTGGCGACCCTGCTCAATTACCTGCACCACGCACATTGC
TAACTAAGGGCACACTAGAACCAGAATATTTCAATTCAGTGTGTAGACTTATG
AAAACTATAGGTCCAGACATGTTCCTCGGAACTTGTCGGCGTTGTCCTGCTG
AAATTGTTGACACTGTGAGTGCTTTGGTTTATGATAATAAGCTTAAAGCACAT
AAAGACAAATCAGCTCAATGCTTTAAAATGTTTTATAAGGGTGTTATCACGCA
TGATGTTTCATCTGCAATTAACAGGCCACAAATAGGCGTGGTAAGAGAATTCC
TTACACGTAACCCTGCTTGGAGAAAAGCTGTCTTTATTTCACCTTATAATTCAC
AGAATGCTGTAGCCTCAAAGATTTTGGGACTACCAACTCAAACTGTTGATTC
ATCACAGGGCTCAGAATATGACTATGTCATATTCACTCAAACCACTGAAACAG
CTCACTCTTGTAATGTAAACAGATTTAATGTTGCTATTACCAGAGCAAAAGTA
GGCATACTTTGCATAATGTCTGATAGAGACCTTTATGACAAGTTGCAATTTACA
AGTCTTGAAATTCCACGTAGGAATGTGGCAACTTTACAAGCTGAAAATGTAA
CAGGACTCTTTAAAGATTGTAGTAAGGTAATCACTGGGTTACATCCTACACAG
GCACCTACACACCTCAGTGTTGACACTAAATTCAAAACTGAAGGTTTATGTG
TTGACATACCTGGCATACCTAAGGACATGACCTATAGAAGACTCATCTCTATG
ATGGGTTTTAAAATGAATTATCAAGTTAATGGTTACCCTAACATGTTTATCACC
CGCGAAGAAGCTATAAGACATGTACGTGCATGGATTGGCTTCGATGTCGAGG
GGTGTCATGCTACTAGAGAAGCTGTTGGTACCAATTTACCTTTACAGCTAGGT
TTTTCTACAGGTGTTAACCTAGTTGCTGTACCTACAGGTTATGTTGATACACCT
AATAATACAGATTTTTCCAGAGTTAGTGCTAAACCACCGCCTGGAGATCAATT
TAAACACCTCATACCACTTATGTACAAAGGACTTCCTTGGAATGTAGTGCGTA
TAAAGATTGTACAAATGTTAAGTGACACACTTAAAAATCTCTCTGACAGAGT
CGTATTTGTCTTATGGGCACATGGCTTTGAGTTGACATCTATGAAGTATTTTGT
GAAAATAGGACCTGAGCGCACCTGTTGTCTATGTGATAGACGTGCCACATGC
TTTTCCACTGCTTCAGACACTTATGCCTGTTGGCATCATTCTATTGGATTTGAT
TACGTCTATAATCCGTTTATGATTGATGTTCAACAATGGGGTTTTACAGGTAAC
CTACAAAGCAACCATGATCTGTATTGTCAAGTCCATGGTAATGCACATGTAGC
TAGTTGTGATGCAATCATGACTAGGTGTCTAGCTGTCCACGAGTGCTTTGTTA
AGCGTGTTGACTGGACTATTGAATATCCTATAATTGGTGATGAACTGAAGATT
AATGCGGCTTGTAGAAAGGTTCAACACATGGTTGTTAAAGCTGCATTATTAGC
AGACAAATTCCCAGTTCTTCACGACATTGGTAACCCTAAAGCTATTAAGTGTG
TACCTCAAGCTGATGTAGAATGGAAGTTCTATGATGCACAGCCTTGTAGTGAC
AAAGCTTATAAAATAGAAGAATTATTCTATTCTTATGCCACACATTCTGACAAA
TTCACAGATGGTGTATGCCTATTTTGGAATTGCAATGTCGATAGATATCCTGCT
AATTCCATTGTTTGTAGATTTGACACTAGAGTGCTATCTAACCTTAACTTGCCT
GGTTGTGATGGTGGCAGTTTGTATGTAAATAAACATGCATTCCACACACCAGC
TTTTGATAAAAGTGCTTTTGTTAATTTAAAACAATTACCATTTTTCTATTACTCT
GACAGTCCATGTGAGTCTCATGGAAAACAAGTAGTGTCAGATATAGATTATGT
ACCACTAAAGTCTGCTACGTGTATAACACGTTGCAATTTAGGTGGTGCTGTCT
GTAGACATCATGCTAATGAGTACAGATTGTATCTCGATGCTTATAACATGATGA
TCTCAGCTGGCTTTAGCTTGTGGGTTTACAAACAATTTGATACTTATAACCTCT
GGAACACTTTTACAAGACTTCAGAGTTTAGAAAATGTGGCTTTTAATGTTGTA
AATAAGGGACACTTTGATGGACAACAGGGTGAAGTACCAGTTTCTATCATTA
ATAACACTGTTTACACAAAAGTTGATGGTGTTGATGTAGAATTGTTTGAAAAT
AAAACAACATTACCTGTTAATGTAGCATTTGAGCTTTGGGCTAAGCGCAACAT
TAAACCAGTACCAGAGGTGAAAATACTCAATAATTTGGGTGTGGACATTGCT
GCTAATACTGTGATCTGGGACTACAAAAGAGATGCTCCAGCACATATATCTAC

Fig. 80 continued

TATTGGTGTTTGTTCTATGACTGACATAGCCAAGAAACCAACTGAAACGATTT
GTGCACCACTCACTGTCTTTTTTGATGGTAGAGTTGATGGTCAAGTAGACTTA
TTTAGAAATGCCCGTAATGGTGTTCTTATTACAGAAGGTAGTGTTAAAGGTTT
ACAACCATCTGTAGGTCCCAAACAAGCTAGTCTTAATGGAGTCACATTAATTG
GAGAAGCCGTAAAAACACAGTTCAATTATTATAAGAAAGTTGATGGTGTTGT
CCAACAATTACCTGAAACTTACTTTACTCAGAGTAGAAATTTACAAGAATTTA
AACCCAGGAGTCAAATGGAAATTGATTTCTTAGAATTAGCTATGGATGAATTC
ATTGAACGGTATAAATTAGAAGGCTATGCCTTCGAACATATCGTTTATGGAGAT
TTTAGTCATAGTCAGTTAGGTGGTTTACATCTACTGATTGGACTAGCTAAACG
TTTTAAGGAATCACCTTTTGAATTAGAAGATTTTATTCCTATGGACAGTACAGT
TAAAAACTATTTCATAACAGATGCGCAAACAGGTTCATCTAAGTGTGTGTGTT
CTGTTATTGATTTATTACTTGATGATTTTGTTGAAATAATAAAATCCCAAGATTT
ATCTGTAGTTTCTAAGGTTGTCAAAGTGACTATTGACTATACAGAAATTTCATT
TATGCTTTGGTGTAAAGATGGCCATGTAGAAACATTTTACCCAAAATTACAAT
CTAGTCAAGCGTGGCAACCGGGTGTTGCTATGCCTAATCTTTACAAAATGCAA
AGAATGCTATTAGAAAAGTGTGACCTTCAAAATTATGGTGATAGTGCAACATT
ACCTAAAGGCATAATGATGAATGTCGCAAAATATACTCAACTGTGTCAATATTT
AAACACATTAACATTAGCTGTACCCTATAATATGAGAGTTATACATTTTGGTGC
TGGTTCTGATAAAGGAGTTGCACCAGGTACAGCTGTTTTAAGACAGTGGTTG
CCTACGGGTACGCTGCTTGTCGATTCAGATCTTAATGACTTTGTCTCTGATGC
AGATTCAACTTTGATTGGTGATTGTGCAACTGTACATACAGCTAATAAATGGG
ATCTCATTATTAGTGATATGTACGACCCTAAGACTAAAAATGTTACAAAAGAA
AATGACTCTAAAGAGGGTTTTTTCACTTACATTTGTGGGTTTATACAACAAAA
GCTAGCTCTTGGAGGTTCCGTGGCTATAAAGATAACAGAACATTCTTGGAATG
CTGATCTTTATAAGCTCATGGGACACTTCGCATGGTGGACAGCCTTTGTTACT
AATGTGAATGCCTCATCATCTGAAGCATTTTTAATTGGATGTAATTATCTTGGC
AAACCACGCGAACAAATAGATGGTTATGTCATGCATGCAAATTACATATTTTG
GAGGAATACAAATCCAATTCAGTTGTCTTCCTATTCTTTATTTGACATGAGTAA
ATTTCCCCTTAAATTAAGGGGTACTGCTGTTATGTCTTTAAAAGAAGGTCAAA
TCAATGATATGATTTTATCTCTTCTTAGTAAAGGTAGACTTATAATTAGAGAAA
ACAACAGAGTTGTTATTTCTAGTGATGTTCTTGTTAACAACTAAACGAACAAT
GTTTGTTTTTCTTGTTTTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTAC
AACCAGAACTCAATTACCCCCTGCATACACTAATTCTTTCACACGTGGTGTTT
ATTACCCTGACAAAGTTTTCAGATCCTCAGTTTTACATTCAACTCAGGACTTG
TTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGG
ACCAATGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGT
TTATTTTGCTTCCACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTA
CTACTTTAGATTCGAAGACCCAGTCCCTACTTATTGTTAATAACGCTACTAATG
TTGTTATTAAAGTCTGTGAATTTCAATTTTGTAATGATCCATTTTTGGGTGTTTA
TTACCACAAAAAACAACAAAAGTTGGATGGAAAGTGAGTTCAGAGTTTATTCT
AGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCCTTTTCTTATGGACCTT
GAAGGAAAACAGGGTAATTTCAAAAATCTTAGGGAATTTGTGTTTAAGAATA
TTGATGGTTATTTTAAAATATATTCTAAGCACACGCCTATTAATTTAGTGCGTG
ATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTTGCCAATAGGT
ATTAACATCACTAGGTTTCAAACTTTACTTGCTTTACATAGAAGTTATTTGACT
CCTGGTGATTCTTCTTCAGGTTGGACAGCTGGTGCTGCAGCTTATTATGTGGG
TTATCTTCAACCTAGGACTTTTCTATTAAAATATAATGAAAATGGAACCATTAC

Fig. 80 continued

AGATGCTGTAGACTGTGCACTTGACCCTCTCTCAGAAACAAAGTGTACGTTG
AAATCCTTCACTGTAGAAAAAGGAATCTATCAAACTTCTAACTTTAGAGTCCA
ACCAACAGAATCTATTGTTAGATTTCCTAATATTACAAACTTGTGCCCTTTTGG
TGAAGTTTTTAACGCCACCAGATTTGCATCTGTTTATGCTTGGAACAGGAAGA
GAATCAGCAACTGTGTTGCTGATTATTCTGTCCTATATAATTCCGCATCATTTTC
CACTTTTAAGTGTTATGGAGTGTCTCCTACTAAATTAAATGATCTCTGCTTTAC
TAATGTCTATGCAGATTCATTTGTAATTAGAGGTGATGAAGTCAGACAAATCG
CTCCAGGGCAAACTGGAAAGATTGCTGATTATAATTATAAATTACCAGATGAT
TTTACAGGCTGCGTTATAGCTTGGAATTCTAACAATCTTGATTCTAAGGTTGGT
GGTAATTATAATTACCTGTATAGATTGTTTAGGAAGTCTAATCTCAAACCTTTT
GAGAGAGATATTTCAACTGAAATCTATCAGGCCGGTAGCACACCTTGTAATGG
TGTTGAAGGTTTTAATTGTTACTTTCCTTTACAATCATATGGTTTCCAACCCAC
TAATGGTGTTGGTTACCAACCATACAGAGTAGTAGTACTTTCTTTTGAACTTC
TACATGCACCAGCAACTGTTTGTGGACCTAAAAAGTCTACTAATTTGGTTAAA
AACAAATGTGTCAATTTCAACTTCAATGGTTTAACAGGCACAGGTGTTCTTAC
TGAGTCTAACAAAAAGTTTCTGCCTTTCCAACAATTTGGCAGAGACATTGCT
GACACTACTGATGCTGTCCGTGATCCACAGACACTTGAGATTCTTGACATTAC
ACCATGTTCTTTTGGTGGTGTCAGTGTTATAACACCAGGAACAAATACTTCTA
ACCAGGTTGCTGTTCTTTATCAGGGTGTTAACTGCACAGAAGTCCCTGTTGCT
ATTCATGCAGATCAACTTACTCCTACTTGGCGTGTTTATTCTACAGGTTCTAAT
GTTTTTCAAACACGTGCAGGCTGTTTAATAGGGGCTGAACATGTCAACAACT
CATATGAGTGTGACATACCCATTGGTGCAGGTATATGCGCTAGTTATCAGACTC
AGACTAATTCTCCTCGGCGGGCACGTAGTGTAGCTAGTCAATCCATCATTGCC
TACACTATGTCACTTGGTGCAGAAAATTCAGTTGCTTACTCTAATAACTCTATT
GCCATACCCACAAATTTTACTATTAGTGTTACCACAGAAATTCTACCAGTGTCT
ATGACCAAGACATCAGTAGATTGTACAATGTACATTTGTGGTGATTCAACTGA
ATGCAGCAATCTTTTGTTGCAATATGGCAGTTTTTGTACACAATTAAACCGTG
CTTTAACTGGAATAGCTGTTGAACAAGACAAAAACACCCAAGAAGTTTTTGC
ACAAGTCAAACAAATTTACAAAACACCACCAATTAAAGATTTTGGTGGTTTT
AATTTTTCACAAATATTACCAGATCCATCAAAACCAAGCAAGAGGTCATTTAT
TGAAGATCTACTTTTCAACAAAGTGACACTTGCAGATGCTGGCTTCATCAAA
CAATATGGTGATTGCCTTGGTGATATTGCTGCTAGAGACCTCATTTGTGCACA
AAAGTTTAACGGCCTTACTGTTTTGCCACCTTTGCTCACAGATGAAATGATTG
CTCAATACACTTCTGCACTGTTAGCGGGTACAATCACTTCTGGTTGGACCTTT
GGTGCAGGTGCTGCATTACAAATACCATTTGCTATGCAAATGGCTTATAGGTTT
AATGGTATTGGAGTTACACAGAATGTTCTCTATGAGAACCAAAAATTGATTGC
CAACCAATTTAATAGTGCTATTGGCAAAATTCAAGACTCACTTTCTTCCACAG
CAAGTGCACTTGGAAAACTTCAAGATGTGGTCAACCAAAATGCACAAGCTTT
AAACACGCTTGTTAAACAACTTAGCTCCAATTTTGGTGCAATTTCAAGTGTTT
TAAATGATATCCTTTCACGTCTTGACAAAGTTGAGGCTGAAGTGCAAATTGAT
AGGTTGATCACAGGCAGACTTCAAAGTTTGCAGACATATGTGACTCAACAAT
TAATTAGAGCTGCAGAAATCAGAGCTTCTGCTAATCTTGCTGCTACTAAAATG
TCAGAGTGTGTACTTGGACAATCAAAAAGAGTTGATTTTTGTGGAAAGGGCT
ATCATCTTATGTCCTTCCCTCAGTCAGCACCTCATGGTGTAGTCTTCTTGCATG
TGACTTATGTCCCTGCACAAGAAAAGAACTTCACAACTGCTCCTGCCATTTG
TCATGATGGAAAAGCACACTTTCCTCGTGAAGGTGTCTTTGTTTCAAATGGC
ACACACTGGTTTGTAACACAAAGGAATTTTTATGAACCACAAATCATTACTAC

Fig. 80 continued

AGACAACACATTTGTGTCTGGTAACTGTGATGTTGTAATAGGAATTGTCAACA
ACACAGTTTATGATCCTTTGCAACCTGAATTAGACTCATTCAAGGAGGAGTTA
GATAAATATTTTAAGAATCATACATCACCAGATGTTGATTTAGGTGACATCTCT
GGCATTAATGCTTCAGTTGTAAACATTCAAAAAGAAATTGACCGCCTCAATG
AGGTTGCCAAGAATTTAAATGAATCTCTCATCGATCTCCAAGAACTTGGAAA
GTATGAGCAGTATATAAAATGGCCATGGTACATTTGGCTAGGTTTTATAGCTGG
CTTGATTGCCATAGTAATGGTGACAATTATGCTTTGCTGTATGACCAGTTGCTG
TAGTTGTCTCAAGGGCTGTTGTTCTTGTGGATCCTGCTGCAAATTTGATGAAG
ACGACTCTGAGCCAGTGCTCAAAGGAGTCAAATTACATTACACATAAACGAA
CTTATGGATTTGTTTATGAGAATCTTCACAATTGGAACTGTAACTTTGAAGCA
AGGTGAAATCAAGGATGCTACTCCTTCAGATTTTGTTCGCGCTACTGCAACG
ATACCGATACAAGCCTCACTCCCTTTCGGATGGCTTATTGTTGGCGTTGCACT
TCTTGCTGTTTTTCAGAGCGCTTCCAAAATCATAACCCTCAAAAAGAGATGG
CAACTAGCACTCTCCAAGGGTGTTCACTTTGTTTGCAACTTGCTGTTGTTGTT
TGTAACAGTTTACTCACACCTTTTGCTCGTTGCTGCTGGCCTTGAAGCCCCTT
TTCTCTATCTTTATGCTTTAGTCTACTTCTTGCAGAGTATAAACTTTGTAAGAA
TAATAATGAGGCTTTGGCTTTGCTGGAAATGCCGTTCCAAAAACCCATTACTT
TATGATGCCAACTATTTTCTTTGCTGGCATACTAATTGTTACGACTATTGTATAC
CTTACAATAGTGTAACTTCTTCAATTGTCATTACTTCAGGTGATGGCACAACA
AGTCCTATTTCTGAACATGACTACCAGATTGGTGGTTATACTGAAAAATGGGA
ATCTGGAGTAAAAGACTGTGTTGTATTACACAGTTACTTCACTTCAGACTATT
ACCAGCTGTACTCAACTCAATTGAGTACAGACACTGGTGTTGAACATGTTAC
CTTCTTCATCTACAATAAAATTGTTGATGAGCCTGAAGAACATGTCCAAATTC
ACACAATCGACGGTTCATCCGGAGTTGTTAATCCAGTAATGGAACCAATTTAT
GATGAACCGACGACGACTACTAGCGTGCCTTTGTAAGCACAAGCTGATGAGT
ACGAACTTATGTACTCATTCGTTTCGGAAGAGACAGGTACGTTAATAGTTAAT
AGCGTACTTCTTTTTCTTGCTTTCGTGGTATTCTTGCTAGTTACACTAGCCATC
CTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATATTGTTAACGTGAGTCTT
GTAAAACCTTCTTTTTACGTTTACTCTCGTGTTAAAAATCTGAATTCTTCTAGA
GTTCCTGATCTTCTGGTCTAAACGAACTAAATATTATATTAGTTTTTCTGTTTG
GAACTTTAATTTTAGCCATGGCAGATTCCAACGGTACTATTACCGTTGAAGAG
CTTAAAAAGCTCCTTGAACAATGGAACCTAGTAATAGGTTTCCTATTCCTTAC
ATGGATTTGTCTTCTACAATTTGCCTATGCCAACAGGAATAGGTTTTTGTATAT
AATTAAGTTAATTTTCCTCTGGCTGTTATGGCCAGTAACTTTAGCTTGTTTTGT
GCTTGCTGCTGTTTACAGAATAAATTGGATCACCGGTGGAATTGCTATCGCAA
TGGCTTGTCTTGTAGGCTTGATGTGGCTCAGCTACTTCATTGCTTCTTTCAGA
CTGTTTGCGCGTACGCGTTCCATGTGGTCATTCAATCCAGAAACTAACATTCT
TCTCAACGTGCCACTCCATGGCACTATTCTGACCAGACCGCTTCTAGAAAGT
GAACTCGTAATCGGAGCTGTGATCCTTCGTGGACATCTTCGTATTGCTGGACA
CCATCTAGGACGCTGTGACATCAAGGACCTGCCTAAAGAAATCACTGTTGCT
ACATCACGAACGCTTTCTTATTACAAATTGGGAGCTTCGCAGCGTGTAGCAG
GTGACTCAGGTTTTGCTGCATACAGTCGCTACAGGATTGGCAACTATAAATTA
AACACAGACCATTCCAGTAGCAGTGACAATATTGCTTTGCTTGTACAGTAAGT
GACAACAGATGTTTCATCTCGTTGACTTTCAGGTTACTATAGCAGAGATATTA
CTAATTATTATGAGGACTTTTAAAGTTTCCATTTGGAATCTTGATTACATCATAA
ACCTCATAATTAAAAATTTATCTAAGTCACTAACTGAGAATAAATATTCTCAAT
TAGATGAAGAGCAACCAATGGAGATTGATTAAACGAACATGAAAATTATTCTT

Fig. 80 continued

TTCTTGGCACTGATAACACTCGCTACTTGTGAGCTTTATCACTACCAAGAGTG
TGTTAGAGGTACAACAGTACTTTTAAAAGAACCTTGCTCTTCTGGAACATACG
AGGGCAATTCACCATTTCATCCTCTAGCTGATAACAAATTTGCACTGACTTGC
TTTAGCACTCAATTTGCTTTTGCTTGTCCTGACGGCGTAAAACACGTCTATCA
GTTACGTGCCAGATCAGTTTCACCTAAACTGTTCATCAGACAAGAGGAAGTT
CAAGAACTTTACTCTCCAATTTTTCTTATTGTTGCGGCAATAGTGTTTATAACA
CTTTGCTTCACACTCAAAAGAAAGACAGAATGATTGAACTTTCATTAATTGAC
TTCTATTTGTGCTTTTTAGCCTTTCTGCTATTCCTTGTTTTAATTATGCTTATTAT
CTTTTGGTTCTCACTTGAACTGCAAGATCATAATGAAACTTGTCACGCCTAAA
CGAACATGAAATTTCTTGTTTTCTTAGGAATCATCACAACTGTAGCTGCATTT
CACCAAGAATGTAGTTTACAGTCATGTACTCAACATCAACCATATGTAGTTGA
TGACCCGTGTCCTATTCACTTCTATTCTAAATGGTATATTAGAGTAGGAGCTAG
AAAATCAGCACCTTTAATTGAATTGTGCGTGGATGAGGCTGGTTCTAAATCAC
CCATTCAGTACATCGATATCGGTAATTATACAGTTTCCTGTTTACCTTTTACAAT
TAATTGCCAGGAACCTAAATTGGGTAGTCTTGTAGTGCGTTGTTCGTTCTATG
AAGACTTTTTAGAGTATCATGACGTTCGTGTTGTTTTAGATTTCATCTAAACGA
ACAAACTAAAATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGC
ATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGAGAAC
GCAGTGGGGCGCGATCAAAACAACGTCGGCCCCAAGGTTTACCCAATAATAC
TGCGTCTTGGTTCACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATTC
CCTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATGACCAAA
TTGGCTACTACCGAAGAGCTACCAGACGAATTCGTGGTGGTGACGGTAAAAT
GAAAGATCTCAGTCCAAGATGGTATTTCTACTACCTAGGAACTGGGCCAGAA
GCTGGACTTCCCTATGGTGCTAACAAAGACGGCATCATATGGGTTGCAACTG
AGGGAGCCTTGAATACACCAAAAGATCACATTGGCACCCGCAATCTTGCTAA
CAATGCTGCAATCGTGCTACAACTTCCTCAAGGAACAACATTGCCAAAAGGC
TTCTACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCAT
CACGTAGTCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAAACGAA
CTTCTCCTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCTG
CTGCTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTGGTAAAGGCCAAC
AACAACAAGGCCAAACTGTCACTAAGAAATCTGCTGCTGAGGCTTCTAAGA
AGCCTCGGCAAAAACGTACTGCCACTAAAGCATACAATGTAACACAAGCTTT
CGGCAGACGTGGTCCAGAACAAACCCAAGGAAATTTTGGGGACCAGGAACT
AATCAGACAAGGAACTGATTACAAACATTGGCCGCAAATTGCACAATTTGCC
CCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGCATTGGCATGGAAGTCACAC
CTTCGGGAACGTGGTTGACCTACACAGGTGCCATCAAATTGGATGACAAAGA
TCCAAATTTCAAAGATCAAGTCATTTTGCTGAATAAGCATATTGACGCATACA
AAACATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAAGAAGGCTGATG
AAACTCAAGCCTTACCGCAGAGACAGAAGAAACAGCAAACTGTGACTCTTC
TTCCTGCTGCAGATTTGGATGATTTCTCCAAACAATTGCAACAATCCATGAGC
AGTGCTGACTCAACTCAGGCCTAAACTCATGCAGACCACACAAGGCAGATG
GGCTATATAAACGTTTTCGCTTTTCCGTTTACGATATATAGTCTACTCTTGTGCA
GAATGAATTCTCGTAACTACATAGCACAAGTAGATGTAGTTAACTTTAATCTC
ACATAGCAATCTTTAATCAGTGTTTAACATTAGGGAGGACTTGAAAGAGCCA
CCACATTTTCACCGAGGCCACGCGGAGTACGATCGAGTGTACAGTGAACAAT
GCTAGGGAGAGCTGCCTATATGGAAGAGCCCTAATGTGTAAAATTAATTTTAG
TAGTGCTATCCCCATGTGATTTTAATAGCTTCTTAGGAGAATGACAAAAAAAA

Fig. 80 continued

AAAAAAAAAAAAAAAAAAAAAAAAA

COLD-ADAPTED, LIVE ATTENUATED SARS-COV-2 VACCINE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of the filing date of U.S. Provisional Application No. 63/453,645, filed on Mar. 21, 2023, and U.S. Provisional Application No. 63/579,686, filed on Aug. 30, 2023, and U.S. Provisional Application No. 63/557,284, filed on Feb. 23, 2024, the disclosures of each of which are incorporated by reference herein.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] This application contains a Sequence Listing which has been submitted electronically in ST26 format and is hereby incorporated by reference in its entirety. Said ST26 file, created on Mar. 19, 2024, is named "800128WO1.xml" and is 291,583 bytes in size.

BACKGROUND

[0003] Coronaviruses are single positive stranded RNA viruses that have emerged occasionally from zoonotic sources to infect human populations. Most of the infections in humans cause mild respiratory symptoms, though some recent coronavirus infections in the last decade have resulted in severe morbidity and mortality. These include the severe acute respiratory syndrome coronavirus (SARS-CoV), middle east respiratory syndrome coronavirus (MERS-CoV) and the currently ongoing pandemic of SARS-CoV-2. Infection with these viruses can lead to acute respiratory distress resulting in a high mortality rate. SARS-CoV originated in 2002 in South China and its global spread led to 8096 cases and 774 deaths. The first case of MERS-CoV emerged in 2012 in Saudi Arabia and since then a total of 2494 cases and 858 associated deaths have been reported. 2019 SARS CoV-2 emerged in Wuhan, China at the end of December 2019 and by March 8th 2020 had resulted in 118,096 cases including 4262 deaths globally. The rapid spread of 2019 SARS-CoV2 resulted in the World Health Organization declaring a global pandemic of international concern.

[0004] At least 7 different SARS-CoV-2 vaccines are being used worldwide including the Pfizer-BioNTech COVID-19 vaccine (BNT162b2) and two versions of the AstraZeneca/Oxford COVID-19 vaccine (AZD1222).

[0005] There are many hurdles to overcome in the development of an effective vaccine for CoVs. Firstly, immunity, whether it is natural or artificial, does not necessarily prevent subsequent infection. Secondly, the propensity of the viruses to recombine may pose a problem. Additionally, vaccination with a viral S-protein has been shown to lead to enhanced disease in the case of FIPV (feline infectious peritonitis virus), a highly virulent strain of feline CoV. This enhanced pathogenicity of the disease is caused by non-neutralizing antibodies that facilitate viral entry into host cells in a process called antibody-dependent enhancement (ADE). After primary infection of one strain of a virus, neutralizing antibodies are produced against the same strain of the virus. However, if a different strain infects the host in a secondary infection, non-neutralizing antibodies produced during the first infection, which do not neutralize the virus, instead, bind to the virus and then bind to the IgG Fe receptors on immune cells and mediate viral entry into these cells.

SUMMARY

[0006] The present disclosure relates to cold-adapted coronaviruses, e.g., cold-adapted SARS-CoV-2 viruses. Cold-adapted vaccines trigger immune responses similar to those elicited upon natural infection and have a long history in the control of viral infections in humans. The process of cold-adaptation of a virus at lower temperatures (e.g., 25° C.) results in an attenuated virus characterized with a compromised ability to grow efficiently at higher temperatures (e.g., 37-40° C.).

[0007] A "cold-adapted coronavirus" within the scope of the disclosure includes a virus that is obtained by sequential propagation at progressively lower temperatures, by introduction of an alteration into one or more regions of the viral genome that yields a cold-adapted virus, or is isolated from a sample, e.g., physiological sample, that is at a lower than physiological host temperature. A "cold-adapted virus" produces viral yields that are up to about 100-times, 200-times, 300 times, 400-times, 500-times, 600-times, 700-times, 800-time, 900-times, 1000-times, or more, greater at lower temperatures, e.g., less than 33° C. and greater than 22° C. or less than 31° C. and greater than 24° C., than wild-type virus.

[0008] For example, a coronavirus isolate was passaged 15 times at 37° C. and then passaged progressively lower temperatures from 35° C. to 25° C. with the last 3 passages at 25° C. To demonstrate attenuation, golden Syrian hamsters were infected with the parent virus (before passaging) and the cold-adapted virus after 15 passages. Syrian hamsters are highly susceptible to SARS-CoV-2 infection and present with pathological phenotypes similar to those of infected humans; therefore, Syrian hamsters are a model to evaluate the attenuation of cold-adapted SARS-CoV-2. Animals were infected by intranasal inoculation with 1000 plaque-forming units of virus. While infection with the parental virus caused 10% body weight loss, this reduction in body weight was not observed with the cold-adapted virus. Four days after infection, the amount of virus in the lungs was determined. There was a significant reduction (about 1000-fold) in the amount of virus in the lungs of animals infected with the cold-adapted virus compared to the lungs of animals infected with the parent virus. Given the attenuation in hamsters, cold-adapted versions of a corona virus such as SARS-CoV-2 may be used as a vaccine candidate.

[0009] The disclosure thus provides for isolated cold-adapted coronaviruses, e.g., cold-adapted SARS-CoV-2 viruses, that have one or more mutations relative to a parental virus that is not cold-adapted, e.g., the replication efficiency of the parental virus is restricted under a low temperature, thereby resulting in decreased viral growth, as well as associated methods of making and using such viruses.

[0010] Also provided herein is a method of treating, inhibiting or preventing a coronavirus infection, e.g., a SARS-CoV-2 infection, in a subject in need thereof, comprising administering to the subject a pharmaceutical composition described herein.

[0011] The disclosure thus provides an isolated cold-adapted coronavirus wherein the the genome of the coronavirus is a mutant genome where expression of coronavirus S, E, M, N, ORF1, e.g., ORF 1a, ORF3, e.g., ORF3a, ORF6, ORF7, and/or ORF8, comprises one or more mutations in one or more open reading frames. The one or more muta-

tions include but are not limited to one or more nucleotide deletion(s), substitution(s), insertion(s), or any combination thereof. In embodiments, the one or more nucleotide deletions result in an amino acid deletion in one of the open reading frames. In embodiments, the one or more mutations include a substitution of one or more nucleotides. In embodiments, the one or more substitutions result in one or more amino acid substitutions in polypeptides encoded by the one of the open reading frames. In embodiments, the one or more mutations include an insertion of one or more nucleotides.

[0012] In embodiments, the open reading frame encodes a non-structural protein. In embodiments, the mutation is in an open reading frame for Nsp1, Nsp2, Nsp3, Nsp6, Nsp13, Nsp14, Nsp15, S, ORF6, ORF7b-ORF8, or any combination thereof. In embodiments, the one or more mutations include a deletion of a codon for the amino acid at position 85 in Nsp1. In one embodiment, the one or more mutations include a nucleotide substitution in the gene for Nsp2 at position 1341 or 1495, or both. In one embodiment, the one or more mutations include an amino acid substitution in Nsp3 at position 272 or 528, or both. In one embodiment, the amino acid at position 272 is not Y. In one embodiment, the amino acid at position 272 is N, D, E or Q. In one embodiment, the amino acid at position 528 is not L. In one embodiment, the amino acid at position 528 is F, W or Y. In one embodiment, the one or more mutations include an amino acid substitution in Nsp6 at position 260. In one embodiment, the amino acid at position 260 is not L. In one embodiment, the amino acid at position 260 is F, W or Y. In one embodiment, the one or more mutations include a nucleotide substitution in the gene for Nsp13 at position 16575. In one embodiment, the one or more mutations include an amino acid substitution in Nsp14 at position 360. In one embodiment, the amino acid at position 360 is not A. In one embodiment, the amino acid at position 360 is S, T, L, I or M. In one embodiment, the one or more mutations include an amino acid substitution in Nsp15 at position 233. In one embodiment, the amino acid at position 233 is not E. In one embodiment, the amino acid at position 233 is A, I, L, G, S, or T. In one embodiment, the one or more mutations include an amino acid substitution in S at position 64, 235, 1094, or a combination thereof. In one embodiment, the amino acid at position 64 is not W, position 253 is not D, or position 1094 is not V. In one embodiment, the amino acid at position 64 is R, H or K; position 253 is G, A, L, I, T or S; or position 1094 is F, Y or W. In one embodiment, the one or more mutations include a deletion of the C-terminus of polypeptide encoded by ORF6. In one embodiment, the one or more mutations include a deletion of ORF7b and/or ORF8.

[0013] Also provided is a method of making a cold-adapted coronavirus comprising modifying the genome thereof to include one or more mutations in one or more open reading frames. In one embodiment, the coronavirus is SARS-CoV-2. In one embodiment, the one or more mutations include a deletion of one or more nucleotides. In one embodiment, the one or more nucleotide deletions result in an amino acid deletion in one of the open reading frames. In one embodiment, the one or more mutations include a substitution of one or more nucleotides. In one embodiment, the one or more substitutions result in one or more amino acid substitutions in a polypeptide encoded by one of the open reading frames. In one embodiment, the one or more mutations include an insertion of one or more nucleotides. In one embodiment, the open reading frame encodes a non-structural protein. In one embodiment, in the mutation is in an open reading frame for Nsp1, Nsp2, Nsp3, Nsp6, Nsp13, Nsp14, Nsp15, S, ORF6, ORF7b-ORF8, or any combination thereof. In one embodiment, the one or more mutations include a deletion of a codon for the amino acid at position 85 in Nsp1. In one embodiment, the one or more mutations include a nucleotide substitution in the gene for Nsp2 at position 1341 or 1495, or both. In one embodiment, the one or more mutations include an amino acid substitution in Nsp3 at position 272 or 528, or both. In one embodiment, the amino acid at position 272 is not Y. In one embodiment, the amino acid at position 272 is N, D, E or Q. In one embodiment, the amino acid at position 528 is not L. In one embodiment, the amino acid at position 528 is F, W or Y. In one embodiment, the one or more mutations include an amino acid substitution in Nsp6 at position 260. In one embodiment, the amino acid at position 260 is not L. In one embodiment, the amino acid at position 260 is F, W or Y. In one embodiment, the one or more mutations include a nucleotide substitution in the gene for Nsp13 at position 16575. In one embodiment, the one or more mutations include an amino acid substitution in Nsp14 at position 360. In one embodiment, the amino acid at position 360 is not A. In one embodiment, the amino acid at position 360 is S, T, L, I or M. In one embodiment, the one or more mutations include an amino acid substitution in Nsp15 at position 233. In one embodiment, the amino acid at position 233 is not E. In one embodiment, the amino acid at position 233 is A, I, L, G, S, or T. In one embodiment, the one or more mutations include an amino acid substitution in S at position 64, 253, 1094, or a combination thereof. In one embodiment, the amino acid at position 64 is not W, position 253 is not D, or position 1094 is not V. In one embodiment, the amino acid at position 64 is R, H or K; position 253 is G, A, L, I, T or S; or position 1094 is F, Y or W. In one embodiment, the one or more mutations include a deletion of the C-terminus of polypeptide encoded by ORF6. In one embodiment, the one or more mutations include a deletion of ORF7b and/or ORF8.

[0014] Further provided is a method to immunize an animal, comprising administering to the animal a composition comprising the cold-adapted coronavirus. In one embodiment, the animal is a mammal. In one embodiment, the mammal is a human. In one embodiment, the composition is injected. In one embodiment, the composition is systemically administered. In one embodiment, the composition is intranasally administered. In one embodiment, the composition is subcutaneously administered. In one embodiment, the composition is intramuscularly administered.

[0015] The disclosure also provides a pharmaceutical composition comprising the cold-adapted coronavirus. In one embodiment, the composition further comprises a pharmaceutically acceptable carrier. In one embodiment, the composition further comprises a different immunogen. In one embodiment, the composition further comprises a different virus, e.g., the different virus is a not a coronavirus. In one embodiment, the different virus is an influenza virus. In some embodiments, the pharmaceutical composition having a cold-adapted coronavirus is administered in addition to one or more therapeutics for the coronavirus, e.g., a different coronavirus vaccine, or for a different virus, e.g., influenza virus. In some embodiments, the pharmaceutical composition having a cold-adapted coronavirus disclosed herein is

administered 1-10 weeks before or after a first administration of a coronavirus vaccine. In some embodiments, the pharmaceutical composition is administered 1-6 weeks, 1-6 months or 1-2 years or later after a first administration of a coronavirus vaccine. In some embodiments, the pharmaceutical composition is administered on the same day or simultaneously with an administration of another pharmaceutical composition. In some embodiments, the pharmaceutical composition is co-formulated with a different coronavirus immunogenic composition, e.g., a different SARS-CoV-2 immunogenic composition, or a different viral immunogenic composition. In some embodiments, the pharmaceutical composition having a cold-adapted SARS-CoV-2 is administered before an administration of a different SARS-CoV-2 pharmaceutical composition, such as 2-10 weeks before an administration of the SARS-CoV-2 spike protein pharmaceutical composition.

[0016] In some embodiments, the pharmaceutical composition is administered prophylactically. In some embodiments, the pharmaceutical composition is administered once every 1, 2, 3, 4, 5, 6 or more months, e.g., every 7-14, 14-21, 21-28, or 28-35 months.

[0017] Also provided herein is a use of a composition described herein for preparing a therapeutic for treating, inhibiting or preventing a respiratory viral infection caused by a coronavirus including a SARS CoV-2.

[0018] Also provided herein is a composition described herein or a pharmaceutical composition described herein for use as a medicament.

[0019] Also provided herein is a composition described herein or a pharmaceutical composition described herein for use in the treatment or prevention of a respiratory viral infection caused by a coronavirus such as a SARS CoV-2.

BRIEF DESCRIPTION OF THE FIGURES

[0020] FIG. **1**. Generation of a cold-adapted coronavirus.

[0021] FIG. **2**. Propagation of an exemplary seed (vaccine) virus in Vero cells.

[0022] FIG. **3**. Pathogenicity and immunogenicity (attenuation) of an exemplary seed (vaccine) virus in hamsters.

[0023] FIG. **4**. No body weight loss after infection with an exemplary cold-adapted coronavirus.

[0024] FIG. **5**. Respiratory function test based on WBP (whole-body plethysmography) Penh: Index that combines the box pressure signals from both inspiration (PIF) and expiration (PEF), with the timing comparison of early and late expiration (PAU). Penh=PAU*(PEF/PIF). Rpef: Ratio of time required to reach the maximum expiratory flow (PEF) relative to time of expiration (Te).

[0025] FIG. **6**. Virus titers in the respiratory organs of infected hamsters (3 dpi).

[0026] FIG. **7**. CT images of infected hamster lungs confirming attenuation of the vaccine virus in hamsters

[0027] FIG. **8**. Table of exemplary cold-adapted coronavirus induces neutralizing antibodies against wild-type, Beta, Gamma, Delta and BA.1.

[0028] FIG. **9**. Experimental design of immunization with an exemplary cold-adapted coronavirus and challenge of immunized hamsters with the wild-type virus.

[0029] FIG. **10**. The body weight change of immunized hamsters upon challenge with the wild-type virus was similar between animals previously immunized with either the wild-type or the exemplary cold-adapted coronavirus.

[0030] FIG. **11**. Respiratory functions of hamsters challenged with the wild-type virus were similar between animals previous immunized with either the wild-type or the exemplary cold-adapted coronavirus. Penh: Index that combines the box pressure signals from both inspiration (PIF) and expiration (PEF), with the timing comparison of early and late expiration (PAU). Penh=PAU*(PEF/PIF). Rpef: Ratio of time required to reach the maximum expiratory flow (PEF) relative to time of expiration (Te).

[0031] FIG. **12**. The levels of virus titer reduction in respiratory organs were similar between animals previous immunized with either the wild-type or the exemplary cold-adapted coronavirus.

[0032] FIG. **13**. Micro-CT analysis in the lungs of cold-adapted coronavirus-immunized hamsters showing no lung lesions in hamsters previously immunized with either the wild-type or the exemplary cold-adapted coronavirus upon challenge with the wild-type virus.

[0033] FIG. **14**. Experimental design of immunization with the exemplary cold-adapted coronavirus and challenge of immunized hamsters with the Delta variant.

[0034] FIG. **15**. The body weight change of immunized hamsters upon challenge with the Delta variant was similar between animals previously immunized with either the wild-type or the exemplary cold-adapted coronavirus.

[0035] FIG. **16**. Respiratory functions of hamsters challenged with the Delta variant were similar between animals previous immunized with either the wild-type or the exemplary cold-adapted coronavirus.

[0036] FIG. **17**. The levels of virus titer reduction in respiratory organs were similar between animals previous immunized with either the wild-type or the exemplary cold-adapted coronavirus.

[0037] FIG. **18**. Micro-CT analysis in the lungs of cold-adapted coronavirus-immunized hamsters showing no lung lesions in hamsters previously immunized with either the wild-type or the exemplary cold-adapted coronavirus upon challenge with the Delta variant.

[0038] FIG. **19**. Attenuation stability test in hamsters showing in vivo stability of the attenuated cold-adapted coronavirus that was passaged in the nasal turbinates 3 times or isolated from the lungs of hamsters 3-5 days after infection and then tested for cold adaptation, temperature sensitivity, and pathogenicity.

[0039] FIG. **20**. Attenuation stability test in hamsters showing the attenuated cold-adapted coronavirus in the nasal turbinate did not change during the 3 passages.

[0040] FIG. **21**. The cold-adapted coronavirus passaged 3 times in nasal turbinate remained similar to the original cold-adapted coronavirus.

[0041] FIG. **22**. Pathogenicity of the cold-adapted coronavirus passaged in nasal turbinates.

[0042] FIGS. **23**A-**23**D. Pathogenicity of passaged cold-adapted coronaviruses. Hamsters lost about 5% of body weight in the wild-type group and gained weight in remaining groups (FIG. **23**A). Respiratory function declined in the wild-type group and remained substantially steady in remaining groups (FIG. **23**B). CT scans of lungs of each group are shown in FIG. **23**C. Virus titer in nasal turbinates (NT) and lungs is shown in FIG. **23**D.

[0043] FIG. **24**. Attenuation stability test in hamsters showing cold-adapted coronavirus was detected in the lungs of hamsters at 5 days post-infection.

[0044] FIG. **25**. Seed cold-adapted coronaviruses isolated from hamster lung at 5 days post-infection showing similar growth kinetics at 25, 32, 37, and 39° C. to those of the cold-adapted coronavirus not passaged through hamster lungs.

[0045] FIG. **26**. Pathogenicity of cold-adapted coronaviruses isolated from hamster lung.

[0046] FIGS. **27**A-D. Body weight change (FIG. **27**A), respiratory function for Penh and Rpef (FIG. **27***b*), micro-CT analysis (FIG. **27**C), and virus titers in the nasal turbinates and lungs of the hamsters (FIG. **27**D) showed that the two isolated viruses maintained the attenuated phenotype in the hamsters.

[0047] FIGS. **28**A-D. Experimental design to test attenuation stability in Vero cells (FIG. **28**A). Table of virus titers of cold-adapted coronavirus passaged through Vero cells at different temperatures (FIG. **28**B). Experimental design to test the pathogenicity of the 3 passaged viruses by intranasal inoculation of hamsters with 10^3 PFU of passaged viruses (FIG. **28**C). Body weight change, respiratory function for Penh and Rpef, micro-CT analysis, and virus titers in the nasal turbinates and lungs of the hamsters (FIG. **28**D).

[0048] FIG. **29**. Virus titer of recombinant viruses possessing a single amino acid mutation in Vero cells.

[0049] FIG. **30**. Pathogenicity of single amino acid mutants in hamster. These viruses were generated by reverse genetics using the BAC system.

[0050] FIG. **31**. Graph showing ORF1ab-Y1090N and S-V1094F mutants suppressed the body weight loss of infected hamsters.

[0051] FIG. **32**. Graphs showing respiratory function of ORF1ab-M85del and ORF1ab-Y1090N mutations in cold-adapted coronavirus contributed to attenuation in hamsters.

[0052] FIG. **33**. Micro-CT images showing that ORF1ab-M85del and ORF1ab-Y1090N mutations in cold-adapted coronavirus caused attenuation in hamsters.

[0053] FIG. **34**. Exemplary parental virus genomic sequence (SEQ ID NO:1).

[0054] FIG. **35**. Genomic structure of an exemplary coronavirus (SEQ ID NO:2).

[0055] FIG. **36**. Exemplary SARS CoV-2 genome (Delta variant) for modification (SEQ ID NO:3).

[0056] FIG. **37**. Exemplary SARS CoV-2 genome (Omicron variant) for modification (SEQ ID NO:4).

[0057] FIG. **38**. Experimental design to test the attenuation of the cold-adapted coronavirus and induction of antibodies and cellular immunity in human ACE2-transgenic mice.

[0058] FIG. **39**. Graphs of AC70 and K18 mice body weight vs. days post infection with cold-adapted coronavirus.

[0059] FIG. **40**. Graphs of cold-adapted coronavirus titer in nasal turbinates and lungs of AC70 or K18 mice.

[0060] FIG. **41**. Anti-RBD and Anti-S ELISA titer in K18 mice.

[0061] FIG. **42**. Th1/Th2 balance in K18-tg mice.

[0062] FIG. **43**. Experimental design to assess cellular immunity against the S protein in K18-tg mice.

[0063] FIG. **44**. Graph showing IFNγ- and TNFα-positive CD4 and CD8 T cells were detected in the splenocytes of K18-mice infected with the vaccine cold-adapted coronavirus.

[0064] FIG. **45**. ELISPOT showed that cellular immunity against the spike protein was induced in K18-mice after vaccine cold-adapted coronavirus infection.

[0065] FIG. **46**. Experimental design for a challenge test of cold-adapted coronavirus immunized hamsters with omicron variant BA.1,

[0066] FIG. **47**. Graph of body weight loss of cold-adapted coronavirus-immunized hamsters challenged with Wuhan-like, Delta, and BA.1 virus.

[0067] FIG. **48**. Respiratory function test based on WBP.

[0068] FIG. **49**. Virus titers in the lungs and nasal turbinates of cold-adapted coronavirus-immunized hamsters challenged with Wuhan-like, Delta, and BA.1 virus.

[0069] FIG. **50**. Micro-CT images of challenged immunized hamster lungs.

[0070] FIG. **51**. Molecular Basis for the attenuation of recombinant vaccine cold-adapted coronaviruses.

[0071] FIG. **52**. Experimental design for growing recombinant viruses in Vero cells at 25, 32, and 37° C.

[0072] FIG. **53**. Graph of virus titer of recombinant viruses in Vero cells.

[0073] FIG. **54**. Graph of virus titer of reverse engineered wild type and cold-adapted coronavirus in Vero cells.

[0074] FIG. **55**. Graph of virus titer of nsp-M85ins and nsp3-Y272N recombinant viruses in Vero cells.

[0075] FIG. **56**. Graph of virus titer of nsp6-F260L and S-F1094V recombinant viruses in Vero cells.

[0076] FIG. **57**. Experimental design to test the pathogenicity of the recombinant viruses.

[0077] FIG. **58**. Body weight changes in hamsters.

[0078] FIG. **59**. Respiratory function in hamsters.

[0079] FIG. **60**. Recombinant virus titer in nasal turbinates and lung of hamsters.

[0080] FIGS. **61**A-B. Mutations introduced into wild-type SARS-Cov-2/UT-HPCo-038/human/2020/Tokyo (SEQ ID NO:78) (FIG. **61**A). Mutations introduced into vaccine cold-adapted coronavirus (FIG. **61**B).

[0081] FIGS. **62**A-B. Schematic of genome of vaccine cold-adapted coronavirus and recombinant vaccine cold-adapted coronavirus with the S gene replaced with the S gene of XBB.1.5 virus (FIG. **61**A). Sequence of SARS CoV-2 virus, "EG.5.1" (FIG. **61**B; SEQ ID NO: 6).

[0082] FIG. **63**. Experimental design for testing virus titers of vaccine cold-adapted coronaviruses possessing the S gene of XBB.1.5 harboring the V1094F substitution or the W64R, D253G, and V1094F substitutions.

[0083] FIG. **64**. Graph showing vaccine cold-adapted coronaviruses possessing the XBB.1.5 S gene showed similar growth kinetics to those of the vaccine cold-adapted coronavirus (LAV).

[0084] FIG. **65**. Experimental design to test the pathogenicity of the three viruses possessing the XBB.1.5 S gene.

[0085] FIG. **66**. Graph of body weight change of hamsters infected with the three viruses possessing the XBB.1.5 S gene.

[0086] FIG. **67**. Graph of respiratory function of hamsters infected with the three viruses possessing the XBB.1.5 S gene.

[0087] FIG. **68**. Graph of virus titers in nasal turbinates and lung of hamsters infected with the three viruses possessing the XBB.1.5 S gene.

[0088] FIGS. **69**A-B. Experimental design to test boost immunization with vaccine cold-adapted coronavirus in hamsters that received mRNA vaccination once (FIG. **69**A) or twice (FIG. **69**B).

[0089] FIG. **70**. Graph of antibody titers after the boost immunization with the vaccine cold-adapted coronavirus.

[0090] FIG. **71**. Experimental design to test vaccine cold-adapted coronavirus attenuation in non-human primates (macaques).

[0091] FIG. **72**. Graph of body weight of macaques infected with vaccine cold-adapted coronavirus or wild-type virus.

[0092] FIG. **73**. Graphs of vaccine see cold-adapted coronavirus virus loads in the nasal, oral, and rectal swabs of macaques.

[0093] FIG. **74**. Graph showing vaccine cold-adapted coronavirus RNA was not detected in any organ tested, whereas wild-type virus RNA was detected in the olfactory bulb, turbinates, ileums, and trachea of macaques.

[0094] FIG. **75**. Experiment design to show vaccine cold-adapted coronavirus efficacy in macaques.

[0095] FIG. **76**. Graph of body weight change of immunized macaques after challenge infection.

[0096] FIG. **77**. Graphs of viral loads in the nasal, oral, and rectal swabs after challenge infection.

[0097] FIG. **78**. Graph of virus titers in organs after challenge infection.

[0098] FIG. **79**. Exemplary parental virus genomic sequence Wuhan-Hu-1 (SEQ ID NO: 5).

[0099] FIG. **80**. Exemplary parental virus genomic sequence SARS-Cov-2/UT-HPCo-038/human/2020/Tokyo (SEQ ID NO:78).

DETAILED DESCRIPTION

[0100] Currently, vaccines against SARS-CoV-2 are only directed against the spike protein. A cold-adapted, live attenuated coronavirus vaccine may elicit a different or enhanced immune response against the virus, resulting in superior protection with one immunization.

Definitions

[0101] The term "isolated" when used in relation to a nucleic acid, peptide, polypeptide or virus refers to a nucleic acid sequence, peptide, polypeptide or virus that is identified and separated from at least one contaminant nucleic acid, polypeptide or other biological component with which it is ordinarily associated in its natural source, e.g., so that it is not associated with in vivo substances, or is substantially purified from in vitro substances. Isolated nucleic acid, peptide, polypeptide or virus is present in a form or setting that is different from that in which it is found in nature. For example, a given DNA sequence (e.g., a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with numerous other mRNAs that encode a multitude of proteins. The isolated nucleic acid molecule may be present in single-stranded or double-stranded form. When an isolated nucleic acid molecule is to be utilized to express a protein, the molecule will contain at a minimum the sense or coding strand (i.e., the molecule may single-stranded), but may contain both the sense and anti-sense strands (i.e., the molecule may be double-stranded).

[0102] As used herein, "substantially purified" means the object species is the predominant species, e.g., on a molar basis it is more abundant than any other individual species in a composition, and preferably is at least about 80% of the species present, and optionally 90% or greater, e.g., 95%, 98%, 99% or more, of the species present in the composition.

[0103] As used herein, "substantially free" means below the level of detection for a particular infectious agent using standard detection methods for that agent.

[0104] A "recombinant" virus is one which has been manipulated in vitro, e.g., using recombinant DNA techniques, to introduce changes to the viral genome. Viruses can be prepared by recombinant or nonrecombinant techniques.

[0105] As used herein, the term "recombinant nucleic acid" or "recombinant DNA sequence or segment" refers to a nucleic acid, e.g., to DNA, that has been derived or isolated from a source, that may be subsequently chemically altered in vitro, so that its sequence is not naturally occurring, or corresponds to naturally occurring sequences that are not positioned as they would be positioned in the native genome. An example of DNA "derived" from a source, would be a DNA sequence that is identified as a useful fragment, and which is then chemically synthesized in essentially pure form. An example of such DNA "isolated" from a source would be a useful DNA sequence that is excised or removed from said source by chemical means, e.g., by the use of restriction endonucleases, so that it can be further manipulated, e.g., amplified, for use in the disclosure, by the methodology of genetic engineering.

[0106] The term "heterologous" as it relates to nucleic acid sequences such as gene sequences encoding a protein and control sequences, denotes sequences that are not normally joined together, and/or are not normally associated with a particular cell, e.g., are from different sources (for instance, sequences from a virus are heterologous to sequences in the genome of an uninfected cell). Thus, a "heterologous" region of a nucleic acid construct or a vector is a segment of nucleic acid within or attached to another nucleic acid molecule that is not found in association with the other molecule in nature. For example, a heterologous region of a nucleic acid construct could include a coding sequence flanked by sequences not found in association with the coding sequence in nature, i.e., a heterologous promoter. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Similarly, a cell transformed with a construct which is not normally present in the cell would be considered heterologous for purposes of this disclosure.

[0107] By "DNA" is meant a polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in double-stranded or single-stranded form found, inter alia, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having the sequence complementary to the mRNA). The term captures molecules that include the four bases adenine, guanine, thymine, or cytosine, as well as molecules that include base analogues which are known in the art.

[0108] As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (i.e., a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "A-G-T," is complemen-

tary to the sequence "T-C-A." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

[0109] DNA molecules are said to have "5' ends" and "3' ends" because mononucleotides are reacted to make oligonucleotides or polynucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage. Therefore, an end of an oligonucleotide or polynucleotide is referred to as the "5' end" if its 5' phosphate is not linked to the 3' oxygen of a mononucleotide pentose ring and as the "3' end" if its 3' oxygen is not linked to a 5' phosphate of a subsequent mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide or polynucleotide, also may be said to have 5' and 3' ends. In either a linear or circular DNA molecule, discrete elements are referred to as being "upstream" or 5' of the "downstream" or 3' elements. This terminology reflects the fact that transcription proceeds in a 5' to 3' fashion along the DNA strand. The promoter and enhancer elements that direct transcription of a linked gene are generally located 5' or upstream of the coding region. However, enhancer elements can exert their effect even when located 3' of the promoter element and the coding region. Transcription termination and polyadenylation signals are located 3' or downstream of the coding region.

[0110] A "gene," "polynucleotide," "coding region," "sequence," "segment," "fragment" or "transgene" which "encodes" a particular protein, is a nucleic acid molecule which is transcribed and optionally also translated into a gene product, e.g., a polypeptide, in vitro or in vivo when placed under the control of appropriate regulatory sequences. The coding region may be present in either a cDNA, genomic DNA, or RNA form. When present in a DNA form, the nucleic acid molecule may be single-stranded (i.e., the sense strand) or double-stranded. The boundaries of a coding region are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A gene can include, but is not limited to, cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or eukaryotic DNA, and synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the gene sequence.

[0111] The term "control elements" refers collectively to promoter regions, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, splice junctions, and the like, which collectively provide for the replication, transcription, post-transcriptional processing and translation of a coding sequence in a recipient cell. Not all of these control elements need always be present so long as the selected coding sequence is capable of being replicated, transcribed and translated in an appropriate host cell.

[0112] The term "promoter" is used herein in its ordinary sense to refer to a nucleotide region comprising a DNA regulatory sequence, wherein the regulatory sequence is derived from a gene which is capable of binding RNA polymerase and initiating transcription of a downstream (3' direction) coding sequence. By "enhancer" is meant a nucleic acid sequence that, when positioned proximate to a promoter, confers increased transcription activity relative to the transcription activity resulting from the promoter in the absence of the enhancer domain.

[0113] By "operably linked" with reference to nucleic acid molecules is meant that two or more nucleic acid molecules (e.g., a nucleic acid molecule to be transcribed, a promoter, and an enhancer element) are connected in such a way as to permit transcription of the nucleic acid molecule. "Operably linked" with reference to peptide and/or polypeptide molecules is meant that two or more peptide and/or polypeptide molecules are connected in such a way as to yield a single polypeptide chain, i.e., a fusion polypeptide, having at least one property of each peptide and/or polypeptide component of the fusion. The fusion polypeptide may be chimeric, i.e., composed of heterologous molecules.

[0114] The term "sequence homology" means the proportion of base matches between two nucleic acid sequences or the proportion amino acid matches between two amino acid sequences. When sequence homology is expressed as a percentage, e.g., 50%, the percentage denotes the proportion of matches over the length of a selected sequence that is compared to some other sequence. Gaps (in either of the two sequences) are permitted to maximize matching; gap lengths of 15 bases or less are usually used, 6 bases or less or 2 bases or less. When using oligonucleotides as probes or treatments, the sequence homology between the target nucleic acid and the oligonucleotide sequence is generally not less than 17 target base matches out of 20 possible oligonucleotide base pair matches (85%); e.g., not less than 9 matches out of 10 possible base pair matches (90%), or not less than 19 matches out of 20 possible base pair matches (95%). The correspondence between one sequence and to another can be determined by techniques known in the art. For example, homology can be determined by a direct comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single strand-specific nuclease(s), and size determination of the digested fragments. Two DNA, or two polypeptide, sequences are "substantially homologous" to each other when at least about 80%, e.g., at least about 90%, such as at least about 95% of the nucleotides, or amino acids, respectively match over a defined length of the molecules, as determined using the methods above.

[0115] The term "selectively hybridize" means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments of the disclosure selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments of the disclosure and a nucleic acid sequence of interest is at least 65%, and more typically

with increasing homologies of at least about 70%, about 90%, about 95%, about 98%, and 100%.

[0116] Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less or 2 or less. Alternatively, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. The two sequences or parts thereof may be homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program.

[0117] The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (e.g., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence that encodes a polypeptide or its complement, or that a polypeptide sequence is identical in sequence or function to a reference polypeptide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

[0118] The following terms are used to describe the sequence relationships between two or more polynucleotides: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing, or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 20 nucleotides in length, frequently at least 25 nucleotides in length, and often at least 50 nucleotides in length. Since two polynucleotides may each (1) comprise a sequence (i.e., a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) may further comprise a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity.

[0119] A "comparison window", as used herein, refers to a conceptual segment of at least 20 contiguous nucleotides and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by using local homology algorithms or by a search for similarity method, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA Genetics Software Package or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

[0120] The term "sequence identity" means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denote a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 85 percent sequence identity, e.g., at least 90 to 95 percent sequence identity, or at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 20 nucleotide positions, frequently over a window of at least 20-50 nucleotides, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the window of comparison.

[0121] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least about 80% sequence identity, at least about 90% sequence identity, at least about 95% percent sequence identity, or at least about 99% sequence identity.

[0122] By "mammal" is meant any member of the class Mammalia including, without limitation, humans and non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats, rabbits and guinea pigs, and the like.

[0123] By "derived from" is meant that a nucleic acid molecule was either made or designed from a parent nucleic acid molecule, the derivative retaining substantially the same functional features of the parent nucleic acid molecule, e.g., encoding a gene product with substantially the same activity as the gene product encoded by the parent nucleic acid molecule from which it was made or designed.

[0124] By "expression construct" or "expression cassette" is meant a nucleic acid molecule that is capable of directing transcription. An expression construct includes, at the least, a promoter. Additional elements, such as an enhancer, and/or a transcription termination signal, may also be included.

[0125] The term "exogenous," when used in relation to a protein, gene, nucleic acid, or polynucleotide in a cell or organism refers to a protein, gene, nucleic acid, or polynucleotide which has been introduced into the cell or organism by artificial or natural means. An exogenous nucleic acid may be from a different organism or cell, or it may be one or more additional copies of a nucleic acid which occurs naturally within the organism or cell. By way of a non-limiting example, an exogenous nucleic acid is in a chro-

mosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature.

[0126] The term "wild-type" or "native" refers to a gene or gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designated the "normal" or "wild-type" form of the gene. In contrast, the term "modified" or "mutant" refers to a gene or gene product that displays modifications in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally-occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wild-type gene or gene product.

[0127] The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule expressed from a recombinant DNA molecule. In contrast, the term "native protein" is used herein to indicate a protein isolated from a naturally occurring (i.e., a nonrecombinant) source. Molecular biological techniques may be used to produce a recombinant form of a protein with identical properties as compared to the native form of the protein.

[0128] Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent identity between any two sequences can be accomplished using a mathematical algorithm.

[0129] Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Alignments using these programs can be performed using the default parameters. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The algorithm may involve first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold. These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when the cumulative alignment score falls off by the quantity X from its maximum achieved value, the cumulative score goes to zero or below due to the accumulation of one or more negative-scoring residue alignments, or the end of either sequence is reached.

[0130] In addition to calculating percent sequence identity, the BLAST algorithm may also perform a statistical analysis of the similarity between two sequences. One measure of similarity provided by the BLAST algorithm may be the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a test nucleic acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0131] The BLASTN program (for nucleotide sequences) may use as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=−4, and a comparison of both strands. For amino acid sequences, the BLASTP program may use as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix. See ncbi.nlm.nih.gov. Alignment may also be performed manually by inspection.

[0132] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0133] A "conservative amino acid substitution" is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate peptide function are well-known in the art.

[0134] A "vector" or "construct" (sometimes referred to as gene delivery or gene transfer "vehicle") refers to a macromolecule or complex of molecules comprising a polynucleotide or virus to be delivered to a host cell, either in vitro or in vivo. The polynucleotide or virus to be delivered may comprise a coding sequence of interest for gene therapy. Vectors include, for example, viral vectors (such as coronavirus, filovirus, adenovirus, adeno-associated virus (AAV), lentivirus, herpesvirus and retrovirus vectors), liposomes and other lipid-containing complexes, and other macromolecular complexes capable of mediating delivery of a polynucleotide to a host cell. Vectors can also comprise other components or functionalities that further modulate gene delivery and/or gene expression, or that otherwise provide beneficial properties to the targeted cells. Such other components include, for example, components that influence binding or targeting to cells (including components that mediate cell-type or tissue-specific binding); components that influence uptake of the vector nucleic acid by the cell; components that influence localization of the polynucleotide within the cell after uptake (such as agents mediating nuclear localization); and components that influence expression of the polynucleotide. Such components also might include markers, such as detectable and/or selectable markers that can be used to detect or select for cells that have

taken up and are expressing the nucleic acid delivered by the vector. Such components can be provided as a natural feature of the vector (such as the use of certain viral vectors which have components or functionalities mediating binding and uptake), or vectors can be modified to provide such functionalities. A large variety of such vectors are known in the art and are generally available. When a vector is maintained in a host cell, the vector can either be stably replicated by the cells during mitosis as an autonomous structure, incorporated within the genome of the host cell, or maintained in the host cell's nucleus or cytoplasm.

[0135] A "recombinant viral vector" refers to a viral vector comprising one or more modifications, including deletions, insertions, substitutions, and/or heterologous genes or sequences. Since many viral vectors exhibit size constraints associated with packaging, the heterologous genes or sequences are typically introduced by replacing one or more portions of the viral genome. Such viruses may become replication-defective or replication-incompetent, e.g., requiring the deleted function(s) to be provided in trans during viral replication and encapsidation (by using, e.g., a helper virus or a packaging cell line carrying genes for replication and/or encapsidation). Modified viral vectors in which a polynucleotide to be delivered is carried on the outside of the viral particle have also been described.

[0136] As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and optionally a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more than about 85%, about 90%, about 95%, and about 99%. For example, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

[0137] The term "subject" refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, canines, felines, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

[0138] The terms "effective amount" or "therapeutically effective amount" or "therapeutic effect" refer to an amount of a therapeutic effective to "treat" a disease or disorder in a subject or mammal. The therapeutically effective amount has a therapeutic effect and as such casn prevent the development of a disease or disorder; slow down the development of a disease or disorder; slow down the progression of a disease or disorder; relieve to some extent one or more of the symptoms associated with a disease or disorder; reduce morbidity and mortality; improve quality of life; or a combination of such effects.

[0139] The terms "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to both 1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and 2) prophylactic or preventative measures that prevent or slow the development of a targeted pathologic condition or disorder. Thus, those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented.

[0140] As used in the present disclosure and embodiments, the singular forms "a", "an" and "the" include plural forms unless the context clearly dictates otherwise.

[0141] The term "therapeutic" refers a composition that is used to treat, inhibit or prevent a disease or a condition, such as viral infection, e.g., coronaviral infection. For example, a therapeutic may be vaccine. A therapeutic may be a drug, e.g., a small molecule drug. A therapeutic may be administered to a subject in need thereof, to prevent a disease or an infection, or to reduce or ameliorate one or more symptoms associated with a disease. A therapeutic may also be considered to treat at least a symptom of the disease.

[0142] "Transfected," "transformed" or "transgenic" is used herein to include any host cell or cell line, which has been altered or augmented by the presence of at least one recombinant DNA sequence. The host cells of the present disclosure are typically produced by transfection with a DNA sequence in a plasmid expression vector, as an isolated linear DNA sequence, or infection with a recombinant viral vector.

[0143] The basic organization of the coronavirus genome is shared with other members of the Nidovirus order (the torovirus genus, also in the family Coronaviridae, and members of the family Arteriviridae) in that the nonstructural proteins involved in proteolytic processing, genome replication, and subgenomic mRNA synthesis (transcription) (an estimated 14-16 end products for coronaviruses) are encoded within the 50-proximal two-thirds of the genome on gene 1 and the (mostly) structural proteins are encoded within the 30-proximal one-third of the genome (8-9 genes for coronaviruses). Genes for the major structural proteins in all coronaviruses occur in 5' to 3' order as S (spike), E (envelope), M, and N. The genome sizes range from 27,317 nt for HCoV-229E to 31,357 nt for MHV-A59.

[0144] In general, coronavirus 5' UTRs range in length from 209 to 528 nt and contain a similarly positioned short, AUG-initiated open reading frame (ORF) relative to the 5' end. The short AUG-initiated ORFs (except for HCoV-229E) begin in a suboptimal Kozak context for translation and potentially encode peptides of 3-11 amino acids. The 3' UTRs range from 288 to 506 nt and possess an octameric sequence of GGAAGAGC beginning at base 73 to 80 upstream from the poly(A) tail, and 30-terminal poly(A) tail.

[0145] In general, the coronaviruses have a large gene 1 (separated into ORFs 1a and 1b and extending over approximately two-thirds of the genome) encoding nonstructural proteins involved in proteolytic processing of the gene 1 polyprotein products, virus genome replication, and sgmRNA synthesis (transcription). Gene 1 is translated as ORFs 1a and 1ab, with 1ab resulting from a pseudoknot-induced −1 ribosomal frame shifting event at a slippery sequence of UUUAAAC at the ORF 1a/1b junction.

[0146] Within the 3'-proximal one-third of the genome, besides ORFs encoding the structural proteins, a variable number of other ORFs appearing to be virus- or group-specific, many apparently encoding nonstructural proteins, are also found here. These (and their potential products) include ORF 3a (7.7-kDa protein), ORF 3b (27.7-kDa protein), and ORF 7 [0.7-kDa hydrophobic protein (HP)] in TGEV; ORF 3 (25.3-kDa protein) in PEDV; ORF 4a (15. 3-kDa protein) and ORF 4b (10.2-kDa protein) in HCoV-

229E; ORF 2a (32-kDa protein), ORF 2b [65-kDa complete or 34.6-kDa truncated hemagglutininesterase (HE) protein, depending on the strain], ORF 4 (17.8-kDa protein), ORF 5a (13.1-kDa protein), and an ORF internal to gene 7 [23-kDa internal (I) protein] in MHV; ORF 2a (32-kDa protein), ORF 2b (65-kDa HE protein), ORF 4a (4.9-kDa protein), ORF 4b (4.8-kDa protein), ORF 5 (12.7-kDa protein), and an ORF internal to gene 7 (23-kDa I protein) in BCoV; and ORF 3a (6.7-kDa protein), ORF 3b (7.4-kDa protein), ORF 5a (7.5-kDa protein), and ORF 5b (9.5-kDa protein) in IBV. Some of these, such as ORFs 3a and 3b in TGEV and ORFs 2a, 2b (HE), 4, 5a, and I in MHV, have been shown to be nonessential for replication in cell culture.

[0147] Coronaviruses (order Nidovirales, family Coronaviridae, and subfamily Orthocoronavirinae) are spherical (125 nm diameter), and enveloped with club-shaped spikes on the surface giving the appearance of a solar corona. Within the helically symmetrical nucleocapsid is the large positive sense, single stranded RNA. With genome sizes ranging from 26 to 32 kilobases (kb) in length, CoVs have the largest genome for RNA viruses. Of the four coronavirus genera (α, β, γ, δ), human coronaviruses (HCoVs) are classified under α-CoV and β-CoV. SARS-CoV-2 is a β-CoV.

[0148] Replication of coronaviruses begins with attachment and entry. Attachment of the virus to the host cell is initiated by interactions between the S protein and its specific receptor. Following receptor binding, the virus enters host cell cytosol via cleavage of S protein by a protease enzyme, followed by fusion of the viral and cellular membranes. The next step is the translation of the replicase gene from the virion genomic RNA and then translation and assembly of the viral replicase complexes. Following replication and subgenomic RNA synthesis, encapsidation occurs resulting in the formation of the mature virus. Following assembly, virions are transported to the cell surface in vesicles and released by exocytosis.

[0149] For SARS-Cov-2, next-generation sequencing also shows 79% homology to SARS-CoV and 50% to MERS-CoV. Phylogenetic analysis has placed SARS-CoV-2 under the subgenus Sarbecovirus of the genus Betacoronavirus.

[0150] The organization of the coronavirus genome is 5'-leader-UTR-replicase-S (Spike)-E (Envelope)-M (Membrane)-N(Nucleocapsid)-3'UTR-poly(A) tail with accessory genes interspersed within the structural genes at the 3' end of the genome.

[0151] The four structural proteins are required by most CoVs to produce a structurally complete viral particle, suggesting that some CoVs may encode additional proteins with overlapping compensatory functions. While each of the major protein plays a primary role in the structure of the virus particle, they are also involved in other aspects of the replication cycle.

[0152] The S protein (about 150 kDa) mediates attachment of the virus to the host cell surface receptors resulting in fusion and subsequent viral entry. In some CoVs, the S protein also mediate cell-cell fusion between infected and adjacent, uninfected cells resulting in formation of multinucleated giant cells, a strategy that allows direct viral spread between cells while avoiding virus-neutralizing antibodies.

[0153] The S protein utilizes an N-terminal signal sequence to gain access into the endoplasmic reticulum (ER), and is heavily N-linked glycosylated. Homotrimers of the virus-encoded S protein make up the distinctive spike-like structure. This trimeric S glycoprotein is a class I fusion protein that mediates attachment to the host receptor. In most coronaviruses, S is cleaved by a host cell furin-like protease into two separate polypeptides, namely S1 and S2. S1 makes up the large receptor-binding domain of the S protein and S2 forms the stalk of the spike.

[0154] The M protein (about 25-30 kDa) with three transmembrane domains is the most abundant structural protein and defines the shape of the viral envelope. It has a small N-terminal glycosylated ectodomain and a much larger C-terminal endodomain that extends 6-8 nm into the viral particle. Studies have shown that the M protein exists as a dimer, and may adopt two different conformations allowing it to promote membrane curvature as well as bind to the nucleocapsid. Interaction of S with M protein is necessary for retention of S in the ER-Golgi intermediate compartment (ERGIC)/Golgi complex and its incorporation into new virions, but is not required for the assembly process.44 Binding of M to N protein stabilises the nucleocapsid (N protein-RNA complex), as well as the internal core of virions, and, ultimately, helps complete the viral assembly. Together, M and E proteins make up the viral envelope and their interaction is sufficient for the production and release of virus-like particles (VLPs).

[0155] The E protein (about 8-12 kDa) is the smallest of the major structural proteins. This transmembrane protein has a N-terminal ectodomain and a C-terminal endodomain with ion channel activity. During the replication cycle, E is abundantly expressed inside the infected cell, but only a small portion is incorporated into the virus envelope. The majority of the protein participates in viral assembly and budding. Recombinant CoVs without E have been shown to exhibit significantly reduced viral titres, crippled viral maturation, or yield incompetent progeny, thereby demonstrating the importance of E protein in virus production and maturation.

[0156] The N protein is the only one that binds to the RNA genome. The protein is composed of two separate domains, an N-terminal domain (NTD) and a C-terminal domain (CTD). It has been suggested that optimal RNA binding requires contribution from both these domains. It is also involved in viral assembly and budding, resulting in complete virion formation.

[0157] The SARS-CoV-2 genome is similar to that of typical CoVs and contains at least ten open reading frames (ORFs). The 5'-terminal two-thirds of the genome ORF1a/b encodes two large polyproteins, which form the viral replicase transcriptase complex. The other ORFs of SARSCoV-2 on the one-third of the genome encode the same four main structural proteins: spike(S), envelope (E), nucleocapsid (N) and membrane (M) proteins, as well as several accessory proteins with unknown functions which do not participate in viral replication.

[0158] Attachment of the virus to the host cell is initiated by interactions between the S protein and its receptor. The site of receptor binding domains (RBD) within the S1 region of a coronavirus S protein varies for each coronavirus. MHV have the RBD at the N-terminus, whereas SARS-CoV have the RBD at the C-terminus. The S-protein/receptor interaction is the primary determinant to infect a host species and also controls viral tissue tropism. Many coronaviruses uti-

lize peptidases as their cellular receptor however, SARS-CoV and HCoV-NL63 use angiotensin-converting enzyme 2 (ACE2) as their receptor.

[0159] Following receptor binding, the virus enters host cell cytosol via acid-dependent proteolytic cleavage of S protein by a cathepsin, TMPRRS2 or another protease, followed by fusion of the viral and cellular membranes. S protein cleavage occurs at two sites within the S2 portion of the protein, with the first cleavage for separating the RBD and fusion domains of the S protein, and the second to expose the fusion peptide (cleavage at S2'). Cleavage at S2' exposes a fusion peptide that inserts into the membrane, followed by the joining of two heptad repeats in S2 forming an antiparallel six-helix bundle. The formation of this bundle results in fusion and ultimate release of the viral genome into the cytoplasm.

[0160] The next step in the coronavirus lifecycle is the translation of the replicase gene from the virion genomic RNA. Coronaviruses encode either two or three proteases that cleave the replicase polyproteins. Next, many of the nonstructural proteins (nsps) assemble into the replicase-transcriptase complex (RTC) to create an environment suitable for RNA synthesis, and ultimately are responsible for RNA replication and transcription of the sub-genomic RNAs. The nsps also contain other enzyme domains and functions.

[0161] Viral RNA synthesis follows the translation and assembly of the viral replicase complexes. Viral RNA synthesis produces both genomic and sub-genomic RNAs. Sub-genomic RNAs serve as mRNAs for the structural and accessory genes which reside downstream of the replicase polyproteins. All positive-sense sub-genomic RNAs are 3' co-terminal with the full-length viral genome and thus form a set of nested RNAs, a distinctive property of the order Nidovirales. Both genomic and sub-genomic RNAs are produced through negative-strand intermediates. These negative-strand intermediates are only about 1% as abundant as their positive-sense counterparts and contain both poly-uridylate and anti-leader sequences.

[0162] Coronaviruses are also known for their ability to recombine using both homologous and non-homologous recombination. The ability of these viruses to recombine is tied to the strand switching ability of the RNA-dependent RNA polymerase (RdRp). It is likely that recombination plays a significant role in viral evolution and is the basis for targeted RNA recombination, a reverse genetics tool used to engineer viral recombinants at the 3' end of the genome.

[0163] Following replication and subgenomic RNA synthesis, the S, E, and M proteins are translated and inserted into the endoplasmic reticulum (ER). These proteins move along the secretory pathway into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). In the compartment, the viral genomes that are encapsidated by the N protein, will bud into the membrane resulting in formation of the mature virus.

[0164] The M protein directs most of the protein-protein interactions required for coronaviruses assembly. However, virus-like particles (VLPs) can only be formed when M protein is expressed along with E protein, suggesting the need for these two proteins to produce coronavirus envelope. Additional roles of the E protein include inducing membrane curvature and preventing M protein aggregation. Following assembly, virions are transported to the cell surface in vesicles and released by exocytosis.

[0165] Some subsets of b-CoVs also comprise a fifth structural protein, hemagglutinin-esterase (HE), which enhances S protein-mediated cell entry and viral spread through the mucosa via its acetyl-esterase activity. Homotrimers of the S glycoprotein make up the distinctive spike structure on the surface of the virus. These trimers are a class I fusion protein, mediating virus attachment to the host receptor by interaction of the S protein and its receptor. In most CoVs, S is cleaved by host cell protease into two separate polypeptides-S1 and S2. S1 contains the receptor-binding domain (RBD) of the S protein (the exact positioning of the RBD varies depending on the viral strain), while S2 forms the stem of the spike molecule.

[0166] The S domain, which comprises S1 and S2 domains, responsible for receptor binding and cell membrane fusion respectively.

Methods

[0167] In one embodiment, coronavirus is serially passaged in cells at progressively lower temperatures. The cells may be infected and then cultured for 1, 2, 3, 4 or more days at one temperature, e.g., 35-37° C., supernatant is collected then used to infect fresh cells at a lower temperature, e.g., from 0.5 to 2 degrees lower such as from 33° C. to 34° C. and cultured for 1, 2, 3, 4 or more days at that temperature. That is repeated until a specific temperature or temperature range is reached, e.g., 25-27° C.

[0168] In one embodiment, prior to infection with coronavirus, the cells are cold adapted. In one embodiment, the cells are mammalian cells such as Vero cells, HT-29 cells, CaCO-2 cells, HeLa cells, HEP-2 cells, HCT-8 cells, HL-60 cells, A549 cells, VeroE6-TMPRSS2 cells, or VeroE6-TMPRSS2-ACE2.

[0169] There is also provided a vector comprising a nucleic acid molecule of the invention having a coronavirus genome from a cold-adapted coronavirus or a portion thereof.

[0170] Optionally a vector of the disclosure comprises a nucleic acid molecule comprising a coronavirus genome comprising a nucleic acid sequence which has at least 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% nucleic acid sequence identity over its entire length to one of SEQ ID Nos. 1-4, and comprises one or more mutations relative to one of SEQ ID Nos. 1-4, that is/are disclosed herein.

[0171] Viral vaccine vectors may use live viruses to deliver nucleic acid (for example, DNA or RNA) into human or non-human animal cells. The nucleic acid contained in the virus encodes one or more antigens that, once expressed in the infected human or non-human animal cells, elicit an immune response. Both humoral and cell-mediated immune responses can be induced by viral vaccine vectors. Viral vaccine vectors combine many of the positive qualities of nucleic acid vaccines with those of live attenuated vaccines. Like nucleic acid vaccines, viral vaccine vectors carry nucleic acid into a host cell for production of antigenic proteins that can be tailored to stimulate a range of immune responses, including antibody, T helper cell (CD4+ T cell), and cytotoxic T lymphocyte (CTL, CD8+ T cell) mediated immunity. Viral vaccine vectors, unlike nucleic acid vaccines, also have the potential to actively invade host cells and replicate, much like a live attenuated vaccine, further activating the immune system like an adjuvant. A viral

vaccine vector therefore generally comprises a live attenuated virus that is genetically engineered to carry nucleic acid (for example, DNA or RNA) encoding protein antigens. Although viral vaccine vectors may produce stronger immune responses than nucleic acid vaccines, for some diseases viral vectors are used in combination with other vaccine technologies in a strategy called heterologous prime-boost. In this system, one vaccine is given as a priming step, followed by vaccination using an alternative vaccine as a booster. The heterologous prime-boost strategy aims to provide a stronger overall immune response. Viral vaccine vectors may be used as both prime and boost vaccines as part of this strategy.

[0172] There is also provided a method of inducing an immune response to a coronavirus in a subject, which comprises administering to the subject an effective amount of a cold-adapted coronavirus or a portion thereof, a nucleic acid having a nucleotide sequence of a cold-adapted coronavirus or a portion thereof, a vector having a nucleotide sequence of a cold-adapted coronavirus or a portion thereof, or a pharmaceutical composition having a cold-adapted coronavirus or a portion thereof.

[0173] There is also provided a method of immunizing a subject against a cold-adapted coronavirus or a portion thereof, which comprises administering to the subject an effective amount of a cold-adapted coronavirus, a nucleic acid a nucleotide sequence of a cold-adapted coronavirus or a portion thereof, a vector having a nucleotide sequence of a cold-adapted coronavirus or a portion thereof, or a pharmaceutical composition having a cold-adapted coronavirus or a portion thereof.

[0174] There is further provided a cold-adapted coronavirus, a nucleotide sequence of a cold-adapted coronavirus or a portion thereof, a vector having a nucleotide sequence of a cold-adapted coronavirus or a portion thereof, or a pharmaceutical composition having a cold-adapted coronavirus or a portion thereof, for use as a medicament.

[0175] There is further provided a cold-adapted coronavirus or a portion thereof, a nucleotide sequence of a cold-adapted coronavirus or a portion thereof, a vector having a nucleotide sequence of a cold-adapted coronavirus or a portion thereof, or a pharmaceutical composition having a cold-adapted coronavirus or a portion thereof, for use in the prevention, treatment, or amelioration of a coronavirus infection.

[0176] There is also provided use of a cold-adapted coronavirus or a portion thereof, a nucleotide sequence of a cold-adapted coronavirus or a portion thereof, a vector having a nucleotide sequence of a cold-adapted coronavirus or a portion thereof, or a pharmaceutical composition having a cold-adapted coronavirus or a portion thereof, in the manufacture of a medicament for the prevention, treatment, or amelioration of a coronavirus infection.

Administration

[0177] Any suitable route of administration may be used. Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, parenteral, intravenous, subcutaneous, vaginal, rectal, intranasal, inhalation or oral. Parenteral administration, such as subcutaneous, intravenous or intramuscular administration, is generally achieved by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions.

[0178] Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Administration can be systemic or local.

[0179] Compositions may be administered in any suitable manner, such as with pharmaceutically acceptable carriers. Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Preparations for parenteral administration include sterile aqueous or nonaqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

[0180] Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base-addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

[0181] Administration can be accomplished by single or multiple doses. The dose administered to a subject in the context of the present disclosure should be sufficient to induce a beneficial therapeutic response in a subject over time, or to inhibit or prevent infection. The dose required will vary from subject to subject depending on the species, age, weight and general condition of the subject, the severity of the infection being treated, the particular composition being used and its mode of administration. An appropriate dose can be determined by one of ordinary skill in the art using only routine experimentation.

[0182] Pharmaceutically acceptable carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition can be sterile, and the formulation suits the mode of administration. The composition can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, and magnesium carbonate. Any of the common pharmaceu-

tical carriers, such as sterile saline solution or sesame oil, can be used. The medium can also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. Other media that can be used with the compositions and methods provided herein are normal saline and sesame oil.

[0183] In some embodiments, the compositions comprise a pharmaceutically acceptable carrier and/or an adjuvant. For example, the adjuvant can be alum, Freund's complete adjuvant, a biological adjuvant or immunostimulatory oligonucleotides (such as CpG oligonucleotides).

[0184] The pharmaceutically acceptable carriers (vehicles) useful in this disclosure are conventional. Remington's Pharmaceutical Sciences, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of one or more therapeutic compositions, such as one or more vaccines, and additional pharmaceutical agents.

[0185] In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional nontoxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

[0186] Optionally a composition is administered intramuscularly.

[0187] Optionally a composition is administered intramuscularly, intradermally, subcutaneously by needle or by gene gun, or electroporation.

[0188] A vaccine of the disclosure includes an isolated cold-adapted virus of the disclosure, and optionally one or more other isolated viruses including influenza viruses, one or more immunogenic proteins or glycoproteins of one or more isolated viruses or one or more other pathogens, e.g., an immunogenic protein from one or more bacteria, influenza viruses, yeast or fungi, or isolated nucleic acid encoding one or more viral proteins (e.g., DNA vaccines) including one or more immunogenic proteins of the isolated cold-adapted coronavirus of the disclosure. In one embodiment, the cold-adapted coronavirus of the disclosure may be a vaccine vectors for other pathogens.

[0189] A complete virion vaccine may be concentrated by ultrafiltration and then purified by zonal centrifugation or by chromatography. Viruses other than the virus of the disclosure, such as those included in a multivalent vaccine, may be inactivated before or after purification using formalin or beta-propiolactone, for instance.

[0190] A subunit vaccine comprises purified glycoproteins. Such a vaccine may be prepared as follows: using viral suspensions fragmented by treatment with detergent, the surface antigens are purified, by ultracentrifugation for example. The detergent used may be cationic detergent for example, such as hexadecyl trimethyl ammonium bromide, an anionic detergent such as ammonium deoxycholate; or a nonionic detergent such as that commercialized under the name TRITON X100. The hemagglutinin may also be isolated after treatment of the virions with a protease such as bromelin, and then purified. A subunit vaccine may be combined with an attenuated virus of the disclosure in a multivalent vaccine.

[0191] A split vaccine comprises virions which have been subjected to treatment with agents that dissolve lipids. A split vaccine can be prepared as follows: an aqueous suspension of the purified virus obtained as above, inactivated or not, is treated, under stirring, by lipid solvents such as ethyl ether or chloroform, associated with detergents. The dissolution of the viral envelope lipids results in fragmentation of the viral particles. The aqueous phase is recuperated containing the split vaccine, constituted mainly of hemagglutinin and neuraminidase with their original lipid environment removed, and the core or its degradation products. Then the residual infectious particles are inactivated if this has not already been done. A split vaccine may be combined with an attenuated virus of the disclosure in a multivalent vaccine.

[0192] Inactivated virus vaccines are provided by inactivating replicated virus using known methods, such as, but not limited to, formalin or β-propiolactone treatment. Inactivated vaccine types that can be used in the disclosure can include whole-virus (WV) vaccines or subvirion (SV) (split) vaccines. The WV vaccine contains intact, inactivated virus, while the SV vaccine contains purified virus disrupted with detergents that solubilize the lipid-containing viral envelope, followed by chemical inactivation of residual virus. An inactivated virus vaccine may be combined with an attenuated virus of the disclosure in a multivalent vaccine.

[0193] Live, attenuated virus vaccines, such as those including a cold-adapted coronavirus of the disclosure can be used for preventing, inhibiting or treating coronavirus infection. Attenuation may be achieved in a single step by transfer of attenuated genes from an attenuated donor virus to a recipient virus according to known methods. The attenuated genes are derived from an attenuated parent.

[0194] A cold adapted (ca) donor virus can be used for attenuated vaccine production. Live, attenuated virus vaccines can be generated that are: (a) infectious, (b) attenuated for seronegative mammals and immunologically primed mammals, (c) immunogenic and/or (d) genetically stable.

[0195] One or more attenuating mutations can be introduced into virus genes by site-directed mutagenesis to rescue infectious viruses bearing these mutant genes. Attenuating mutations can be introduced into non-coding regions of the genome, as well as into coding regions. Thus, new dviruses can also be generated bearing attenuating mutations introduced by site-directed mutagenesis, and such new viruses can be used in the production of live attenuated vaccine candidates. Similarly, other known and suitable attenuated strains can be employed with one or more of the mutations disclosed herein to obtain attenuated vaccines suitable for use in the vaccination of mammals.

[0196] In one embodiment, such attenuated viruses maintain the genes from the virus that encode antigenic determinants substantially similar to those of the original clinical isolates. This is because the purpose of the attenuated vaccine is to provide substantially the same antigenicity as the original clinical isolate of the virus, while at the same time lacking pathogenicity to the degree that the vaccine

causes minimal chance of inducing a serious disease condition in the vaccinated mammal.

[0197] The viruses in a multivalent vaccine can thus be attenuated or inactivated, formulated and administered, according to known methods, as a vaccine to induce an immune response in an animal, e.g., a mammal. Methods are well-known in the art for determining whether such attenuated or inactivated vaccines have maintained similar antigenicity to that of the clinical isolate or high growth strain derived therefrom.

[0198] In one embodiment, a vaccine comprises a dose of the cold-adapted virus that is 10^2 to 10^5 $TCID_{50}$. In one embodiment, a vaccine comprises a dose of the cold-adapted virus that is 10^3 to 10^6 $TCID_{50}$. In one embodiment, a vaccine comprises a dose of the cold-adapted virus that is 10^4 to 10^7 $TCID_{50}$. In one embodiment, a vaccine comprises a dose of the cold-adapted virus that is 10^5 to 10^8 $TCID_{50}$.

Pharmaceutical Compositions

[0199] Pharmaceutical compositions of the present disclosure, suitable for inoculation, e.g., nasal, parenteral or oral administration, comprise one or more virus isolates, e.g., one or more cold-adapted SARS-CoV-2 viruses, a subunit thereof, isolated protein(s) thereof, and/or isolated nucleic acid encoding one or more proteins thereof, optionally further comprising sterile aqueous or non-aqueous solutions, suspensions, and emulsions. The compositions can further comprise auxiliary agents or excipients, as known in the art. The composition of the disclosure is generally presented in the form of individual doses (unit doses).

[0200] Conventional vaccines generally contain about 0.1 to 200 μg, e.g., 1 to 5 ug, 5 to 20 μg, 10 to 30 μg or 30 to 100 μg, of S protein from each of the strains entering into the composition. The vaccine forming the main constituent of the vaccine composition of the disclosure may comprise a single cold-adapted SARS-CoV-2 virus, or a combination of viruses, for example, at least two or three different viruses, including one or more other coronaviruses.

[0201] Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and/or emulsions, which may contain auxiliary agents or excipients known in the art. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Carriers or occlusive dressings can be used to increase skin permeability and enhance antigen absorption. Liquid dosage forms for oral administration may generally comprise a liposome solution containing the liquid dosage form. Suitable forms for suspending liposomes include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water. Besides the inert diluents, such compositions can also include adjuvants, wetting agents, emulsifying and suspending agents, or sweetening, flavoring, or perfuming agents.

[0202] When a composition of the present disclosure is used for administration to an individual, it can further comprise salts, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the composition. For vaccines, adjuvants, substances which can augment a specific immune response, can be used. Normally, the adjuvant and the composition are mixed prior to presentation to the immune system, or presented separately, but into the same site of the organism being immunized.

[0203] Heterogeneity in a vaccine may be provided by mixing viruses for at least two virus strains, such as 2-20 strains or any range or value therein. Vaccines can be provided for variations in a single strain of virus, using techniques known in the art.

[0204] A pharmaceutical composition according to the present disclosure may further or additionally comprise at least one chemotherapeutic compound, for example, for gene therapy, immunosuppressants, anti-inflammatory agents or immune enhancers, and for vaccines, chemotherapeutics including, but not limited to, gamma globulin, amantadine, guanidine, hydroxybenzimidazole, interferon-α, interferon-β, interferon-γ, tumor necrosis factor-alpha, thiosemicarbarzones, methisazone, rifampin, ribavirin, a pyrimidine analog, a purine analog, foscarnet, phosphonoacetic acid, acyclovir, dideoxynucleosides, a protease inhibitor, or ganciclovir.

[0205] The composition can also contain variable but small quantities of endotoxin-free formaldehyde, and preservatives, which have been found safe and not contributing to undesirable effects in the organism to which the composition is administered.

[0206] The administration of the cold-adapted SARS-CoV-2 virus containing composition (or the antisera that it elicits) may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the compositions of the disclosure which are vaccines are provided before any symptom or clinical sign of a pathogen infection becomes manifest. The prophylactic administration of the composition serves to prevent or attenuate any subsequent infection.

[0207] When provided therapeutically, a viral vaccine is provided upon the detection of a symptom or clinical sign of actual infection. The therapeutic administration of the compound(s) serves to attenuate any actual infection.

[0208] Thus, a vaccine composition of the present disclosure may be provided either before the onset of infection (so as to prevent or attenuate an anticipated infection) or after the initiation of an actual infection.

[0209] A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient mammal. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. A composition of the present disclosure is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient, e.g., enhances at least one primary or secondary humoral or cellular immune response against at least one strain of an infectious coronavirus.

[0210] The "protection" provided need not be absolute, i.e., the coronavirus infection need not be totally prevented or eradicated, if there is a statistically significant improvement compared with a control population or set of mammals. Protection may be limited to mitigating the severity or rapidity of onset of symptoms or clinical signs of the virus infection.

[0211] A composition comprising a cold-adapted SARS-CoV-2 virus may confer resistance to one or more pathogens, e.g., one or more SARS-CoV-2 strains, by either passive immunization or active immunization. In active immunization, an attenuated live vaccine composition is administered prophylactically to a host (e.g., a mammal), and the host's immune response to the administration protects against infection and/or disease. For passive immuni-

zation, the elicited antisera can be recovered and administered to a recipient suspected of having an infection caused by at least one virus strain.

[0212] In one embodiment, the vaccine is provided to a mammalian female (at or prior to pregnancy or parturition), under conditions of time and amount sufficient to cause the production of an immune response which serves to protect both the female and the fetus or newborn (via passive incorporation of the antibodies across the placenta or in the mother's milk).

[0213] The present disclosure thus includes methods for preventing or attenuating a disorder or disease, e.g., an infection by at least one strain of pathogen. As used herein, a vaccine is said to prevent or attenuate a disease if its administration results either in the total or partial attenuation (i.e., suppression) of a clinical sign or condition of the disease, or in the total or partial immunity of the individual to the disease.

[0214] A composition having at least one cold-adapted SARS-CoV-2 virus of the present disclosure, including one which is attenuated and one or more other isolated viruses, one or more isolated viral proteins thereof, one or more isolated nucleic acid molecules encoding one or more viral proteins thereof, or a combination thereof, may be administered by any means that achieve the intended purposes.

[0215] For example, administration of such a composition may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, oral or transdermal routes. Parenteral administration can be accomplished by bolus injection or by gradual perfusion over time.

[0216] A typical regimen for preventing, suppressing, or treating a virus related pathology, comprises administration of an effective amount of a vaccine composition as described herein, administered as a single treatment, or repeated as enhancing or booster dosages, over a period up to and including between one week and about 24 months, or any range or value therein.

[0217] According to the present disclosure, an "effective amount" of a composition is one that is sufficient to achieve a desired effect. It is understood that the effective dosage may be dependent upon the species, age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect wanted. The ranges of effective doses provided below are not intended to limit the disclosure and represent dose ranges.

[0218] The dosage of a live, attenuated or killed virus vaccine for an animal such as a mammalian adult organism may be from about 10^2-10^{15}, e.g., 10^3-10^{12}, plaque forming units (PFU)/kg, or any range or value therein. The dose of inactivated vaccine may range from about 0.1 to 1000, e.g., 2.5 to 10 μg, of S protein. However, the dosage should be a safe and effective amount as determined by conventional methods, using existing vaccines as a starting point.

[0219] The dosage of immunoreactive S in each dose of replicated virus vaccine may be standardized to contain a suitable amount, e.g., 30 to 100 μg or any range or value therein, or the amount recommended by government agencies or recognized professional organizations.

[0220] The dosage of immunoreactive S in each dose of virus vaccine can be standardized to contain a suitable amount, e.g., 1-50 μg or any range or value therein, or the amount recommended by the U.S. Public Health Service (PHS), which is usually 15 μg per component for older children (greater than or equal to 3 years of age), and 7.5 μg per component for children less than 3 years of age. The quantity of NA can also be standardized, however, this glycoprotein can be labile during the processor purification and storage. Each 0.5-ml dose of vaccine may contains approximately 1-50 billion virus particles, for example 10 billion particles.

Exemplary SARS-CoV-2 Genomes and Encoded Products

[0221] An exemplary cold-adapted coronavirus has one or more alterations in one or more of Nsp1, Nsp2, Nsp3, Nsp4, Nsp5, Nsp6, Nsp7, Nsp8, Nsp9, Nsp10, Nsp11, Nsp12, Nsp13, Nsp14, Nsp15, Nsp16, S, E, M, N, or any combination thereof relative to a:

[0222] The Nsp1 amino acid sequence is provided below as SEQ ID NO:20:

MESLVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQHLKD

GTCGLVEVEKGVLPQLEQPYVFIKRSDARTAPHGHVMVELVAELEGIQ

YGRSGETLGVLVPHVGEIPVAYRKVLLRKNGNKGAGGHSYGADLKSFD

LGDELGTDPYEDFQENWNTKHSSGVTRELMRELNGG

[0223] Nsp2 amino acid sequence is provided below as SEQ ID NO:21:

AYTRYVDNNFCGPDGYPLECIKDLLARAGKASCTLSEQLDFIDTK

RGVYCCREHEHEIAWYTERSEKSYELQTPFEIKLAKKFDTFNGECPN

FVFPLNSIIKTIQPRVEKKKLDGFMGRIRSVYPVASPNECNQMCLST

LMKCDHCGETSWQTGDFVKATCEFCGTENLTKEGATTCGYLPQNAVV

KIYCPACHNSEVGPEHSLAEYHNESGLKTILRKGGRTIAFGGCVFSY

VGCHNKCAYWVPRASANIGCNHTGVVGEGSEGLNDNLLEILQKEKVN

INIVGDFKLNEEIAIILASFSASTSAFVETVKGLDYKAFKQIVESCG

NFKVTKGKAKKGAWNIGEQKSILSPLYAFASEAARVVRSIFSRTLET

AQNSVRVLQKAAITILDGISQYSLRLIDAMMFTSDLATNNLVVMAYI

TGGVVQLTSQWLTNIFGTVYEKLKPVLDWLEEKFKEGVEFLRDGWEI

VKFISTCACEIVGGQIVTCAKEIKESVQTFFKLVNKFLALCADSIII

GGAKLKALNLGETFVTHSKGLYRKCVKSREETGLLMPLKAPKEIIFL

EGETLPTEVLTEEVVLKTGDLQPLEQPTSEAVEAPLVGTPVCINGLM

LLEIKDTEKYCALAPNMMVTNNTFTLKGG

[0224] Nsp3 amino acid sequence is provided below as SEQ ID NO:22:

APTKVTFGDDTVIEVQGYKSVNITFELDERIDKVLNEKCSAYTVELGTEV

NEFACVVADAVIKTLQPVSELLTPLGIDLDEWSMATYYLFDESGEFKLAS

HMYCSFYPPDEDEEEGDCEEEEFEPSTQYEYGTEDDYQGKPLEFGATSA

ALQPEEEQEEDWLDDDSQQTVGQQDGSEDNQTTTIQTIVEVQPQLEMEL

TPVVQTIEVNSFSGYLKLTDNVYIKNADIVEEAKKVKPTVVVNAANVYL

KHGGGVAGALNKATNNAMQVESDDYIATNGPLKVGGSCVLSGHNLAK

-continued

HCLHVVGPNVNKGEDIQLLKSAYENFNQHEVLLAPLLSAGIFGADPIHSL

RVCVDTVRTNVYLAVFDKNLYDKLVSSFLEMKSEKQVEQKIAEIPKEEV

KPFITESKPSVEQRKQDDKKIKACVEEVTTTLEETKFLTENLLLYIDING

NLHPDSATLVSDIDITFLKKDAPYIVGDVVQEGVLTAVVIPTKKAGGTTE

MLAKALRKVPTDNYITTYPGQGLNGYTVEEAKTVLKKCKSAFYILPSIIP

NEKQEILGTVSWNLREMLAHAEETRKLMPVCVETKAIVSTIQRKYKGIKI

QEGVVDYGARFYFYTSKTTVASLINTLNDLNETLVTMPLGYVTHGLNLEE

AARYMRSLKVPATVSVSSPDAVTAYNGYLTSSSKTPEEHFIETISLAGSY

KDWSYSGQSTQLGIEFLKRGDKSVYYT

SNPTTFHLDGEVITFDNLKTLLSLREVRTIKVFTTVDNINLHTQVVDMSM

TYGQQFGPTYLDGADVTKIKPHNSHEGKTFYVLPNDDTLRVEAFEYYHT

TDPSFLGRYMSALNHTKKWKYPQVNGLTSIKWADNNCYLATALLTLQQ

IELKFNPPALQDAYYRARAGEAANFCALILAYCNKTVGELGDVRETMSY

LFQHANLDSCKRVLNVVCKTCGQQQTTLKGVEAVMYMGTLSYEQFKK

GVQIPCTCGKQATKYLVQQESPFVMMSAPPAQYELKHGTFTCASEYTG

NYQCGHYKHITSKETLYCIDGALLTKSSEYKGPITDVFYKENSYTTTIKP

VTYKLDGVVCTEIDPKLDNYYKKDNSYFTEQPIDLVPNQPYPNASFDNF

KFVCDNIKFADDLNQLTGYKKPASRELKVTFFPDLNGDVVAIDYKHYTP

SFKKGAKLLHKPIVWHVNNATNKATYKPNTWCIRCLWSTKPVETSNSF

DVLKSEDAQGMDNLACEDLKPVSEEVVENPTIQKDVLECNVKTTEVVG

DIILKPANNSLKITEEVGHTDLMAAYVDNSSLTIKKPNELSRVLGLKTLA

THGLAAVNSVPWDTIANYAKPFLNKVVSTTTNIVTRCLNRVCTNYMPYF

FTLLLQLCTFTRSTNSRIKASMPTTIAKNTVKSVGKFCLEASFNYLKSPN

FSKLINIIIWFLLLSVCLGSLIYSTAALGVLMSNLGMPSYCTGYREGYLN

STNVTIATYCTGSIPCSVCLSGLDSLDTYPSLETIQITISSFKWDLTAFG

LVAEWFLAYILFTRFFYVLGLAAIMQLFFSYFAVHFISNSWLMWLIINLV

QMAPISAMVRMYIFFASFYYVWKSYVHVVDGCNSSTCMMCYKRNRATRVE

CTTIVNGVRRSFYVYANGGKGFCKLHNWNCVNCDTFCAGSTFISDEVAR

DLSLQFKRPINPTDQSSYIVDSVTVKNGSIHLYFDKAGQKTYERHSLSHF

VNLDNLRANNTKGSLPINVIVFDGKSKCEESSAKSASVYYSQLMCQPILL

LDQALVSDVGDSAEVAVKMFDAYVNTFSSTFNVPMEKLKTLVATAEAE

LAKNVSLDNVLSTFISAARQGFVDSDVETKDVVECLKLSHQSDIEVTGDS

CNNYMLTYNKVENMTPRDLGACIDCSARHINAQVAKSHNIALIWNVKD

FMSLSEQLRKQIRSAAKKNNLPFKLTCATTRQVVNVVTTKIALKGG

[0225] Nsp4 amino acid sequence is provided below as SEQ ID NO:23:

KIVNNWLKQLIKVTLVFLFVAAIFYLITPVHVMSKHTDFSSEIIGYKAID

GGVTRDIASTDTCFANKHADFDTWFSQRGGSYTNDKACPLIAAVITREVG

-continued

FVVPGLPGTILRTTNGDFLHFLPRVFSAVGNICYTPSKLIEYTDFATSAC

VLAAECTIFKDASGKPVPYCYDTNVLEGSVAYESLRPDTRYVLMDGSIIQ

FPNTYLEGSVRVVTTFDSEYCRHGTCERSEAGVCVSTSGRWVLNNDYYR

SLPGVFCGVDAVNLLTNMFTPLIQPIGALDISASIVAGGIVAIVVTCLAY

YFMRFRRAFGEYSHVVAFNTLLFLMSFTVLCLTPVYSFLPGVYSVIYLYL

TFYLTNDVSFLAHIQWMVMFTPLVPFWITIAYIICISTKHFYWFFSNYLK

RRVVFNGVSFSTFEEAALCTFLLNKEMYLKLRSDVLLPLTQYNRYLALYN

KYKYFSGAMDTTSYREAACCHLAKALNDFSNSGSDVLYQPPQTSITSAVL

Q

[0226] Nsp5 amino acid sequence is provided below as SEQ ID NO:24:

SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRHVICTSED

MLNPNYEDLLIRKSNHNFLVQAGNVQLRVIGHSMQNCVLKLKVDTANP

KTPKYKFVRIQSGQTFSVLACYNGSPSGVYQCAMRPNFTIKGSFLNGSC

GSVGFNIDYDCVSFCYMHHMELPTGVHAGTDLEGNFYGPFVDRQTAQAA

GTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNYEPL

TQDHVDILGPLSAQTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEF

TPFDVVRQCSGVTFQ

[0227] Nsp6 amino acid sequence is provided below as SEQ ID NO:25:

SAVKRTIKGTHHWLLLTILTSLLVLVQSTQWSLFFFLYENAFLPFAMGI

IAMSAFAMMFVKHKHAFLCLFLLPSLATVAYFNMVYMPASWVMRIMTWL

DMVDTSLSGFKLKDCVMYASAVVLLILMTARTVYDDGARRVWTLMNV

LTLVYKVYYGNALDQAISMWALIISVTSNYSGVVTTVMFLARGIVFMCV

EYCPIFFITGNTLQCIMLVYCFLGYFCTCYFGLFCLLNRYFRLTLGVYD

YLVSTQEFRYMNSQGLLPPKNSIDAFKLNIKLLGVGGKPCIKVATVQ

[0228] Nsp7 amino acid sequence is provided below as SEO ID NO: 26:

SKMSDVKCTSVVLLSVLQQLRVESSSKLWAQCVQLHNDILLAKDTTEA

FEKMVSLLSVLLSMQGAVDINKLCEEMLDNRATLQ

[0229] Nsp8 amino acid sequence is provided below as SEQ ID NO:27:

AIASEFSSLPSYAAFATAQEAYEQAVANGDSEVVLKKLKKSLNVAKS

EFDRDAAMQRKLEKMADQAMTQMYKQARSEDKRAKVTSAMQTMLFTM

LRKLDNDALNNIINNARDGCVPLNIIPLTTAAKLMVVIPDYNTYKNTC

DGTTFTYASALWEIQQVVDADSKIVQLSEISMDNSPNLAWPLIVTALR

ANSAVKLQ

[0230] Nsp9 amino acid sequence is provided below as SEQ ID NO:28:

NNELSPVALRQMSCAAGTTQTACTDDNALAYYNTTKGGRFVLALLSDL

QDLKWARFPKSDGTGTIYTELEPPCRFVTDTPKGPKVKYLYFIKGLNN

LNRGMVLGSLAATVRLQ

[0231] Nsp10 amino acid sequence is provided below as SEQ ID NO:29:

AGNATEVPANSTVLSFCAFAVDAAKAYKDYLASGGQPITNCVKMLCTH

TGTGQAITVTPEANMDQESFGGASCCLYCRCHIDHPNPKGFCDLKGKY

VQIPTTCANDPVGFTLKNTVCTVCGMWKGYGCSCDQLREPMLQ

[0232] Nsp11 amino acid sequence is provided below as SEQ ID NO:30:

SADAQSFLNGFAV

[0233] Nsp12 amino acid sequence is provided below as SEQ ID NO:31:

SADAQSFLNRVCGVSAARLTPCGTGTSTDVVYRAFDIYNDKVAGFAKFL

KTNCCRFQEKDEDDNLIDSYFVVKRHTFSNYQHEETIYNLLKDCPAVAK

HDFFKFRIDGDMVPHISRQRLTKYTMADLVYALRHFDEGNCDTLKEILV

TYNCCDDDYFNKKDWYDFVENPDILRVYANLGERVRQALLKTVQFCD

AMRNAGIVGVLTLDNQDLNGNWYDFGDFIQTTPGSGVPVVDSYYSLLM

PILTLTRALTAESHVDTDLTKPYIKWDLLKYDFTEERLKLFDRYFKYWD

QTYHPNCVNCLDDRCILHCANFNVLFSTVFPLTSFGPLVRKIFVDGVPF

VVSTGYHFRELGVVHNQDVNLHSSRLSFKELLVYAADPAMHAASGNLLL

DKRTTCFSVAALTNNVAFQTVKPGNFNKDFYDFVVSKGFFKEGSSVELK

HFFFAQDGNAAISDYDYYRYNLPTMCDIRQLLFVVEVVDKYFDCYDGG

CINANQVIVNNLDKSAGFPFNKWGKARLYYDSMSYEDQDALFAYTKRN

VIPTITQMNLKYAISAKNRARTVAGVSICSTMTNRQFHQKLLKSIAATR

GATVVIGTSKFYGGWHNMLKTVYSDVENPHLMGWDYPKCDRAMPNML

RIMASLVLARKHTTCCSLSHRFYRLANECAQVLSEMVMCGGSLYVKPG

GTSSGDATTAYANSVFNICQAVTANVNALLSTDGNKIADKYVRNLQHR

LYECLYRNRDVDTDFVNEFYAYLRKHFSMMILSDDAVVCFNSTYASQG

LVASIKNFKSVLYYQNNVFMSEAKCWTETDLTKGPHEFCSQHTMLVKQ

GDDYVYLPYPDPSRILGAGCFVDDIVKTDGTLMIERFVSLAIDAYPLTK

HPNQEYADVFHLYLQYIRKLHDELTGHMLDMYSVMLINDNTSRYWEPEF

YEAMYTPHTVLQ

[0234] Nsp13 amino acid sequence is provided below as SEQ ID NO:32:

AVGACVLCNSQTSLRCGACIRRPFLCCKCCYDHVISTSHKLVLSVNPYV

CNAPGCDVTDVTQLYLGGMSYYCKSHKPPISFPLCANGQVFGLYKNTC

VGSDNVTDFNAIATCDWTNAGDYILANTCTERLKLFAAETLKATEETFK

LSYGIATVREVLSDRELHLSWEVGKPRPPLNRNYVFTGYRVTKNSKVQI

GEYTFEKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTVMPLSAPTLVPQ

EHYVRITGLYPTLNISDEFSSNVANYQKVGMQKYSTLQGPPGTGKSHFA

IGLALYYPSARIVYTACSHAAVDALCEKALKYLPIDKCSRIIPARARVE

CFDKFKVNSTLEQYVFCTVNALPETTADIVVFDEISMATNYDLSVVNAR

LRAKHYVYIGDPAQLPAPRTLLTKGTLEPEYFNSVCRLMKTIGPDMFLG

TCRRCPAEIVDTVSALVYDNKLKAHKDKSAQCFKMFYKGVITHDVSSAI

NRPQIGVVREFLTRNPAWRKAVFISPYNSQNAVASKILGLPTQTVDSSQ

GSEYDYVIFTQTTETAHSCNVNRFNVAITRAKVGILCIMSDRDLYDKLQ

FTSLEIPRRNVATLQ

[0235] Nsp14 amino acid sequence is provided below as SEQ ID NO:33:

AENVTGLFKDCSKVITGLHPTQAPTHLSVDTKFKTEGLCVDIPGIPKDM

TYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATR

EAVGTNLPLQLGFSTGVNLVAVPTGYVDTPNNTDFSRVSAKPPPGDQFK

HLIPLMYKGLPWNVVRIKIVQMLSDTLKNLSDRVVFVLWAHGFELTSM

KYFVKIGPERTCCLCDRRATCFSTASDTYACWHHSIGFDYVYNPFMIDV

QQWGFTGNLQSNHDLYCQVHGNAHVASCDAIMTRCLAVHECFVKRVD

WTIEYPIIGDELKINAACRKVQHMVVKAALLADKFPVLHDIGNPKAIKC

VPQADVEWKFYDAQPCSDKAYKIEELFYSYATHSDKFTDGVCLFWNCN

VDRYPANSIVCRFDTRVLSNLNLPGCDGGSLYVNKHAFHTPAFDKSAFV

NLKQLPFFYYSDSPCESHGKQVVSDIDYVPLKSATCITRQNLGGAVCRH

HANEYRLYLDAYNMMISAGFSLWVYKQFDTYNLWNTFTRLQ

[0236] Nsp15 amino acid sequence is provided below as SEQ ID NO:34:

SLENVAFNVVNKGHFDGQQGEVPVSIINNTVYTKVDGVDVELFENKTT

LPVNVAFELWAKRNIKPVPEVKILNNLGVDIAANTVIWDYKRDAPAHI

STIGVCSMTDIAKKPTETICAPLTVFFDGRVDGQVDLFRNARNGVLIT

EGSVKGLQPSVGPKQASLNGVTLIGEAVKTQFNYYKKVDGVVQQLPET

YFTQSRNLQEFKPRSQMEIDFLELAMDEFIERYKLEGYAFEHIVYGDF

SHSQLGGLHLLIGLAKRFKESPFELEDFIPMDSTVKNYFITDAQTGSS

KCVCSVIDLLLDDFVEIIKSQDLSVVSKVVKVTIDYTEISFMLWCKDG

HVETFYPKLQ

[0237] Nsp16 amino acid sequence is provided below as SEQ ID NO:35:

SSQAWQPGVAMPNLYKMQRMLLEKCDLQNYGDSATLPKGIMMNVAK
YTQLCQYLNTLTLAVPYNMRVIHFGAGSDKGVAPGTAVLRQWLPTGTL
LVDSDLNDFVSDADSTLIGDCATVHTANKWDLIISDMYDPKTKNVTKE
NDSKEGFFTYICGFIQQKLALGGSVAIKITEHSWNADLYKLMGHFAWW
TAFVTNVNASSSEAFLIGCNYLGKPREQIDGYVMHANYIFWRNTNPIQ
LSSYSLFDMSKFPLKLRGTAVMSLKEGQINDMILSLLSKGRLIIRENN
RVVISSDVLVNN

[0238] S amino acid sequence is provided below as SEQ ID NO:36:

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVL
HSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTE
KSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVY
YHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREF
VFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDA
VDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLC
PFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSP
TKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGC
VIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPC
NGVEGENCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPK
KSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAV
RDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAI
HADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICA
SYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTI
SVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALT
GIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRS
FIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPL
LTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVT
QNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALN
TLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYV
TQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSA
PHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFV
TQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEEL
DKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL
QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCC
SCGSCCKFDEDDSEPVLKGVKLHYT

[0239] ORF3a amino acid sequence is provided below as SEO ID NO. 37.

MDLFMRIFTIGTVTLKQGEIKDATPSDFVRATATIPIQASLPFGWLIV
GVALLAVFQSASKIITLKKRWQLALSKGVHFVCNLLLLFVTVYSHLLL
VAAGLEAPFLYLYALVYFLQSINFVRIIMRLWLCWKCRSKNPLLYDAN
YFLCWHTNCYDYCIPYNSVTSSIVITSGDGTTSPISEHDYQIGGYTEK
WESGVKDCVVLHSYFTSDYYQLYSTQLSTDTGVEHVTFFIYNKIVDEP
EEHVQIHTIDGSSGVVNPVMEPIYDEPTTTTSVPL

[0240] E amino acid sequence is provided below as SEQ ID NO:38:

MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVN
VSLVKPSFYVYSRVKNLNSSRVPDLLV

[0241] M amino acid sequence is provided below as SEQ ID NO:39:

MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYI
IKLIFLWLLWPVTLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYF
IASFRLFARTRSMWSFNPETNILLNVPLHGTILTRPLLESELVIGAVI
LRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYKLGASQRVAGDSG
FAAYSRYRIGNYKLNTDHSSSSDNIALLVQ

[0242] ORF6 amino acid sequence is provided below as SEQ ID NO:40:

MFHLVDFQVTIAEILLIIMRTFKVSIWNLDYIINLIIKNLSKSLTENK
YSQLDEEQPMEID

[0243] ORF7a amino acid sequence is provided below as SEO ID NO: 41:

MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHP
LADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQEL
YSPIFLIVAAIVFITLCFTLKRKTE

[0244] ORF7b amino acid sequence is provided below as SEQ ID NO:42:

MIELSLIDFYLCFLAFLLFLVLIMLIIFWFSLELQDHNETCHA

[0245] ORF8 amino acid sequence is provided below as SEQ ID NO:43:

MKFLVFLGIITTVAAFHQECSLQSCTQHQPYVVDDPCPIHFYSKWYIRV
GARKSAPLIELCVDEAGSKSPIQYIDIGNYTVSCLPFTINCQEPKLGSL
VVRCSFYEDFLEYHDVRVVLDFI

[0246] N amino acid sequence is provided below as SEQ ID NO:44:

MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNT

ASWFTALTQHGKEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGD

GKMKDLSPRWYFYYLGTGPEAGLPYGANKDGIIWVATEGALNTPKDHI

GTRNLANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSR

NSTPGSSKRTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQ

TVTKKSAAEASKKPRQKRTATKAYNVTQAFGRRGPEQTQGNFGDQELI

RQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYTGAIKLDD

KDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQ

TVTLLPAADLDDFSKQLQQSMSSADSTQA

[0247] ORF10 amino acid sequence is provided below as SEQ ID NO:45:

MGYINVFAFPFTIYSLLLCRMNSRNYIAQVDVVNFNLT

[0248] or a polypeptide having at least 80%, 82%, 84%, 85%, 87%, 89%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% amino acid sequence identity to any of Nsp1, Nsp2, Nsp3, Nsp4, Nsp5, Nsp6, Nsp7, Nsp8, Nsp9, Nsp10, Nsp11, Nsp12, Nsp13, Nsp14, Nsp15, Nsp16, S, E, M, N, ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10.

[0249] In one embodiment, a cold-adapted coronavirus has a mutation in one or more of genes for Nsp1, Nsp2, Nsp3, Nsp6, Nsp13, Nsp14, Nsp15, ORF6, ORF7b, ORF8, or any combination thereof. In one embodiment, a cold-adapted coronavirus comprise nucleic acids encoding a polypeptide for Nsp1, Nsp2, Nsp3, Nsp6, Nsp13, Nsp14, Nsp15, or a polypeptide encoded by ORF6, with at least 80%, 82%, 84%, 85%, 87%, 89%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% amino acid sequence identity to one of SEQ ID Nos. 20-22, 25, 32-34, or 40.

[0250] An exemplary cold-adapted coronavirus has a genome with one or more alterations in nucleotide sequences for one or more of Nsp1, Nsp2, Nsp3, Nsp4, Nsp5, Nsp6, Nsp7, Nsp8, Nsp9, Nsp10, Nsp11, Nsp12, Nsp13, Nsp14, Nsp15, Nsp16, S, E, M, N, or any combination thereof, which alteration may be a deletion of one or more nucleotides, a substitution of one or more nucleotides, an insertion of one or more nucleotides, or any combination thereof, relative to a genome having SEQ ID NO:1 (FIG. **34**), SEQ ID NO:2 (FIG. **35**), SEQ ID NO:3, or SEQ ID NO: 4, or a nucleic acid sequence with at least 80%, 82%, 84%, 85%, 87%, 89%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% nucleic acid sequence identity thereto.

[0251] An exemplary cold-adapted coronavirus has one or more alterations in one or more of Nsp1, Nsp2, Nsp3, Nsp4, Nsp5, Nsp6, Nsp7, Nsp8, Nsp9, Nsp10, Nsp11, Nsp12, Nsp13, Nsp14, Nsp15, Nsp16, S, E, M or N relative to proteins encoded by severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1 (NCBI Reference Sequence: NC_045512.2) (SEQ ID NO: 5; FIG. **79**), such as a genome that encodes the following polypeptides.

[0252] ORF1ab amino acid sequence is provided below as (SEQ ID NO:51):

MESLVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQHLKD

GTCGLVEVEKGVLPQLEQPYVFIKRSDARTAPHGHVMVELVAELEGIQY

GRSGE

TLGVLVPHVGEIPVAYRKVLLRKNGNKGAGGHSYGADLKSFDLGDELG

TDPYEDFQENWNTKHSSGVTRELMRELNGGAYTRYVDNNFCGPDGYPL

ECIKDLLARAGKASCTLSEQLDFIDTKRGVYCCREHEHEIAWYTERSEKS

YELQTPFEIKLAKKFDTFNGECPNFVFPLNSIIKTIQPRVEKKKLDGFMG

RIRSVYPVASPNECNQMCLSTLMKCDHCGETSWQTGDFVKATCEFCGTE

NLTKEGATTCGYLPQNAVVKIYCPACHNSEVGPEHSLAEYHNESGLKTI

LRKGGRTIAFGGCVFSYVGCHNKCAYWVPRASANIGCNHTGVVGEGSE

GLNDNLLEILQKEKVNINIVGDFKLNEEIAIILASFSASTSAFVETVKGL

DYKAFKQIVESCGNFKVTKGKAKKGAWNIGEQKSILSPLYAFASEAARVV

RSIFSRTLETAQNSVRVLQKAAITILDGISQYSLRLIDAMMFTSDLATNN

LVVMAYITGGVVQLTSQWLTNIFGTVYEKLKPVLDWLEEKFKEGVEFLRD

GWEIVKFISTCACEIVGGQIVTCAKEIKESVQTFFKLVNKFLALCADSII

IGGAKLKALNLGETFVTHSKGLYRKCVKSREETGLLMPLKAPKEIIFLEG

ETLPTEVLTEEVVLKTGDLQPLEQPTSEAVEAPLVGTPVCINGLMLLEIK

DTEKYCALAPNMMVTNNTFTLKGGAPTKVTFGDDTVIEVQGYKSVNITFE

LDERIDKVLNEKCSAYTVELGTEVNEFACVVADAVIKTLQPVSELLTPLG

IDLDEWSMATYYLFDESGEFKLASHMYCSFYPPDEDEEEGDCEEEEFEPS

TQYEYGTEDDYQGKPLEFGATSAALQPEEEQEEDWLDDDSQQTVGQQD

GSEDNQTTTIQTIVEVQPQLEMELTPVVQTIEVNSFSG

YLKLTDNVYIKNADIVEEAKKVKPTVVVNAANVYLKHGGGVAGALNK

ATNNAMQVESDDYIATNGPLKVGGSCVLSGHNLAKHCLHVVGPNVNK

GEDIQLLKSAYENFNQHEVLLAPLLSAGIFGADPIHSLRVCVDTVRTNVY

LAVFDKNLYDKLVSSFLEMKSEKQVEQKIAEIPKEEVKPFITESKPSVEQ

RKQDDKKIKACVEEVTTTLEETKFLTENLLLYIDINGNLHPDSATLVSDI

DITFLKKDAPYIVGDVVQEGVLTAVVIPTKKAGGTTEMLAKALRKVPTDN

YITTYPGQGLNGYTVEEAKTVLKKCKSAFYILPSIISNEKQEILGTVSWN

LREMLAHAEETRKLMPVCVETKAIVSTIQRKYKGIKIQEGVVDYGARFYF

YTSKTTVASLINTLNDLNETLVTMPLGYVTHGLNLEEAARYMRSLKVPA

TVSVSSPDAVTAYNGYLTSSSKTPEEHFIETISLAGSYKDWSYSGQSTQL

GIEFLKRGDKSVYYTSNPTTFHLDGEVITFDNLKTLLSLREVRTIKVFTT

VDNINLHTQVVDMSMTYGQQFGPTYLDGADVTKIKPHNSHEGKTFYVLP

NDDTLRVEAFEYYHTTDPSFLGRYMSALNHTKKWKYPQVNGLTSIKWA

DNNCYLATALLTLQQIELKFNPPALQDAYYRARAGEAANFCALILAYCN

KTVGELGDVRETMSYLFQHANLDSCKRVLNVVCKTCGQQQTTLKGVE

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AVMYMGTLSYEQFKKGVQIPCTCGKQATKYLVQQESPFVMMSAPPAQ

YELKHGTFTCASEYTGNYQCGHYKHITSKETLYCIDGALLTKSSEYKGPI

TDVFYKENSYTTTIKPVTYKLDGVVCTEIDPKLDNYYKKDNSYFTEQPID

LVPNQPYPNASFDNFKFVCDNIKFADDLNQLTGYKKPASRELKVTFFPD

LNGDVVAIDYKHYTPSFKKGAKLLHKPIVWHVNNATNKATYKPNTWCI

RCLWSTKPVETSNSFDVLKSEDAQGMDNLACEDLKPVSEEVVENPTIQK

DVLECNVKTTEVVGDIILKPANNSLKITEEVGHTDLMAAYVDNSSLTIKK

PNELSRVLGLKTLATHGLAAVNSVPWDTIANYAKPFLNKVVSTTTNIVT

RCLNRVCTNYMPYFFTLLLQLCTFTRSTNSRIKASMPTTIAKNTVKSVGK

FCLEASFNYLKSPNFSKLINIIIWFLLLSVCLGSLIYSTAALGVLMSNLG

MPSYCTGYREGYLNSTNVTIATYCTGSIPCSVCLSGLDSLDTYPSLETIQ

ITISSFKWDLTAFGLVAEWFLAYILFTRFFYVLGLAAIMQLFFSYFAVHF

ISNSWLMWLIINLVQMAPISAMVRMYIFFASFYYVWKSYVHVVDGCNSST

CMMCYKRNRATRVECTTIVNGVRRSFYVYANGGKGFCKLHNWNCVNCDT

FCAGSTFISDEVARDLSLQFKRPINPTDQSSYIVDSVTVKNGSIHLYFDK

AGQKTYERHSLSHFVNLDNLRANNTKGSLPINVIVFDGKSKCEESSAKSA

SVYYSQLMCQPILLLDQALVSDVGDSAEVAVKMFDAYVNTFSSTFNVPM

EKLKTLVATAEAELAKNVSLDNVLSTFISAARQGFVDSDVETKDVVECL

KLSHQSDIEVTGDSCNNYMLTYNKVENMTPRDLGACIDCSARHINAQV

AKSHNIALIWNVKDFMSLSEQLRKQIRSAAKKNNLPFKLTCATTRQVVN

VVTTKIALKGGKIVNNWLKQLIKVTLVFLFVAAIFYLITPVHVMSKHTDF

SSEIIGYKAIDGGVTRDIASTDTCFANKHADFDTWFSQRGGSYTNDKACP

LIAAVITREVGFVVPGLPGTILRTTNGDFLHFLPRVFSAVGNICYTPSKL

IEYTDFATSACVLAAECTIFKDASGKPVPYCYDTNVLEGSVAYESLRPDT

RYVLMDGSIIQFPNTYLEGSVRVVTTFDSEYCRHGTCERSEAGVCVSTSG

RWVLNNDYYRSLPGVFCGVDAVNLLTNMFTPLIQPIGALDISASIVAGGI

VAIVVTCLAYYFMRFRRAFGEYSHVVAFNTLLFLMSFTVLCLTPVYSFLP

GVYSVIYLYLTFYLTNDVSFLAHIQWMVMFTPLVPFWITIAYIICISTKH

FYWFFSNYLKRRVVFNGVSFSTFEEAALCTFLLNKEMYLKLRSDVLLPLT

QYNRYLALYNKYKYFSGAMDTTSYREAACCHLAKALNDESNSGSDVLY

QPPQTSITSAVLQSGFRKMAFPSGKVEGCMVQVTCGTTTLNG

LWLDDVVYCPRHVICTSEDMLNPNYEDLLIRKSNHNFLVQAGNVQLRVI

GHSMQNCVLKLKVDTANPKTPKYKFVRIQPGQTFSVLACYNGSPSGVY

QCAMRPNFTIKGSFLNGSCGSVGFNIDYDCVSFCYMHHMELPTGVHAGT

DLEGNFYGPFVDRQTAQAAGTDTTITVNVLAWLYAAVINGDRWFLNRF

TTTLNDFNLVAMKYNYEPLTQDHVDILGPLSAQTGIAVLDMCASLKELL

QNGMNGRTILGSALLEDEFTPFDVVRQCSGVTFQSAVKRTIKGTHHWLL

LTILTSLLVLVQSTQWSLFFFLYENAFLPFAMGIIAMSAFAMMFVKHKHA

FLCLFLLPSLATVAYFNMVYMPASWVMRIMTWLDMVDTSLSGFKLKDC

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VMYASAVVLLILMTARTVYDDGARRVWTLMNVLTLVYKVYYGNALD

QAISMWALIISVTSNYSGVVTTVMFLARGIVFMCVEYCPIFFITGNTLQC

IMLVYCFLGYFCTCYFGLFCLLNRYFRLTLGVYDYLVSTQEFRYMNSQG

LLPPKNSIDAFKLNIKLLGVGGKPCIKVATVQSKMSDVKCTSVVLLSVLQ

QLRVESSSKLWAQCVQLHNDILLAKDTTEAFEKMVSLLSVLLSMQGAV

DINKLCEEMLDNRATLQAIASEFSSLPSYAAFATAQEAYEQAVANGDSE

VVLKKLKKSLNVAKSEFDRDAAMQRKLEKMADQAMTQMYKQARSED

KRAKVTSAMQTMLFTMLRKLDNDALNNIINNARDGCVPLNIIPLTTAAK

LMVVIPDYNTYKNTCDGTTFTYASALWEIQQVVDADSKIVQLSEISMDN

SPNLAWPLIVTALRANSAVKLQNNELSPVALRQMSCAAGTTQTACTDD

NALAYYNTTKGGRFVLALLSDLQDLKWARFPKSDGTGTIYTELEPPCRF

VTDTPKGPKVKYLYFIKGLNNLNRGMVLGSLAATVRLQAGNATEVPAN

STVLSFCAFAVDAAKAYKDYLASGGQPITNCVKMLCTHTGTGQAITVTP

EANMDQESFGGASCCLYCRCHIDHPNPKGFCDLKGKYVQIPTTCANDPV

GFTLKNTVCTVCGMWKGYGCSCDQLREPMLQSADAQSFLNRVCGVSA

ARLTPCGTGTSTDVVYRAFDIYNDKVAGFAKFLKTNCCRFQEKDEDDN

LIDSYFVVKRHTFSNYQHEETIYNLLKDCPAVAKHDFFKFRIDGDMVPHI

SRQRLTKYTMADLVYALRHFDEGNCDTLKEILVTYNCCDDDYFNKKD

WYDFVENPDILRVYANLGERVRQALLKTVQFCDAMRNAGIVGVLTLDN

QDLNGNWYDFGDFIQTTPGSGVPVVDSYYSLLMPILTLTRALTAESHVD

TDLTKPYIKWDLLKYDFTEERLKLFDRYFKYWDQTYHPNCVNCLDDRC

ILHCANFNVLFSTVFPPTSFGPLVRKIFVDGVPFVVSTGYHFRELGVVHN

QDVNLHSSRLSFKELLVYAADPAMHAASGNLLLDKRTTCFSVAALTNN

VAFQTVKPGNFNKDFYDFAVSKGFFKEGSSVELKHFFFAQDGNAAISDY

DYYRYNLPTMCDIRQLLFVVEVVDKYFDCYDGGCINANQVIVNNLDKS

AGFPFNKWGKARLYYDSMSYEDQDALFAYTKRNVIPTITQMNLKYAIS

AKNRARTVAGVSICSTMTNRQFHQKLLKSIAATRGATVVIGTSKFYGGW

HNMLKTVYSDVENPHLMGWDYPKCDRAMPNMLRIMASLVLARKHTTC

CSLSHRFYRLANECAQVLSEMVMCGGSLYVKPGGTSSGDATTAYANSV

FNICQAVTANVNALLSTDGNKIADKYVRNLQHRLYECLYRNRDVDTDF

VNEFYAYLRKHFSMMILSDDAVVCFNSTYASQGLVASIKNFKSVLYYQN

NVFMSEAKCWTETDLTKGPHEFCSQHTMLVKQGDDYVYLPYPDPSRIL

GAGCFVDDIVKTDGTLMIERFVSLAIDAYPLTKHPNQEYADVFHLYLQYI

RKLHDELTGHMLDMYSVMLTNDNTSRYWEPEFYEAMYTPHTVLQAVG

ACVLCNSQTSLRCGACIRRPFLCCKCCYDHVISTSHKLVLSVNPYVCNAP

GCDVTDVTQLYLGGMSYYCKSHKPPISFPLCANGQVFGLYKNTCVGSD

NVTDFNAIATCDWTNAGDYILANTCTERLKLFAAETLKATEETFKLSYGI

ATVREVLSDRELHLSWEVGKPRPPLNRNYVFTGYRVTKNSKVQIGEYTF

EKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTVMPLSAPTLVPQEHYVR

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ITGLYPTLNISDEFSSNVANYQKVGMQKYSTLQGPPGTGKSHFAIGLALY

YPSARIVYTACSHAAVDALCEKALKYLPIDKCSRIIPARARVECFDKFKV

NSTLEQYVFCTVNALPETTADIVVFDEISMATNYDLSVVNARLRAKHYV

YIGDPAQLPAPRTLLTKGTLEPEYFNSVCRLMKTIGPDMFLGTCRRCPAE

IVDTVSALVYDNKLKAHKDKSAQCFKMFYKGVITHDVSSAINRPQIGVV

REFLTRNPAWRKAVFISPYNSQNAVASKILGLPTQTVDSSQGSEYDYVIF

TQTTETAHSCNVNRFNVAITRAKVGILCIMSDRDLYDKLQFTSLEIPRRN

VATLQAENVTGLFKDCSKVITGLHPTQAPTHLSVDTKFKTEGLCVDIPGI

PKDMTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEG

CHATREAVGTNLPLQLGFSTGVNLVAVPTGYVDTPNNTDFSRVSAKPPP

GDQFKHLIPLMYKGLPWNVVRIKIVQMLSDTLKNLSDRVVFVLWAHGF

ELTSMKYFVKIGPERTCCLCDRRATCFSTASDTYACWHHSIGFDYVYNP

FMIDVQQWGFTGNLQSNHDLYCQVHGNAHVASCDAIMTRCLAVHECF

VKRVDWTIEYPIIGDELKINAACRKVQHMVVKAALLADKFPVLHDIGNP

KAIKCVPQADVEWKFYDAQPCSDKAYKIEELFYSYATHSDKFTDGVCLF

WNCNVDRYPANSIVCRFDTRVLSNLNLPGCDGGSLYVNKHAFHTPAFD

KSAFVNLKQLPFFYYSDSPCESHGKQVVSDIDYVPLKSATCITRQNLGGA

VCRHHANEYRLYLDAYNMMISAGFSLWVYKQFDTYNLWNTFTRLQSL

ENVAFNVVNKGHFDGQQGEVPVSIINNTVYTKVDGVDVELFENKTTLPV

NVAFELWAKRNIKPVPEVKILNNLGVDIAANTVIWDYKRDAPAHISTIGV

CSMTDIAKKPTETICAPLTVFFDGRVDGQVDLFRNARNGVLITEGSVKGL

QPSVGPKQASLNGVTLIGEAVKTQFNYYKKVDGVVQQLPETYFTQSRNL

QEFKPRSQMEIDFLELAMDEFIERYKLEGYAFEHIVYGDFSHSQLGGLHL

LIGLAKRFKESPFELEDFIPMDSTVKNYFITDAQTGSSKCVCSVIDLLLD

DFVEIIKSQDLSVVSKVVKVTIDYTEISFMLWCKDGHVETFYPKLQSSQA

WQPGVAMPNLYKMQRMLLEKCDLQNYGDSATLPKGIMMNVAKYTQLC

QYLNTLTLAVPYNMRVIHFGAGSDKGVAPGTAVLRQWLPTGTLLVDSD

LNDFVSDADSTLIGDCATVHTANKWDLIISDMYDPKTKNVTKENDSKEG

FFTYICGFIQQKLALGGSVAIKITEHSWNADLYKLMGHFAWWTAFVTNV

NASSSEAFLIGCNYLGKPREQIDGYVMHANYIFWRNTNPIQLSSYSLFDM

SKFPLKLRGTAVMSLKEGQINDMILSLLSKGRLIIRENNRVVISSDVLVN

N

[0253] a polypeptide having residues 266 to 805 of ORF1ab (nsp1) is provided below as SEQ ID NO:52:

MESLVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQHLKD

GTCGLVEVEKGVLPQLEQPYVFIKRSDARTAPHGHVMVELVAELEGIQY

GRSGETLGVLVPHVGEIPVAYRKVLLRKNGNKGAGGHSYGADLKSFDL

GDELGTDPYEDFQENWNTKHSSGVTRELMRELNGG

[0254] a polypeptide having residues 806 to 2719 of ORF1ab (nsp2) is provided below as SEQ ID NO:53:

AYTRYVDNNFCGPDGYPLECIKDLLARAGKASCTLSEQLDFIDTKRGV

YCCREHEHEIAWYTERSEKSYELQTPFEIKLAKKFDTFNGECPNFVFP

LNSIIKTIQPRVEKKKLDGFMGRIRSVYPVASPNECNQMCLSTLMKCD

SHCGETWQTGDFVKATCEFCGTENLTKEGATTCGYLPQNAVVKIYCPA

CHNSEVGPEHSLAEYHNESGLKTILRKGGRTIAFGGCVFSYVGCHNKC

AYWVPRASANIGCNHTGVVGEGSEGLNDNLLEILQKEKVNINIVGDFK

LNEEIAIILASFSASTSAFVETVKGLDYKAFKQIVESCGNFKVTKGKA

KKGAWNIGEQKSILSPLYAFASEAARVVRSIFSRTLETAQNSVRVLQK

AAITILDGISQYSLRLIDAMMFTSDLATNNLVVMAYITGGVVQLTSQW

LTNIFGTVYEKLKPVLDWLEEKFKEGVEFLRDGWEIVKFISTCACEIV

GGQIVTCAKEIKESVQTFFKLVNKFLALCADSIIIGGAKLKALNLGET

FVTHSKGLYRKCVKSREETGLLMPLKAPKEIIFLEGETLPTEVLTEEV

VLKTGDLQPLEQPTSEAVEAPLVGTPVCINGLMLLEIKDTEKYCALAP

NMMVTNNTFTLKGG

[0255] a polypeptide having residues 2720 to 554 of ORF1ab (nsp3) is provided below as SEQ ID NO:54:

APTKVTFGDDTVIEVQGYKSVNITFELDERIDKVLNEKCSAYTVELGT

EVNEFACVVADAVIKTLQPVSELLTPLGIDLDEWSMATYYLFDESGEF

KLASHMYCSFYPPDEDEEEGDCEEEEFEPSTQYEYGTEDDYQGKPLEF

GATSAALQPEEEQEEDWLDDDSQQTVGQQDGSEDNQTTTIQTIVEVQP

QLEMELTPVVQTIEVNSFSGYLKLTDNVYIKNADIVEEAKKVKPTVVV

NAANVYLKHGGGVAGALNKATNNAMQVESDDYIATNGPLKVGGSCVLS

GHNLAKHCLHVVGPNVNKGEDIQLLKSAYENFNQHEVLLAPLLSAGIF

GADPIHSLRVCVDTVRTNVYLAVFDKNLYDKLVSSFLEMKSEKQVEQK

IAEIPKEEVKPFITESKPSVEQRKQDDKKIKACVEEVTTTLEETKFLT

ENLLLYIDINGNLHPDSATLVSDIDITFLKKDAPYIVGDVVQEGVLTA

VVIPTKKAGGTTEMLAKALRKVPTDNYITTYPGQGLNGYTVEEAKTVL

KKCKSAFYILPSIISNEKQEILGTVSWNLREMLAHAEETRKLMPVCVE

TKAIVSTIQRKYKGIKIQEGVVDYGARFYFYTSKTTVASLINTLNDLN

ETLVTMPLGYVTHGLNLEEAARYMRSLKVPATVSVSSPDAVTAYNGYL

TSSSKTPEEHFIETISLAGSYKDWSYSGQSTQLGIEFLKRGDKSVYYT

SNPTTFHLDGEVITFDNLKTLLSLREVRTIKVFTTVDNINLHTQVVDM

SMTYGQQFGPTYLDGADVTKIKPHNSHEGKTFYVLPNDDTLRVEAFEY

YHTTDPSFLGRYMSALNHTKKWKYPQVNGLTSIKWADNNCYLATALLT

LQQIELKFNPPALQDAYYRARAGEAANFCALILAYCNKTVGELGDVRE

TMSYLFQHANLDSCKRVLNVVCKTCGQQQTTLKGVEAVMYMGTLSYEQ

FKKGVQIPCTCGKQATKYLVQQESPFVMMSAPPAQYELKHGTFTCASE

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YTGNYQCGHYKHITSKETLYCIDGALLTKSSEYKGPITDVFYKENSYT

TTIKPVTYKLDGVVCTEIDPKLDNYYKKDNSYFTEQPIDLVPNQPYPN

ASFDNFKFVCDNIKFADDLNQLTGYKKPASRELKVTFFPDLNGDVVAI

DYKHYTPSFKKGAKLLHKPIVWHVNNATNKATYKPNTWCIRCLWSTKP

VETSNSFDVLKSEDAQGMDNLACEDLKPVSEEVVENPTIQKDVLECNV

KTTEVVGDIILKPANNSLKITEEVGHTDLMAAYVDNSSLTIKKPNELS

RVLGLKTLATHGLAAVNSVPWDTIANYAKPFLNKVVSTTTNIVTRCLN

RVCTNYMPYFFTLLLQLCTFTRSTNSRIKASMPTTIAKNTVKSVGKFC

LEASFNYLKSPNFSKLINIIIWFLLLSVCLGSLIYSTAALGVLMSNLG

MPSYCTGYREGYLNSTNVTIATYCTGSIPCSVCLSGLDSLDTYPSLET

IQITISSFKWDLTAFGLVAEWFLAYILFTRFFYVLGLAAIMQLFFSYF

AVHFISNSWLMWLIINLVQMAPISAMVRMYIFFASFYYVWKSYVHVVD

GCNSSTCMMCYKRNRATRVECTTIVNGVRRSFYVYANGGKGFCKLHNW

NCVNCDTFCAGSTFISDEVARDLSLQFKRPINPTDQSSYIVDSVTVKN

GSIHLYFDKAGQKTYERHSLSHFVNLDNLRANNTKGSLPINVIVFDGK

SKCEESSAKSASVYYSQLMCQPILLLDQALVSDVGDSAEVAVKMFDAY

VNTFSSTFNVPMEKLKTLVATAEAELAKNVSLDNVLSTFISAARQGFV

DSDVETKDVVECLKLSHQSDIEVTGDSCNNYMLTYNKVENMTPRDLGA

CIDCSARHINAQVAKSHNIALIWNVKDFMSLSEQLRKQIRSAAKKNNL

PFKLTCATTRQVVNVVTTKIALKGG

[0256] a polypeptide having residues 8555 to 10054 of ORF1ab (nsp4) is provided below as SEQ ID NO:55:

KIVNNWLKQLIKVTLVFLFVAAIFYLITPVHVMSKHTDFSSEIIGYKA

IDGGVTRDIASTDTCFANKHADFDTWFSQRGGSYTNDKACPLIAAVIT

REVGFVVPGLPGTILRTTNGDFLHFLPRVFSAVGNICYTPSKLIEYTD

FATSACVLAAECTIFKDASGKPVPYCYDTNVLEGSVAYESLRPDTRYV

LMDGSIIQFPNTYLEGSVRVVTTFDSEYCRHGTCERSEAGVCVSTSGR

WVLNNDYYRSLPGVFCGVDAVNLLTNMFTPLIQPIGALDISASIVAGG

IVAIVVTCLAYYFMRFRRAFGEYSHVVAFNTLLFLMSFTVLCLTPVYS

FLPGVYSVIYLYLTFYLTNDVSFLAHIQWMVMFTPLVPFWITIAYIIC

ISTKHFYWFFSNYLKRRVVFNGVSFSTFEEAALCTFLLNKEMYLKLRS

DVLLPLTQYNRYLALYNKYKYFSGAMDTTSYREAACCHLAKALNDFSN

SGSDVLYQPPQTSITSAVLQ

[0257] a polypeptide having residues 10055 to 10972 of ORF1ab (nsp5A_3CLpro and nsp5B_3CLpro) is provided below as SEQ ID NO:56:

SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRHVICTSED

MLNPNYEDLLIRKSNHNFLVQAGNVQLRVIGHSMQNCVLKLKVDTANP

-continued

KTPKYKFVRIQPGQTFSVLACYNGSPSGVYQCAMRPNFTIKGSFLNGS

SCGVGFNIDYDCVSFCYMHHMELPTGVHAGTDLEGNFYGPFVDRQTAQ

AAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNYE

PLTQDHVDILGPLSAQTGIAVLDMCASLKELLQNGMNGRTILGSALLE

DEFTPFDVVRQCSGVTFQ

[0258] a polypeptide having residues 10973 to 11842 of ORF 1ab (nsp6) is provided below as SEQ ID NO:57:

SAVKRTIKGTHHWLLLTILTSLLVLVQSTQWSLFFFLYENAFLPFAMG

IIAMSAFAMMFVKHKHAFLCLFLLPSLATVAYFNMVYMPASWVMRIMT

WLDMVDTSLSGFKLKDCVMYASAVVLLILMTARTVYDDGARRVWTLMN

VLTLVYKVYYGNALDQAISMWALIISVTSNYSGVVTTVMFLARGIVFM

CVEYCPIFFITGNTLQCIMLVYCFLGYFCTCYFGLFCLLNRYFRLTLG

VYDYLVSTQEFRYMNSQGLLPPKNSIDAFKLNIKLLGVGGKPCIKVAT

VQ

[0259] a polypeptide having residues 11843 to 12091 of ORF 1ab (nsp7) is provided below SEQ ID NO:58:

SKMSDVKCTSVVLLSVLQQLRVESSSKLWAQCVQLHNDILLAKDTTEA

FEKMVSLLSVLLSMQGAVDINKLCEEMLDNRATLQ

[0260] a polypeptide having residues 12092 to 12685 of PRF 1ab (nsp8) is provided below as SEQ ID NO:59:

AIASEFSSLPSYAAFATAQEAYEQAVANGDSEVVLKKLKKSLNVAKSE

FDRDAAMQRKLEKMADQAMTQMYKQARSEDKRAKVTSAMQTMLFTM

LRKLDNDALNNIINNARDGCVPLNIIPLTTAAKLMVVIPDYNTYKNTC

DCTTFTYASALWEIQQVVDADSKIVQLSEISMDNSPNLAWPLIVTALR

ANSAVKLQ

[0261] a polypeptide having residues 12686 to 13024 of ORF 1ab (nsp9) is provided below as SEQ ID NO:60:

NNELSPVALRQMSCAAGTTQTACTDDNALAYYNTTKGGRFVLALLSDL

QDLKWARFPKSDGTGTIYTELEPPCRFVTDTPKGPKVKYLYFIKGLNN

LNRGMVLGSLAATVRLQ

[0262] a polypeptide having residues 13025 to 13441 of ORF1ab (nsp10) is provided below as SEQ ID NO:61:

AGNATEVPANSTVLSFCAFAVDAAKAYKDYLASGGQPITNCVKMLCTH

TGTGQAITVTPEANMDQESFGGASCCLYCRCHIDHPNPKGFCDLKGKY

VQIPTTCANDPVGFTLKNTVCTVCGMWKGYGCSCDQLREPMLQ

[0263] a polypeptide having residues 13442 to 13480 of ORF1ab (nsp11) is provided below as SEQ ID NO:62:

SADAQSFLNGFAV

[0264] a polypeptide having residues 13442 to 13468 and 13468 to 16236 of ORF1ab (nsp12; RNA-dependent RNA polymerase) is provided below as SEQ ID NO: 63:

SADAQSFLNRVCGVSAARLTPCGTGTSTDVVYRAFDIYNDKVAGFAK
FLKTNCCRFQEKDEDDNLIDSYFVVKRHTFSNYQHEETIYNLLKDCP
AVAKHDFFKFRIDGDMVPHISRQRLTKYTMADLVYALRHFDEGNCDT
LKEILVTYNCCDDDYFNKKDWYDFVENPDILRVYANLGERVRQALLK
TVQFCDAMRNAGIVGVLTLDNQDLNGNWYDFGDFIQTTPGSGVPVVD
SYYSLLMPILTLTRALTAESHVDTDLTKPYIKWDLLKYDFTEERLKL
FDRYFKYWDQTYHPNCVNCLDDRCILHCANFNVLFSTVFPPTSFGPL
VRKIFVDGVPFVVSTGYHFRELGVVHNQDVNLHSSRLSFKELLVYAA
DPAMHAASGNLLLDKRTTCFSVAALTNNVAFQTVKPGNFNKDFYDFA
VSKGFFKEGSSVELKHFFFAQDGNAAISDYDYYRYNLPTMCDIRQLL
FVVEVVDKYFDCYDGGCINANQVIVNNLDKSAGFPFNKWGKARLYYD
SMSYEDQDALFAYTKRNVIPTITQMNLKYAISAKNRARTVAGVSICS
TMTNRQFHQKLLKSIAATRGATVVIGTSKFYGGWHNMLKTVYSDVEN
PHLMGWDYPKCDRAMPNMLRIMASLVLARKHTTCCSLSHRFYRLANE
CAQVLSEMVMCGGSLYVKPGGTSSGDATTAYANSVFNICQAVTANVN
ALLSTDGNKIADKYVRNLQHRLYECLYRNRDVDTDFVNEFYAYLRKH
FSMMILSDDAVVCFNSTYASQGLVASIKNFKSVLYYQNNVFMSEAKC
WTETDLTKGPHEFCSQHTMLVKQGDDYVYLPYPDPSRILGAGCFVDD
IVKTDGTLMIERFVSLAIDAYPLTKHPNQEYADVFHLYLQYIRKLHD
ELTGHMLDMYSVMLINDNTSRYWEPEFYEAMYTPHTVLQ

[0265] a polypeptide having residues 16237 to 18039 (nsp13; helicase) is provided below as SEQ ID NO:64:

AVGACVLCNSQTSLRCGACIRRPFLCCKCCYDHVISTSHKLVLSVNP
YVCNAPGCDVTDVTQLYLGGMSYYCKSHKPPISFPLCANGQVFGLYK
NTCVGSDNVTDFNAIATCDWTNAGDYILANTCTERLKLFAAETLKAT
EETFKLSYGIATVREVLSDRELHLSWEVGKPRPPLNRNYVFTGYRVT
KNSKVQIGEYTFEKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTVMPL
SAPTLVPQEHYVRITGLYPTLNISDEFSSNVANYQKVGMQKYSTLQG
PPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCEKALKYLPIDK
CSRIIPARARVECFDKFKVNSTLEQYVFCTVNALPETTADIVVFDEI
SMATNYDLSVVNARLRAKHYVYIGDPAQLPAPRTLLTKGTLEPEYFN
SVCRLMKTIGPDMFLGTCRRCPAEIVDTVSALVYDNKLKAHKDKSAQ

-continued

CFKMFYKGVITHDVSSAINRPQIGVVREFLTRNPAWRKAVFISPYNS
QNAVASKILGLPTQTVDSSQGSEYDYVIFTQTTETAHSCNVNRFNVA
ITRAKVGILCIMSDRDLYDKLQFTSLEIPRRNVATLQ

[0266] a polypeptide having residues 18040 to 19620 (nsp14A2 and nsp14; 3'-to-5' exonuclease) is provided below as SEQ ID NO:65:

AENVTGLFKDCSKVITGLHPTQAPTHLSVDTKFKTEGLCVDIPGIPK
DMTYRRLISMMGFKMNYQVNGYPNMFITREEAIRHVRAWIGFDVEGC
HATREAVGTNLPLQLGFSTGVNLVAVPTGYVDTPNNTDFSRVSAKPP
PGDQFKHLIPLMYKGLPWNVVRIKIVQMLSDTLKNLSDRVVFVLWAH
GFELTSMKYFVKIGPERTCCLCDRRATCFSTASDTYACWHHSIGFDY
VYNPFMIDVQWGFTGNLQSNHDLYCQVHGNAHVASCDAIMTRCLAVH
ECFVKRVDWTIEYPIIGDELKINAACRKVQHMVVKAALLADKFPVLH
DIGNPKAIKCVPQADVEWKFYDAQPCSDKAYKIEELFYSYATHSDKF
DGVCLFWNCNVDRYPANSIVCRFDTRVLSNLNLPGCDGGSLYVNKHA
FHTPAFDKSAFVNLKQLPFFYYSDSPCESHGKQVVSDIDYVPLKSAT
CITRQNLGGAVCRHHANEYRLYLDAYNMMISAGFSLWVYKQFDTYNL
WNTFTRLQ

[0267] a polypeptide having residues 19621 to 20658 (nsp15A1 and nsp15B; endoRNAse) is provided below as SEQ ID NO:66:

SLENVAFNVVNKGHFDGQQGEVPVSIINNTVYTKVDGVDVELFENKTT
LPVNVAFELWAKRNIKPVPEVKILNNLGVDIAANTVIWDYKRDAPAHI
STIGVCSMTDIAKKPTETICAPLTVFFDGRVDGQVDLFRNARNGVLIT
EGSVKGLQPSVGPKQASLNGVTLIGEAVKTQFNYYKKVDGVVQQLPET
YFTQSRNLQEFKPRSQMEIDFLELAMDEFIERYKLEGYAFEHIVYGDF
SHSQLGGLHLLIGLAKRFKESPFELEDFIPMDSTVKNYFITDAQTGSS
KCVCSVIDLLLDDFVEIIKSQDLSVVSKVVKVTIDYTEISFMLWCKDG
HVETFYPKLQ

[0268] a polypeptide having residues 20659 to 21552 (nsp16; 2'-O-ribose methyltransferase) is provided below as SEQ ID NO:67:

SSQAWQPGVAMPNLYKMQRMLLEKCDLQNYGDSATLPKGIMMNVAKYT
QLCQYLNTLTLAVPYNMRVIHFGAGSDKGVAPGTAVLRQWLPTGTLLV
DSDLNDFVSDADSTLIGDCATVHTANKWDLIISDMYDPKTKNVTKEND
SKEGFFTYICGFIQQKLALGGSVAIKITEHSWNADLYKLMGHFAWWTA
FVTNVNASSSEAFLIGCNYLGKPREQIDGYVMHANYIFWRNTNPIQLS
SYSLFDMSKFPLKLRGTAVMSLKEGQINDMILSLLSKGRLIIRENNRV
VISSDVLVNN

[0269] S amino acid sequence is provided below as (SEQ ID NO:68):

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSV
LHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFAS
TEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFL
GVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKN
LREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINI
TRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNEN
GTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRF
PNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFST
FKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNY
KLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDIST
EIYQAGSTPCNGVEGENCYFPLQSYGFQPTNGVGYQPYRVVVLSFEL
LHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQ
FGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVL
YQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNN
SYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSV
AYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNL
LLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGG
FNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAA
RDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAA
LQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSS
TASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKV
EAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVL
GQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAI
CHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDV
VIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINAS
VVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIA
GLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVK
LHYT

[0270] ORF3a amino acid sequence is provided below as (SEQ ID NO:69):

MDLFMRIFTIGTVTLKQGEIKDATPSDFVRATATIPIQASLPFGWLI
VGVALLAVFQSASKIITLKKRWQLALSKGVHFVCNLLLLFVTVYSHL
LLVAAGLEAPFLYLYALVYFLQSINFVRIIMRLWLCWKCRSKNPLLY
DANYFLCWHTNCYDYCIPYNSVTSSIVITSGDGTTSPISEHDYQIGG
YTEKWESGVKDCVVLHSYFTSDYYQLYSTQLSTDTGVEHVTFFIYNK
IVDEPEEHVQIHTIDGSSGVVNPVMEPIYDEPTTTTSVPL

[0271] E amino acid sequence is provided below as (SEQ ID NO:70):

MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSL
VKPSFYVYSRVKNLNSSRVPDLLV

[0272] M amino acid sequence is provided below as (SEQ ID NO:71):

MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIK
LIFLWLLWPVTLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYFIASF
RLFARTRSMWSFNPETNILLNVPLHGTILTRPLLESELVIGAVILRGHLR
IAGHHLGRCDIKDLPKEITVATSRTLSYYKLGASQRVAGDSGFAAYSRYR
IGNYKLNTDHSSSSDNIALLVQ

[0273] ORF6 amino acid sequence is provided below as (SEQ ID NO:72):

MFHLVDFQVTIAEILLIIMRTFKVSIWNLDYIINLIIKNLSKSLTENKY
SQLDEEQPMEID

[0274] ORF7a amino acid sequence is provided below as (SEQ ID NO:73):

MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPL
ADNKFALTCFSTQFAFACPDGVKHVYQLRARSVSPKLFIRQEEVQELYS
PIFLIVAAIVFITLCFTLKRKTE

[0275] ORF7b amino acid sequence is provided below as (SEQ ID NO:74):

MIELSLIDFYLCFLAFLLFLVLIMLIIFWFSLELQDHNETCHA

[0276] ORF8 amino acid sequence is provided below as (SEQ ID NO:75):

MKFLVFLGIITTVAAFHQECSLQSCTQHQPYVVDDPCPIHFYSKWYIRV
GARKSAPLIELCVDEAGSKSPIQYIDIGNYTVSCLPFTINCQEPKLGSL
VVRCSFYEDFLEYHDVRVVLDFI

[0277] N amino acid sequence is provided below as (SEQ ID NO:76):

MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNT
ASWFTALTQHGKEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGD
GKMKDLSPRWYFYYLGTGPEAGLPYGANKDGIIWVATEGALNTPKDHIG
TRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRN
STPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQT
VTKKSAAEASKKPRQKRTATKAYNVTQAFGRRGPEQTQGNFGDQELIRQ
GTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYTGAIKLDDKD

-continued
PNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTV

TLLPAADLDDFSKQLQQSMSSADSTQA

[0278] ORF10 amino acid sequence is provided below as (SEQ ID NO:77):

MGYINVFAFPFTIYSLLLCRMNSRNYIAQVDVVNFNLT

[0279] or a polypeptide having at least 80%, 82%, 84%, 85%, 87%, 89%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99& % amino acid sequence identity thereto.

[0280] In one embodiment, a cold-adapted coronavirus has a mutation in one or more of genes for Nsp1, Nsp2, Nsp3, Nsp6, Nsp13, Nsp14, Nsp15, ORF6, ORF7b, ORF8, or any combination thereof. In one embodiment, a cold-adapted coronavirus has a Nsp1, Nsp2, Nsp3, Nsp6, Nsp13, Nsp14, Nsp15, polypeptide encoded by ORF6, with at least 80%, 82%, 84%, 85%, 87%, 89%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99& % amino acid sequence identity to one of SEQ ID Nos. 20-22, 25, 33-34, 72, 74, or 75.

[0281] The similarity between amino acid or nucleic acid sequences is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are. Homologs or variants of a given gene or protein will possess a relatively high degree of sequence identity when aligned using standard methods. Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, Adv. Appl. Math. 2:482, 1981; Needleman and Wunsch, J. Mol. Biol. 48:443, 1970; Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85:2444, 1988; Higgins and Sharp, Gene 73:237-244, 1988; Higgins and Sharp, CABIOS 5:151-153, 1989; Corpet et al., Nucleic Acids' Research 16:10881-10890, 1988; and Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85:2444, 1988. Altschul et al., Nature Genet. 6:119-129, 1994. The NCBI Basic Local Alignment Search Tool (BLAST™) (Altschul et al., J. Mol. Biol. 215:403-410, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx.

[0282] Sequence identity between nucleic acid sequences, or between amino acid sequences, can be determined by comparing an alignment of the sequences. When an equivalent position in the compared sequences is occupied by the same nucleotide, or amino acid, then the molecules are identical at that position. Scoring an alignment as a percentage of identity is a function of the number of identical nucleotides or amino acids at positions shared by the compared sequences. When comparing sequences, optimal alignments may require gaps to be introduced into one or more of the sequences to take into consideration possible insertions and deletions in the sequences. Sequence comparison methods may employ gap penalties so that, for the same number of identical molecules in sequences being compared, a sequence alignment with as few gaps as possible, reflecting higher relatedness between the two compared sequences, will achieve a higher score than one with many gaps. Calculation of maximum percent identity involves the production of an optimal alignment, taking into consideration gap penalties.

[0283] Suitable computer programs for carrying out sequence comparisons are widely available in the commercial and public sector. Examples include MatGat (Campanella et al., 2003, BMC Bioinformatics 4:29; program available from bitincka.com/ledion/matgat), Gap (Needleman & Wunsch, 1970, J. Mol. Biol. 48:443-453), FASTA (Altschul et al., 1990, J. Mol. Biol. 215:403-410; program available from ebi.ac.uk/fasta), Clustal W 2.0 and X 2.0 (Larkin et al., 2007, Bioinformatics 23:2947-2948; program available from ebi.uk/tools/clustalw2) and EMBOSS Pairwise Alignment Algorithms (Needleman & Wunsch, 1970, supra; Kruskal, 1983, In: Time warps, string edits and macromolecules: the theory and practice of sequence comparison, Sankoff & Kruskal (eds), pp 1-44, Addison Wesley; programs available from ebi.ac.uk/tools/emboss/align. All programs may be run using default parameters.

[0284] For example, sequence comparisons may be undertaken using the "needle" method of the EMBOSS Pairwise Alignment Algorithms, which determines an optimum alignment (including gaps) of two sequences when considered over their entire length and provides a percentage identity score. Default parameters for amino acid sequence comparisons ("Protein Molecule" option) may be Gap Extend penalty: 0.5, Gap Open penalty: 10.0, Matrix: Blosum 62.

[0285] The sequence comparison may be performed over the full length of the reference sequence.

[0286] Sequences described herein include reference to an amino acid sequence comprising an amino acid residue "at a position corresponding to an amino acid residue position" of another sequence. Such corresponding positions may be identified, for example, from an alignment of the sequences using a sequence alignment method described herein, or another sequence alignment method known to the person of ordinary skill in the art.

[0287] The subject for the vaccine may be any animal, including a human and non-human animals. Non-human animals include all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dogs, cats, cows, horses, chickens, amphibians, and reptiles, e.g., mammals, such as humans or non-human primates, sheep, dogs, cats, cows and horses. The subject may also be livestock such as, cattle, swine, sheep, poultry, and horses, or pets, such as dogs and cats.

[0288] The methods described herein can be employed for subjects of any species, gender, age, ethnic population, or genotype. Accordingly, the term subject includes males and females, and it includes elderly, elderly-to-adult transition age subjects adults, adult-to-pre-adult transition age subjects, and pre-adults, including adolescents, children, and infants.

[0289] Examples of human ethnic populations include Caucasians, Asians, Hispanics, Africans, African Americans, Native Americans, Semites, and Pacific Islanders. The methods may be more appropriate for some ethnic populations such as Caucasians, especially northern European populations, as well as Asian populations.

[0290] The term subject also includes subjects of any genotype or phenotype as long as they are in need of the compositions, as described above. In addition, the subject can have the genotype or phenotype for any hair color, eye color, skin color or any combination thereof.

[0291] The term subject includes a subject of any body height, body weight, or any organ or body part size or shape.

[0292] The invention will be further described by the following non-limiting examples.

EXAMPLES

[0293] The invention will be described by the following non-limiting examples.

Example 1: Identification of Mutations Associated with Attenuation and Temperature-Sensitive, Cold-Adaption of Live Attenuated Coronavirus

[0294] A clinical isolate of wild-type SARS-Cov-2/UT-HPCo038/human/2020/Tokyo (FIG. **80**, SEQ ID NO:78) was passaged 15 times at 37° C. in Vero cells. After cloning the virus by plaque purification, cold adaptation was initiated at progressively lower temperatures from 35° C. to 25° C. with the last 3 passages at 25° C. (FIGS. **1** and **2**). When viruses grew well in Vero cells at 25° C., plaque purification was performed again to obtain cold-adapted coronavirus. The cold-adapted coronavirus replicated well at 25° C. and 32° C. but failed to propagate at 37° C. and 39° C. compared to the wild-type virus.

[0295] The cells are cultured at 30° C. for 1 day before infection. To demonstrate attenuation, golden Syrian hamsters were infected with the parent virus (before passaging) and the cold-adapted virus after 15 passages. Syrian hamsters are highly susceptible to SARS-CoV-2 infection and present with pathological phenotypes similar to those of infected humans; therefore, Syrian hamsters are a model to evaluate the attenuation of cold-adapted SARS-CoV-2.

[0296] To test the pathogenicity of the cold-adapted coronavirus, hamsters were intranasally inoculated with 10^3 PFU of cold-adapted coronavirus (FIG. **3**). Body weight and respiratory function were monitored for 8-9 days post-infection. Lung and nasal titers were measured 3 days after infection. Micro-CT analysis was performed at 7 days post-infection.

[0297] The body weight of hamsters infected with cold-adapted coronavirus gradually increased, similar to PBS controls, whereas that of hamsters infected with wild-type virus was significantly reduced (FIG. **4**). Respiratory function was tested by whole-body plethysmography (WBP) before infection and at 1-, 3-, 5-, and 8-days post infection (dpi). Whole body plethysmography (WBP) showed that the cold-adapted coronavirus did not cause a decrease in respiratory function based on Penh and Rpef (FIG. **5**). Penh: Index that combines the box pressure signals from both inspiration (PIF) and expiration (PEF), with the timing comparison of early and late expiration (PAU). Penh=PAU* (PEF/PIF). Rpef: Ratio of time required to reach the maximum expiratory flow (PEF) relative to time of expiration (Te).

[0298] Hamsters infected with wild-type virus exhibited a decline in respiratory function relative to the cold-adapted coronavirus. Four days after infection, the amount of virus in the lungs was determined. Virus titers in the lungs of hamsters infected with cold-adapted coronavirus were significantly reduced compared with those of hamsters infected with wild-type virus; however, virus titers in the nasal turbinates were similar for cold-adapted coronavirus- and wild-type virus-infected hamsters (FIG. **6**). Computed tomography (CT) scans of the lungs of hamsters administered the phosphate buffered saline control, cold-adapted coronavirus, and wild-type virus were performed (FIG. **7**). No significant inflammatory findings were detected by micro-CT analysis in the lungs of hamsters infected with the cold-adapted coronavirus. Wild-type virus caused emphysema and extensive inflammation in the lungs of infected hamsters.

[0299] Serological assays were performed on the cold-adapted coronavirus and wild-type inoculated hamsters for detecting neutralizing antibodies against SARS-CoV-2 (FIG. **8**). Both seed and wild-type virus induced neutralizing antibodies against wild-type, beta, gamma, delta and BA. 1 variants in infected hamsters.

[0300] Given the attenuation in hamsters, this cold-adapted version of SARS-CoV-2 may be used as a vaccine candidate.

[0301] ORF1ab-Y1090N was the most important mutation for attenuation in the hamster. ORF1ab-M85del also contributed to the attenuation.

[0302] In one embodiment, the cold-adapted virus has at least one of Nsp1-M85del, nsp3 (PLpro)-Y272N, nsp6-L260F, S-V1094F.

TABLE 1

	nsp1	nsp2	nsp2	nsp3 (PLpro)	nsp3 (PLpro)	nsp6	nsp13 (Helicase)
Nucleotide change compared with parental virus	del517-519	c1342t	g1495t	t3533a	c4301t	c11750t	c16575t
Amino acid change	⊿M85	—	—	Y272N	L528F	L260F	—
	nsp14(3'-5' exonuclease)	nsp15 (endoRNase)	S	S	S	ORF6	ORF7b-ORF8
Nucleotide change compared with parental virus	g19117t	a20318c	t21752a	a22320g	g24842t	del27209-27266	del27816-28244

TABLE 1-continued

Amino acid change	A360S	E233A	W64R	D253G	V1094F	C-terminus deleted ORF6	Deletion of ORF8 and ORF7b

Example 2: Challenge of Vaccine Virus Immunized Hamsters with Wild-Type Wuhan-Like (SEQ ID NO: 5) Virus

[0303] The cold-adapted coronavirus produced as described in Example 1 from the wild-type SARS-Cov-2/UT-HPCo038/human/2020/Tokyo (FIG. **80**, SEQ ID NO: 78) was used to immunize hamsters by intranasal inoculation. The immunized hamsters were intranasally challenged with wild-type virus at 10^5 PFU (FIG. **9**). Body weight and respiratory function were monitored for 7 days post-infection. Lung and nasal titers were measured 3 days after infection. Micro-CT analysis was performed at 7 days post-infection.

[0304] Body weight loss of cold-adapted coronavirus-immunized hamsters was limited, whereas PBS-administrated hamsters showed significant body weight loss after challenge infection (FIG. **10**). Whole body plethysmography (WBP) showed that cold-adapted coronavirus-immunized hamsters had no reduction in respiratory function in terms of either Penh or Rpef. In contrast, respiratory function was impaired in PBS-treated hamsters (FIG. **11**). Virus titers in the lungs and nasal turbinates of cold-adapted coronavirus-immunized hamsters were significantly reduced compared with those of the PBS group at 3 and 7 days post-infection (FIG. **12**). In hamsters immunized with wild-type or vaccine (seed) virus, virus titers were detected in nasal turbinates at about 99.9% less than in unimmunized hamsters and undetected in lung on day 3. By day 7, virus was undetectable in the immunized hamsters. CT scans of lungs of each group are shown in FIG. **13**, which showed no significant inflammatory findings as detected by micro-CT analysis in the lungs of cold-adapted coronavirus-immunized hamsters. Extensive inflammation in the lungs of PBS-administrated hamsters was observed.

Example 3: Challenge of Vaccine Virus Immunized Hamsters with Wild-Type Delta Virus

[0305] The cold-adapted coronavirus produced as described in Example 1 from the wild-type SARS-Cov-2/UT-HPCo038/human/2020/Tokyo (FIG. **80**, SEQ ID NO: 78) was used to immunize hamsters by intranasal inoculation (FIG. **14**). Hamsters immunized with the cold-adapted coronavirus were intranasally challenged with wild-type virus at 10^5 PFU. Body weight and respiratory function were monitored for 7 days post-infection. Lung and nasal titers were measured 3 days after infection. Micro-CT analysis was performed at 7 days post-infection.

[0306] Body weight loss of cold-adapted coronavirus-immunized hamsters was limited, whereas PBS-administrated hamsters showed significant body weight loss after challenge infection (FIG. **15**). Whole body plethysmography (WBP) showed that cold-adapted coronavirus-immunized hamsters had no reduction in respiratory function in terms of either Penh or Rpef. In contrast, respiratory function was impaired in PBS-treated hamsters (FIG. **16**). Virus titers in the lungs and nasal turbinates of cold-adapted coronavirus-immunized hamsters were significantly reduced compared with those of the PBS group at 3 and 7 days after infection (FIG. **17**). In hamsters immunized with wild-type or vaccine (seed) virus, virus titers were detected in NT at about 99% less than in unimmunized hamsters and undetected in lung on day 3 with the exception of one hamster. By day 7, virus was undetectable in the immunized hamsters in either NT or lung. CT scans of lungs of each group are shown in FIG. **18**, which showed no significant inflammatory findings were detected by micro-CT analysis in the lungs of cold-adapted coronavirus-immunized hamsters. Extensive inflammation in the lungs of PBS-administrated hamsters was observed.

Example 4: Passage of Cold-Adapted Coronavirus Through Hamster Nasal Turbinate

[0307] To test the in vivo stability of the attenuated cold-adapted coronavirus, the cold-adapted coronavirus was passaged in the nasal turbinates 3 times or it was isolated from the lungs of hamsters 3-5 days after infection and then tested for cold adaptation, temperature sensitivity, and pathogenicity in hamsters (FIG. **19**). Three lineages (Line A, Line B, and Line C) of passaged virus were isolated from lungs at days 3-, 4-, and 5-days post infection. When the cold-adapted coronavirus was passaged in triplicates in the nasal turbinate of hamsters, the virus titers in the nasal turbinate did not change during the 3 passages (FIG. **20**). Three lines of cold-adapted coronaviruses passaged 3 times in the nasal turbinates showed similar growth kinetics at 25, 32, 37, and 39° C. to those of the unpassaged cold-adapted coronavirus (FIG. **21**).

[0308] To test the pathogenicity of Line A, Line B, or Line C of passaged virus, hamsters were intranasally inoculated with 10^3 PFU of passaged viruses. Body weight and respiratory function were monitored for 7 days post-infection. Lung and nasal titers were measured 3 days after infection. Micro-CT analysis was performed at 7 days post-infection (FIG. **22**). Body weight change, respiratory function for Penh and Rpef, micro-CT analysis, and virus titers in the nasal turbinates and lungs of hamsters showed that the three lines of passaged virus maintained the attenuated phenotype in the hamsters (FIGS. **23**A and **23**B). CT scans of lungs of each group are shown in FIG. **23**C. Virus titer in nasal turbinates (NT) and lungs is shown in FIG. **23**D. The vaccine virus is shown to remain attenuated after 3 passages in NT.

[0309] At 5 days post-infection, cold-adapted coronavirus was detected in the lungs of hamsters. Among these 5 samples, cold-adapted coronavirus was isolated from two samples (#3 and #5) (FIG. **24**). The two viruses (#3 and #5) showed similar growth kinetics at 25, 32, 37, and 39° C. to those of the cold-adapted coronavirus (FIG. **25**).

[0310] To test the pathogenicity of the two isolated viruses, hamsters were intranasally inoculated with 10^3 PFU of the viruses. Body weight and respiratory function were monitored for 7 days post-infection. Lung and nasal titers were measured 3 days after infection. Micro-CT analysis was performed at 7 days post-infection (FIG. **26**). Body weight and respiratory function were monitored for 7 days

post infection. Lung and nasal titers were measured 3 days after infection. Micro-CT analysis was performed at 7 days post infection. As shown in FIGS. **27**A-**27**D, body weight change (FIG. **27**A), respiratory function for Penh and Rpef (FIG. **27**B), micro-CT analysis (FIG. **27**C), and virus titers in the nasal turbinates and lungs of hamsters (FIG. **27**D) showed that the two isolated viruses maintained the attenuated phenotype in the hamsters.

[0311] To test the in vitro stability of the cold-adapted coronavirus for the attenuation property, the cold-adapted coronavirus was passaged in Vero cells 5 times at 32, 30, and 27.5° C. at a multiplicity of infection (MOI) of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, or 10^{-6} (FIG. **28**A). When the cold-adapted coronavirus was passaged in +Vero cells at 32, 30, and 27.5° C., the virus titers ranged between ~10^7 and ~10^8 pfu/ml, except for the low MOI condition (10^{-4} and 10^{-5}) at 27.5° C. Three samples passaged at 30° C. and 32° C. at a lower MOI condition (surrounded by brown squares) were tested for cold adaptation, temperature sensitivity, and pathogenicity in hamsters (FIG. **28**B). To test the pathogenicity of the 3 passaged viruses, hamsters were intranasally inoculated with 10^3 PFU of passaged viruses. Body weight and respiratory function were monitored for 7 days post-infection. Lung and nasal titers were measured 3 days after infection. Micro-CT analysis was performed at 7 days post-infection (FIG. **28**C). Body weight change, respiratory function for Penh and Rpef, micro-CT analysis, and virus titers in the nasal turbinates and lungs of the hamsters showed that the three passaged viruses maintained the attenuated phenotype in the hamsters (FIG. **28**D).

Example 5: Determining the Amino Acid Changes that Contribute to Attenuation

[0312] To determine which amino acid changes contribute to the attenuation, cold-adaptation, and temperature-sensitivity, recombinant wild-type viruses possessing a candidate substitution (ORF1ab-M85del, ORF1ab-Y1090N, ORF1ab-L3829F, and S-V1094F) were generated by reverse genetics. ORF1ab-Y1090N contributed to the temperature sensitivity. ORF1ab-M85del, ORF1ab-Y1090N, ORF1ab-L3829F, and S-V1094F were important for cold-adaptation (FIG. **29**).

[0313] To test the pathogenicity of recombinant viruses, hamsters were intranasally inoculated with 10^5 PFU. Body weight and respiratory function were monitored for 7 days post infection. Micro-CT analysis was performed at 7 days post infection (FIG. **30**). ORF1ab-Y1090N and S-V1094F suppressed body weight loss of infected hamsters (FIG. **31**). In the assessment of respiratory function, ORF1ab-M85del and ORF1ab-Y1090N contributed to attenuation in hamsters (FIG. **32**). Micro-CT analysis showed that ORF1ab-M85del and ORF1ab-Y1090N caused attenuation in hamsters (FIG. **33**).

Example 6: Determining the Amino Acid Changes that Contribute to Attenuation

[0314] To test the attenuation of the cold-adapted coronavirus and induction of antibodies and cellular immunity, human ACE2-transgenic mice (strains AC70 and K18) were infected with the vaccine cold-adapted coronavirus at 10, 10^2, 10^3, or 10^4 PFU (FIG. **38**). Body weight change was measured for 2 weeks and virus titers in the nasal turbinate and lungs were determined at 3 days post-infection. At 3 weeks after infection, antibody titers and cellular immunity were detected by ELISA using the receptor-binding domain (RBD) of spike protein and ectodomain of spike protein, intracellular cytokine staining, and ELISPOT.

[0315] AC70 and K18 mice did not lose body weight or die after infection with the vaccine cold-adapted coronavirus at 10^2, 10^3, or 10^4 PFU. Wild-type virus caused body weight loss and the death of all or some of the AC70 and K18 mice at 10^2, 10^3, or 10^4 PFU infection (FIG. **39**). Vaccine cold-adapted coronavirus was not detected in the lungs of AC70 or K18 mice, whereas wild-type virus was detected in the lungs of both strains. In the nasal turbinate, virus titers of the vaccine cold-adapted coronavirus were similar to or lower than those of wild-type virus (FIG. **40**). Vaccine cold-adapted coronavirus induced antibodies against the receptor binding domain (RBD) and S protein at levels similar to those induced by wild-type virus (FIG. **41**).

Example 7: Th1 dependence of induced antibodies IgG1 and IgG2c

[0316] To evaluate the Th1/Th2 balance, the induced antibodies IgG1 and IgG2c were measured by ELISA. A high IgG2c antibody titer was detected, confirming that the immune response was Th1 dependent (FIG. **42**).

Example 8: Cellular Immunity

[0317] To evaluate the cellular immunity, splenocytes collected from K18-mice infected with the vaccine cold-adapted coronavirus were stimulated with the spike protein peptide pool and ELISPOT and intracellular cytokine straining were performed at 3 weeks after infection (FIG. **43**). IFNγ- and TNFα-positive CD4 and CD8 T cells were detected in the splenocytes of vaccine cold-adapted coronavirus (FIG. **44**). Similarly, ELISPOT showed that cellular immunity against the spike protein was induced in K18-mice after vaccine cold-adapted coronavirus infection (FIG. **45**).

Example 9: Challenge of Cold-Adapted Coronavirus Immunized Hamsters with Wild Type Omicron Variant BA.1 Virus

[0318] Hamsters immunized with the cold-adapted coronavirus were intranasally challenged with wild-type omicron variant BA.1 virus at 10^5 PFU (FIG. **46**). Body weight and respiratory function were monitored for 7 days post-infection. Lung and nasal titers were measured 3 days after infection. Micro-CT analysis was performed at 7 days post-infection. Body weight loss of cold-adapted coronavirus-immunized hamsters was limited after BA.1 infection (FIG. **47**).

[0319] Whole body plethysmography (WBP) showed that cold-adapted coronavirus-immunized hamsters challenged with BA.1 showed no reduction in respiratory function in terms of either Penh or Rpef (FIG. **48**). Virus titers in the lungs and nasal turbinates of cold-adapted coronavirus-immunized hamsters after challenge infection were significantly reduced compared with those of the PBS group at 3 days after infection (FIG. **49**). No significant inflammatory findings were detected by micro-CT analysis in the lungs of cold-adapted coronavirus-immunized and PBS-administrated hamsters after BA.1 infection (FIG. **50**).

Example 10: Determining the Amino Acid Changes that Contribute to Attenuation in Cold-Adapted Coronaviruses Possessing an Amino Acid Substitution

[0320] To determine which amino acid changes contribute to the attenuation, cold-adaptation, and temperature sensitivity, recombinant vaccine cold-adapted coronaviruses possessing a substitution (nsp1-M85ins, nsp3-N272Y, nsp6-F260L, and S-F1094V) were generated by reverse genetics (FIG. **51**). These recombinant viruses were inoculated into Vero cells and virus growth was examined at 25, 32, and 37° C. (FIGS. **52** and **53**). The growth of the reverse engineered vaccine cold-adapted coronavirus and wild type virus at 25, 32, and 37° C. is shown in FIG. **54**. The mutant cold-adapted coronaviruses nsp1-M85ins and nsp3-N272Y correlate with temperature sensitivity (FIG. **55**). The mutant cold-adapted coronaviruses with nsp6-F260L and S-F1094V mutations correlate with cold-adaptation (FIG. **56**).

[0321] To test the pathogenicity of the recombinant viruses, hamsters were intranasally inoculated with 10^5 PFU (FIG. **57**). Body weight (FIG. **58**) and respiratory function (FIG. **59**) were monitored for 7 days post-infection. Virus titers were determined at 3 days post-infection (FIG. **60**). The mutant cold-adapted coronaviruses nsp1-M85ins and nsp3-N272Y correlate with attenuation. All four substitutions (nsp1-M85ins, nsp3-N272Y, nsp6-F260L, and S-F1094V) contributed to the attenuation of the cold-adapted coronavirus (FIGS. **61**A-**61**B).

Example 11

[0322] To investigate whether the vaccine cold-adapted coronavirus works as a vaccine backbone, the S gene of the vaccine cold-adapted coronavirus was replaced with the S gene of XBB.1.5 (FIG. **62**A). The genome of the vaccine cold-adapted coronavirus with the substitution of the S gene from XBB.1.5 is shown in FIG. **62**B.

[0323] In addition to the virus shown in FIG. **62**A, two vaccine cold-adapted coronaviruses possessing the S gene of XBB.1.5 harboring the V1094F substitution or the W64R, D253G, and V1094F substitutions were prepared by reverse genetics and their replication in Vero cells was examined at 25, 32, and 37° C. (FIG. **63**). The three viruses possessing the XBB. 1.5 S gene showed similar growth kinetics to those of the vaccine cold-adapted coronavirus (LAV) (FIG. **64**).

[0324] To test the pathogenicity of the three viruses possessing the XBB.1.5 S gene, hamsters were intranasally inoculated with 10^3 PFU (FIG. **65**). Body weight and respiratory function were monitored for 7 days post-infection. Virus titers were determined at 3 and 7 days post-infection. The three viruses possessing the XBB.1.5 S gene did not cause body weight loss (FIG. **66**). The three viruses possessing the XBB.1.5 S gene impaired respiratory functions (FIG. **67**). Virus titers in the lungs of hamsters infected with the three viruses possessing the XBB.1.5 S gene were similar to those of the vaccine cold-adapted coronavirus (LAV) (FIG. **68**).

Example 12

[0325] To test the boost immunization capability of the vaccine cold-adapted coronavirus, hamsters were immunized with the mRNA vaccine once (FIG. **69**A) or twice (FIG. **69**B) at 0.3 or 3 μg per shot before infection with the vaccine cold-adapted coronavirus. Antibody titers against the RBD were measured pre- and post-boost immunization with the vaccine cold-adapted coronavirus. Antibody titers increased after the boost immunization with the vaccine cold-adapted coronavirus, even when antibodies to the RBD were present prior to the boost immunization (FIG. **70**).

[0326] To test attenuation in non-human primates, macaques were inoculated with the vaccine cold-adapted coronavirus and virus loads in nasal, oral, and rectal swabs and body weight were assessed. At 7 days post-infection, virus RNA titers in organs were determined by RT-qPCR (FIG. **71**). Neither the wild-type nor the vaccine cold-adapted coronavirus caused body weight loss (FIG. **72**). Vaccine cold-adapted coronavirus loads in the nasal, oral, and rectal swabs were lower than the wild-type virus loads (FIG. **73**). Vaccine cold-adapted coronavirus RNA was not detected in any organ tested, whereas wild-type virus RNA was detected in the olfactory bulb, turbinates, ileums, and trachea (FIG. **74**).

[0327] Macaques immunized with the vaccine cold-adapted coronavirus were intranasally and intratracheally challenged with a delta variant at 10^6 $TCID_{50}$ (FIG. **75**). Virus loads in nasal, oral, and rectal swabs and body weight were assessed. At 8 days post-infection, virus RNA titers in organs were determined by RT-qPCR. The delta variant did not cause body weight loss in either the immunized or mock macaques (FIG. **76**). The virus loads of the challenge virus in the nasal, oral, and rectal swabs of the immunized macaques were lower than those of the mock animals (FIG. **77**). Challenge virus RNA levels in the organs of the immunized macaques were significantly lower than those in the organs of the mock animals (FIG. **78**).

Statements

[0328] 1. An isolated cold-adapted SARS-CoV-2 virus that has one or more mutations in one or more open reading frames that encode a non-structural protein for Nsp1, Nsp2, Nsp3, Nsp6, Nsp13, Nsp14, Nsp15, S, ORF6, ORF7b, ORF8, or any combination thereof, relative to a parental virus that is not cold-adapted.

[0329] 2. The isolated cold-adapted SARS-CoV-2 virus of statement 1, wherein the one or more mutations includes:

- **[0330]** a deletion of a codon for the amino acid at position 85 in Nsp1;
- **[0331]** a nucleotide substitution in the gene for Nsp2 at position 1341 or 1495, or
  - **[0332]** both, and wherein the substitution is optionally a silent substitution;
- **[0333]** an amino acid substitution in Nsp3 at position 272 or 528, or both; or
- **[0334]** a combination thereof.

[0335] 3. The isolated cold-adapted SARS-CoV-2 virus of statement 2, wherein the amino acid substitution in Nsp3 comprises:

- **[0336]** an amino acid substitution at position 272 that is not Y;
- **[0337]** an amino acid substitution at position 272 is N, D, E or Q;
- **[0338]** an amino acid substitution at position 528 is not L;
- **[0339]** an amino acid substitution at position 528 is F, W or Y; or
- **[0340]** a combination thereof.

[0341] 4. The isolated cold-adapted coronavirus of any one of statements 1 to 3, wherein the one or more mutations

include an amino acid substitution in Nsp6 at position 260, wherein the amino acid substitution is not L or the amino acid substitution is F, W or Y.

[0342] 5. The isolated cold-adapted coronavirus of any one of statements 1 to 4, wherein the one or more mutations include a nucleotide substitution in the gene for Nsp13 at position 16575, which is optionally a silent substitution, or wherein the one or more mutations include an amino acid substitution in Nsp14 at position 360, or a combination thereof.

[0343] 6. The isolated cold-adapted coronavirus of statement 7, wherein the amino acid substitution in Nsp14 at position 360 is not A or the amino acid substitution is S, T, L, I or M.

[0344] 7. The isolated cold-adapted coronavirus of any one of statements 1 to 6, wherein the one or more mutations include an amino acid substitution in Nsp15 at position 233, wherein the amino acid at position 233 is not E or the amino acid at position 233 is A, I, L, G, S, or T.

[0345] 8. The isolated cold-adapted coronavirus of any one of statements 1 to 7, wherein the one or more mutations include an amino acid substitution in S at position 64, 235, 1094, or a combination thereof, wherein the amino acid substitution in S at position 64 is not W, position 235 is not D, or position 1094 is not V or wherein the amino acid substitution in S at position 64 is R, H or K; position 235 is G, A, L, I, T or S; or position 1094 is F, Y or W.

[0346] 9. The isolated cold-adapted coronavirus of any one of statements 1 to 8, wherein the one or more mutations include a deletion of the C-terminus of a polypeptide encoded by ORF6, which deletion optionally inhibits interferon antagonism.

[0347] 10. The isolated cold-adapted coronavirus of any one of statements 1 to 9, wherein the one or more mutations include a deletion of ORF7b and/or ORF8 genes, which optionally results in a deletion in nucleotides from position 27816 to position 28244 in a nucleic acid sequence of the parental virus, which yields a portion of ORF7b protein or the inability to encode a protein from ORF8 or both.

[0348] 11. A method of making a cold-adapted SARS-CoV-2 virus comprising modifying a genome thereof to include one or more mutations in one or more open reading frames that encode for a non-structural protein for Nsp1, Nsp2, Nsp3, Nsp6, Nsp13, Nsp14, Nsp15, S, ORF6, ORF7b, ORF8, or any combination thereof, relative to a parental virus that is not cold-adapted.

[0349] 12. The method of statement 11, wherein the one or more mutations include: a deletion of a codon for the amino acid at position 85 in Nsp1;

[0350] a nucleotide substitution in the gene for Nsp2 at position 1341 or 1495, or both;

[0351] an amino acid substitution in Nsp3 at position 272 or 528, or both; or a combination thereof.

[0352] 13. The method of statement 12, wherein the amino acid substitution in Nsp3 at position 272 is not Y or the amino acid substitution is N, D, E or Q; the amino acid substitution in Nsp3 at position 528 is not L or the amino acid substitution is F, W or Y; or a combination thereof.

[0353] 14. The method of any one of statements 11 to 13, wherein the one or more mutations include an amino acid substitution in Nsp6 at position 260 is not L or the amino acid substitution is F, W or Y; the amino acid substitution in Nsp14 at position 360 is not A or the amino acid substitution is S, T, L, I or M; the amino acid substitution in Nsp15 at position 233 is not E or the substitution is A, I, L, G, S, or T; or a combination thereof.

[0354] 15. The method of any one of statements 11 to 14, wherein the one or more mutations include a nucleotide substitution in the gene for Nsp13 at position 16575.

[0355] 16. The method of any one of statements 11 to 15, wherein the one or more mutations include an amino acid substitution in S at position 64, 235, 1094, or a combination thereof, wherein the amino acid substitution at position 64 is not W, position 235 is not D, or position 1094 is not V, or a combination thereof, or wherein the amino acid substitution at position 64 is R, H or K, position 235 is G, A, L, I, T or S, position 1094 is F, Y or W, or a combination thereof.

[0356] 17. The method of any one of statements 11 to 16, wherein the one or more mutations include a deletion of the C-terminus of polypeptide encoded by ORF6, a deletion of ORF7b, a deletion of ORF8, or a combination thereof.

[0357] 18. A method to immunize an animal, comprising administering to the animal a composition comprising the cold-adapted coronavirus of any one of statements 1 to 10.

[0358] 19. The method of statement 18, wherein the animal is a mammal.

[0359] 20. The method of statement 22, wherein the mammal is a human.

[0360] 21. The method of any one of statements 18-20, wherein the composition is injected, systemically administered, or intranasally administered.

[0361] 22. A pharmaceutical composition comprising the cold-adapted coronavirus of any one of statements 1 to 10.

[0362] 23. The pharmaceutical composition of statement 22 further comprising a pharmaceutically acceptable carrier, a different immunogen, a different virus, or a combination thereof.

[0363] 24. The pharmaceutical composition of statement 23, wherein the different virus is not a coronavirus or the different virus is an influenza virus or both.

[0364] The specific methods, devices and compositions described herein are representative of example embodiments and not intended as limitations on the scope of the technology. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification and are encompassed within the spirit of the technology as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the technology disclosed herein without departing from the scope and spirit of the technology.

[0365] The technology illustratively described herein suitably can be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. The methods and processes illustratively described herein suitably can be practiced in differing orders of steps, and the methods and processes are not necessarily restricted to the orders of steps indicated herein or in the claims.

[0366] Under no circumstances can the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances can the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such

statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

[0367] The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the technology as claimed. Thus, it will be understood that although the present technology has been specifically disclosed by example embodiments and optional features, modification and variation of the concepts herein disclosed can be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this technology as defined by the appended claims and statements of the technology.

[0368] The technology has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the technology. This includes the generic description of the technology with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein. In addition, where features or aspects of the technology are described in terms of Markush groups, those skilled in the art will recognize that the technology is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0369] All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification, this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details herein may be varied considerably without departing from the basic principles of the invention.

SEQUENCE LISTING

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gatgccaact attttctttg ctggcatact aattgttacg actattgtat accttacaat  25800
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aacaacattg ccaaaaggct tctacgcaga agggagcaga ggcggcagtc aagcctcttc  28740
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aacttctcct gctagaatgg ctggcaatgg cggtgatgct gctcttgctt tgctgctgct  28860
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aactgtcact aagaaatctg ctgctgaggc ttctaagaag cctcggcaaa aacgtactgc  28980
cactaaagca tacaatgtaa cacaagcttt cggcagacgt ggtccagaac aaacccaagg  29040
aaattttggg gaccaggaac taatcagaca aggaactgat tacaaacatt ggccgcaaat  29100
tgcacaattt gccccccagcg cttcagcgtt cttcggaatg tcgcgcattg gcatggaagt  29160
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accaacagag cctaaaaagg acaaaaagaa gaaggctgat gaaactcaag ccttaccgca  29340
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caaacaattg caacaatcca tgagccgtgc tgactcaact caggcctaaa ctcatgcaga  29460
ccacacaagg cagatgggct atataaacgt tttcgctttt ccgtttacga tatatagtct  29520
actcttgtgc agaatgaatt ttcgtaacta catagcacaa gtagatgtag ttaactttaa  29580
tctcacatag caatctttaa tcagtgtgta acattaggga ggacttgaaa gagccaccac  29640
attttcacct acagtgaaca atgctaggga gagctgccta tatggaagag ccctaatgtg  29700
taaaattaat tttagtagtg ctatccc                                      29727

SEQ ID NO: 7          moltype =   length =
SEQUENCE: 7
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SEQ ID NO: 8          moltype =   length =
SEQUENCE: 8
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SEQ ID NO: 9          moltype =   length =
SEQUENCE: 9
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SEQ ID NO: 10         moltype =   length =
SEQUENCE: 10
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SEQ ID NO: 11         moltype =   length =
SEQUENCE: 11
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SEQ ID NO: 12         moltype =   length =
SEQUENCE: 12
000

SEQ ID NO: 13         moltype =   length =
SEQUENCE: 13
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SEQ ID NO: 14         moltype =   length =
SEQUENCE: 14
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SEQ ID NO: 15         moltype =   length =
SEQUENCE: 15
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SEQ ID NO: 16         moltype =   length =
SEQUENCE: 16
000

SEQ ID NO: 17         moltype =   length =
SEQUENCE: 17
000

SEQ ID NO: 18         moltype =   length =
SEQUENCE: 18
000

SEQ ID NO: 19         moltype =   length =
SEQUENCE: 19
000

SEQ ID NO: 20         moltype = AA  length = 180
FEATURE               Location/Qualifiers
source                1..180
                      mol_type = protein
                      organism = Coronavirus 2
SEQUENCE: 20
MESLVPGFNE KTHVQLSLPV LQVRDVLVRG FGDSVEEVLS EARQHLKDGT CGLVEVEKGV  60
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LPQLEQPYVF IKRSDARTAP HGHVMVELVA ELEGIQYGRS GETLGVLVPH VGEIPVAYRK  120
VLLRKNGNKG AGGHSYGADL KSFDLGDELG TDPYEDFQEN WNTKHSSGVT RELMRELNGG  180

SEQ ID NO: 21          moltype = AA  length = 638
FEATURE                Location/Qualifiers
source                 1..638
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 21
AYTRYVDNNF CGPDGYPLEC IKDLLARAGK ASCTLSEQLD FIDTKRGVYC CREHEHEIAW  60
YTERSEKSYE LQTPFEIKLA KKFDTFNGEC PNFVFPLNSI IKTIQPRVEK KKLDGFMGRI  120
RSVYPVASPN ECNQMCLSTL MKCDHCGETS WQTGDFVKAT CEFCGTENLT KEGATTCGYL  180
PQNAVVKIYC PACHNSEVGP EHSLAEYHNE SGLKTILRKG GRTIAFGGCV FSYVGCHNKC  240
AYWVPRASAN IGCNHTGVVG EGSEGLNDNL LEILQKEKVN INIVGDFKLN EEIAIILASF  300
SASTSAFVET VKGLDYKAFK QIVESCGNFK VTKGKAKKGA WNIGEQKSIL SPLYAFASEA  360
ARVVRSIFSR TLETAQNSVR VLQKAAITIL DGISQYSLRL IDAMMFTSDL ATNNLVVMAY  420
ITGGVVQLTS QWLTNIFGTV YEKLKPVLDW LEEKFKEGVE FLRDGWEIVK FISTCACEIV  480
GGQIVTCAKE IKESVQTFFK LVNKFLALCA DSIIIGGAKL KALNLGETFV THSKGLYRKC  540
VKSREETGLL MPLKAPKEII FLEGETLPTE VLTEEVVLKT GDLQPLEQPT SEAVEAPLVG  600
TPVCINGLML LEIKDTEKYC ALAPNMMVTN NTFTLKGG                          638

SEQ ID NO: 22          moltype = AA  length = 1945
FEATURE                Location/Qualifiers
source                 1..1945
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 22
APTKVTFGDD TVIEVQGYKS VNITFELDER IDKVLNEKCS AYTVELGTEV NEFACVVADA  60
VIKTLQPVSE LLTPLGIDLD EWSMATYYLF DESGEFKLAS HMYCSFYPPD EDEEEGDCEE  120
EEFEPSTQYE YGTEDDYQGK PLEFGATSAA LQPEEEQEED WLDDDSQQTV GQQDGSEDNQ  180
TTTIQTIVEV QPQLEMELTP VVQTIEVNSF SGYLKLTDNV YIKNADIVEE AKKVKPTVVV  240
NAANVYLKHG GGVAGALNKA TNNAMQVESD DYIATNGPLK VGGSCVLSGH NLAKHCLHVV  300
GPNVNKGEDI QLLKSAYENF NQHEVLLAPL LSAGIFGADP IHSLRVCVDT VRTNVYLAVF  360
DKNLYDKLVS SFLEMKSEKQ VEQKIAEIPK EEVKPFITES KPSVEQRKQD DKKIKACVEE  420
VTTTLEETKF LTENLLLYID INGNLHPDSA TLVSDIDITF LKKDAPYIVG DVVQEGVLTA  480
VVIPTKKAGG TTEMLAKALR KVPTDNYITT YPGQGLNGYT VEEAKTVLKK CKSAFYILPS  540
IIPNEKQEIL GTVSWNLREM LAHAEETRKL MPVCVETKAI VSTIQRKYKG IKIQEGVVDY  600
GARFYFYTSK TTVASLINTL NDLNETLVTM PLGYVTHGLN LEEAARYMRS LKVPATVSVS  660
SPDAVTAYNG YLTSSSKTPE EHFIETISLA GSYKDWSYSG QSTQLGIEFL KRGDKSVYYT  720
SNPTTFHLDG EVITFDNLKT LLSLREVRTI KVFTTVDNIN LHTQVVDMSM TYGQQFGPTY  780
LDGADVTKIK PHNSHEGKTF YVLPNDDTLR VEAFEYYHTT DPSFLGRYMS ALNHTKKWKY  840
PQVNGLTSIK WADNNCYLAT ALLTLQQIEL KFNPPALQDA YYRARAGEAA NFCALILAYC  900
NKTVGELGDV RETMSYLFQH ANLDSCKRVL NVVCKTCGQQ QTTLKGVEAV MYMGTLSYEQ  960
FKKGVQIPCT CGKQATKYLV QQESPFVMMS APPAQYELKH GTFTCASEYT GNYQCGHYKH  1020
ITSKETLYCI DGALLTKSSE YKGPITDVFY KENSYTTTIK PVTYKLDGVV CTEIDPKLDN  1080
YYKKDNSYFT EQPIDLVPNQ PYPNASFDNF KFVCDNIKFA DDLNQLTGYK KPASRELKVT  1140
FFPDLNGDVV AIDYKHYTPS FKKGAKLLHK PIVWHVNNAT NKATYKPNTW CIRCLWSTKP  1200
VETSNSFDVL KSEDAQGMDN LACEDLKPVS EEVVENPTIQ KDVLECNVKT TEVVGDIILK  1260
PANNSLKITE EVGHTDLMAA YVDNSSLTIK KPNELSRVLG LKTLATHGLA AVNSVPWDTI  1320
ANYAKPFLNK VVSTTTNIVT RCLNRVCTNY MPYFFTLLLQ LCTFTRSTNS RIKASMPTTI  1380
AKNTVKSVGK FCLEASFNYL KSPNFSKLIN IIIWFLLLSV CLGSLIYSTA ALGVLMSNLG  1440
MPSYCTGYRE GYLNSTNVTI ATYCTGSIPC SVCLSGLDSL DTYPSLETIQ ITISSFKWDL  1500
TAFGLVAEWF LAYILFTRFF YVLGLAAIMQ LFFSYFAVHF ISNSWLMWLI INLVQMAPIS  1560
AMVRMYIFFA SFYYVWKSYV HVVDGCNSST CMMCYKRNRA TRVECTTIVN GVRRSFYVYA  1620
NGGKGFCKLH NWNCVNCDTF CAGSTFISDE VARDLSLQFK RPINPTDQSS YIVDSVTVKN  1680
GSIHLYFDKA GQKTYERHSL SHFVNLDNLR ANNTKGSLPI NVIVFDGKSK CEESSAKSAS  1740
VYYSQLMCQP ILLLDQALVS DVGDSAEVAV KMFDAYVNTF SSTFNVPMEK LKTLVATAEA  1800
ELAKNVSLDN VLSTFISAAR QGFVDSDVET KDVVECLKLS HQSDIEVTGD SCNNYMLTYN  1860
KVENMTPRDL GACIDCSARH INAQVAKSHN IALIWNVKDF MSLSEQLRKQ IRSAAKKNNL  1920
PFKLTCATTR QVVNVVTTKI ALKGG                                        1945

SEQ ID NO: 23          moltype = AA  length = 500
FEATURE                Location/Qualifiers
source                 1..500
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 23
KIVNNWLKQL IKVTLVFLFV AAIFYLITPV HVMSKHTDFS SEIIGYKAID GGVTRDIAST  60
DTCFANKHAD FDTWFSQRGG SYTNDKACPL IAAVITREVG FVVPGLPGTI LRTTNGDFLH  120
FLPRVFSAVG NICYTPSKLI EYTDFATSAC VLAAECTIFK DASGKPVPYC YDTNVLEGSV  180
AYESLRPDTR YVLMDGSIIQ FPNTYLEGSV RVVTTFDSEY CRHGTCERSE AGVCVSTSGR  240
WVLNNDYYRS LPGVFCGVDA VNLLTNMFTP LIQPIGALDI SASIVAGGIV AIVVTCLAYY  300
FMRFRRAFGE YSHVVAFNTL LFLMSFTVLC LTPVYSFLPG VYSVIYLYLT FYLTNDVSFL  360
AHIQWMVMFT PLVPFWITIA YIICISTKHF YWFFSNYLKR RVVFNGVSFS TFEEAALCTF  420
LLNKEMYLKL RSDVLLPLTQ YNRYLALYNK YKYFSGAMDT TSYREAACCH LAKALNDFSN  480
SGSDVLYQPP QTSITSAVLQ                                              500
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SEQ ID NO: 24          moltype = AA  length = 306
FEATURE                Location/Qualifiers
source                 1..306
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 24
SGFRKMAFPS GKVEGCMVQV TCGTTTLNGL WLDDVVYCPR HVICTSEDML NPNYEDLLIR  60
KSNHNFLVQA GNVQLRVIGH SMQNCVLKLK VDTANPKTPK YKFVRIQSGQ TFSVLACYNG  120
SPSGVYQCAM RPNFTIKGSF LNGSCGSVGF NIDYDCVSFC YMHHMELPTG VHAGTDLEGN  180
FYGPFVDRQT AQAAGTDTTI TVNVLAWLYA AVINGDRWFL NRFTTTLNDF NLVAMKYNYE  240
PLTQDHVDIL GPLSAQTGIA VLDMCASLKE LLQNGMNGRT ILGSALLEDE FTPFDVVRQC  300
SGVTFQ                                                             306

SEQ ID NO: 25          moltype = AA  length = 290
FEATURE                Location/Qualifiers
source                 1..290
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 25
SAVKRTIKGT HHWLLLTILT SLLVLVQSTQ WSLFFFLYEN AFLPFAMGII AMSAFAMMFV  60
KHKHAFLCLF LLPSLATVAY FNMVYMPASW VMRIMTWLDM VDTSLSGFKL KDCVMYASAV  120
VLLILMTART VYDDGARRVW TLMNVLTLVY KVYYGNALDQ AISMWALIIS VTSNYSGVVT  180
TVMFLARGIV FMCVEYCPIF FITGNTLQCI MLVYCFLGYF CTCYFGLFCL LNRYFRLTLG  240
VYDYLVSTQE FRYMNSQGLL PPKNSIDAFK LNIKLLGVGG KPCIKVATVQ             290

SEQ ID NO: 26          moltype = AA  length = 83
FEATURE                Location/Qualifiers
source                 1..83
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 26
SKMSDVKCTS VVLLSVLQQL RVESSSKLWA QCVQLHNDIL LAKDTTEAFE KMVSLLSVLL  60
SMQGAVDINK LCEEMLDNRA TLQ                                          83

SEQ ID NO: 27          moltype = AA  length = 198
FEATURE                Location/Qualifiers
source                 1..198
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 27
AIASEFSSLP SYAAFATAQE AYEQAVANGD SEVVLKKLKK SLNVAKSEFD RDAAMQRKLE  60
KMADQAMTQM YKQARSEDKR AKVTSAMQTM LFTMLRKLDN DALNNIINNA RDGCVPLNII  120
PLTTAAKLMV VIPDYNTYKN TCDGTTFTYA SALWEIQQVV DADSKIVQLS EISMDNSPNL  180
AWPLIVTALR ANSAVKLQ                                                198

SEQ ID NO: 28          moltype = AA  length = 113
FEATURE                Location/Qualifiers
source                 1..113
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 28
NNELSPVALR QMSCAAGTTQ TACTDDNALA YYNTTKGGRF VLALLSDLQD LKWARFPKSD  60
GTGTIYTELE PPCRFVTDTP KGPKVKYLYF IKGLNNLNRG MVLGSLAATV RLQ         113

SEQ ID NO: 29          moltype = AA  length = 139
FEATURE                Location/Qualifiers
source                 1..139
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 29
AGNATEVPAN STVLSFCAFA VDAAKAYKDY LASGGQPITN CVKMLCTHTG TGQAITVTPE  60
ANMDQESFGG ASCCLYCRCH IDHPNPKGFC DLKGKYVQIP TTCANDPVGF TLKNTVCTVC  120
GMWKGYGCSC DQLREPMLQ                                               139

SEQ ID NO: 30          moltype = AA  length = 13
FEATURE                Location/Qualifiers
source                 1..13
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 30
SADAQSFLNG FAV                                                     13

SEQ ID NO: 31          moltype = AA  length = 932
FEATURE                Location/Qualifiers
source                 1..932
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                     mol_type = protein
                     organism = Coronavirus 2
SEQUENCE: 31
SADAQSFLNR VCGVSAARLT PCGTGTSTDV VYRAFDIYND KVAGFAKFLK TNCCRFQEKD  60
EDDNLIDSYF VVKRHTFSNY QHEETIYNLL KDCPAVAKHD FFKFRIDGDM VPHISRQRLT  120
KYTMADLVYA LRHFDEGNCD TLKEILVTYN CCDDDYFNKK DWYDFVENPD ILRVYANLGE  180
RVRQALLKTV QFCDAMRNAG IVGVLTLDNQ DLNGNWYDFG DFIQTTPGSG VPVVDSYYSL  240
LMPILTLTRA LTAESHVDTD LTKPYIKWDL LKYDFTEERL KLFDRYFKYW DQTYHPNCVN  300
CLDDRCILHC ANFNVLFSTV FPLTSFGPLV RKIFVDGVPF VVSTGYHFRE LGVVHNQDVN  360
LHSSRLSFKE LLVYAADPAM HAASGNLLLD KRTTCFSVAA LTNNVAFQTV KPGNFNKDFY  420
DFVVSKGFFK EGSSVELKHF FFAQDGNAAI SDYDYYRYNL PTMCDIRQLL FVVEVVDKYF  480
DCYDGGCINA NQVIVNNLDK SAGFPFNKWG KARLYYDSMS YEDQDALFAY TKRNVIPTIT  540
QMNLKYAISA KNRARTVAGV SICSTMTNRQ FHQKLLKSIA ATRGATVVIG TSKFYGGWHN  600
MLKTVYSDVE NPHLMGWDYP KCDRAMPNML RIMASLVLAR KHTTCCSLSH RFYRLANECA  660
QVLSEMVMCG GSLYVKPGGT SSGDATTAYA NSVFNICQAV TANVNALLST DGNKIADKYV  720
RNLQHRLYEC LYRNRDVDTD FVNEFYAYLR KHFSMMILSD DAVVCFNSTY ASQGLVASIK  780
NFKSVLYYQN NVFMSEAKCW TETDLTKGPH EFCSQHTMLV KQGDDYVYLP YPDPSRILGA  840
GCFVDDIVKT DGTLMIERFV SLAIDAYPLT KHPNQEYADV FHLYLQYIRK LHDELTGHML  900
DMYSVMLTND NTSRYWEPEF YEAMYTPHTV LQ                                932

SEQ ID NO: 32          moltype = AA  length = 601
FEATURE                Location/Qualifiers
source                 1..601
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 32
AVGACVLCNS QTSLRCGACI RRPFLCCKCC YDHVISTSHK LVLSVNPYVC NAPGCDVTDV  60
TQLYLGGMSY YCKSHKPPIS FPLCANGQVF GLYKNTCVGS DNVTDFNAIA TCDWTNAGDY  120
ILANTCTERL KLFAAETLKA TEETFKLSYG IATVREVLSD RELHLSWEVG KPRPPLNRNY  180
VFTGYRVTKN SKVQIGEYTF EKGDYGDAVV YRGTTTYKLN VGDYFVLTSH TVMPLSAPTL  240
VPQEHYVRIT GLYPTLNISD EFSSNVANYQ KVGMQKYSTL QGPPGTGKSH FAIGLALYYP  300
SARIVYTACS HAAVDALCEK ALKYLPIDKC SRIIPARARV ECFDKFKVNS TLEQYVFCTV  360
NALPETTADI VVFDEISMAT NYDLSVVNAR LRAKHYVYIG DPAQLPAPRT LLTKGTLEPE  420
YFNSVCRLMK TIGPDMFLGT CRRCPAEIVD TVSALVYDNK LKAHKDKSAQ CFKMFYKGVI  480
THDVSSAINR PQIGVVREFL TRNPAWRKAV FISPYNSQNA VASKILGLPT QTVDSSQGSE  540
YDYVIFTQTT ETAHSCNVNR FNVAITRAKV GILCIMSDRD LYDKLQFTSL EIPRRNVATL  600
Q                                                                  601

SEQ ID NO: 33          moltype = AA  length = 527
FEATURE                Location/Qualifiers
source                 1..527
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 33
AENVTGLFKD CSKVITGLHP TQAPTHLSVD TKFKTEGLCV DIPGIPKDMT YRRLISMMGF  60
KMNYQVNGYP NMFITREEAI RHVRAWIGFD VEGCHATREA VGTNLPLQLG FSTGVNLVAV  120
PTGYVDTPNN TDFSRVSAKP PPGDQFKHLI PLMYKGLPWN VVRIKIVQML SDTLKNLSDR  180
VVFVLWAHGF ELTSMKYFVK IGPERTCCLC DRRATCFSTA SDTYACWHHS IGFDYVYNPF  240
MIDVQQWGFT GNLQSNHDLY CQVHGNAHVA SCDAIMTRCL AVHECFVKRV DWTIEYPIIG  300
DELKINAACR KVQHMVVKAA LLADKFPVLH DIGNPKAIKC VPQADVEWKF YDAQPCSDKA  360
YKIEELFYSY ATHSDKFTDG VCLFWNCNVD RYPANSIVCR FDTRVLSNLN LPGCDGGSLY  420
VNKHAFHTPA FDKSAFVNLK QLPFFYYSDS PCESHGKQVV SDIDYVPLKS ATCITRCNLG  480
GAVCRHHANE YRLYLDAYNM MISAGFSLWV YKQFDTYNLW NTFTRLQ                527

SEQ ID NO: 34          moltype = AA  length = 346
FEATURE                Location/Qualifiers
source                 1..346
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 34
SLENVAFNVV NKGHFDGQQG EVPVSIINNT VYTKVDGVDV ELFENKTTLP VNVAFELWAK  60
RNIKPVPEVK ILNNLGVDIA ANTVIWDYKR DAPAHISTIG VCSMTDIAKK PTETICAPLT  120
VFFDGRVDGQ VDLFRNARNG VLITEGSVKG LQPSVGPKQA SLNGVTLIGE AVKTQFNYYK  180
KVDGVVQQLP ETYFTQSRNL QEFKPRSQME IDFLELAMDE FIERYKLEGY AFEHIVYGDF  240
SHSQLGGLHL LIGLAKRFKE SPFELEDFIP MDSTVKNYFI TDAQTGSSKC VCSVIDLLLD  300
DFVEIIKSQD LSVVSKVVKV TIDYTEISFM LWCKDGHVET FYPKLQ                  346

SEQ ID NO: 35          moltype = AA  length = 298
FEATURE                Location/Qualifiers
source                 1..298
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 35
SSQAWQPGVA MPNLYKMQRM LLEKCDLQNY GDSATLPKGI MMNVAKYTQL CQYLNTLTLA  60
VPYNMRVIHF GAGSDKGVAP GTAVLRQWLP TGTLLVDSDL NDFVSDADST LIGDCATVHT  120
ANKWDLIISD MYDPKTKNVT KENDSKEGFF TYICGFIQQK LALGGSVAIK ITEHSWNADL  180
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YKLMGHFAWW TAFVTNVNAS SSEAFLIGCN YLGKPREQID GYVMHANYIF WRNTNPIQLS  240
SYSLFDMSKF PLKLRGTAVM SLKEGQINDM ILSLLSKGRL IIRENNRVVI SSDVLVNN    298

SEQ ID NO: 36          moltype = AA  length = 1273
FEATURE                Location/Qualifiers
source                 1..1273
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 36
MFVFLVLLPL VSSQCVNLTT RTQLPPAYTN SFTRGVYYPD KVFRSSVLHS TQDLFLPFFS  60
NVTWFHAIHV SGTNGTKRFD NPVLPFNDGV YFASTEKSNI IRGWIFGTTL DSKTQSLLIV  120
NNATNVVIKV CEFQFCNDPF LGVYYHKNNK SWMESEFRVY SSANNCTFEY VSQPFLMDLE  180
GKQGNFKNLR EFVFKNIDGY FKIYSKHTPI NLVRDLPQGF SALEPLVDLP IGINITRFQT  240
LLALHRSYLT PGDSSSGWTA GAAAYYVGYL QPRTFLLKYN ENGTITDAVD CALDPLSETK  300
CTLKSFTVEK GIYQTSNFRV QPTESIVRFP NITNLCPFGE VFNATRFASV YAWNRKRISN  360
CVADYSVLYN SASFSTFKCY GVSPTKLNDL CFTNVYADSF VIRGDEVRQI APGQTGKIAD  420
YNYKLPDDFT GCVIAWNSNN LDSKVGGNYN YLYRLFRKSN LKPFERDIST EIYQAGSTPC  480
NGVEGFNCYF PLQSYGFQPT NGVGYQPYRV VVLSFELLHA PATVCGPKKS TNLVKNKCVN  540
FNFNGLTGTG VLTESNKKFL PFQQFGRDIA DTTDAVRDPQ TLEILDITPC SFGGVSVITP  600
GTNTSNQVAV LYQGVNCTEV PVAIHADQLT PTWRVYSTGS NVFQTRAGCL IGAEHVNNSY  660
ECDIPIGAGI CASYQTQTNS PRRARSVASQ SIIAYTMSLG AENSVAYSNN SIAIPTNFTI  720
SVTTEILPVS MTKTSVDCTM YICGDSTECS NLLLQYGSFC TQLNRALTGI AVEQDKNTQE  780
VFAQVKQIYK TPPIKDFGGF NFSQILPDPS KPSKRSFIED LLFNKVTLAD AGFIKQYGDC  840
LGDIAARDLI CAQKFNGLTV LPPLLTDEMI AQYTSALLAG TITSGWTFGA GAALQIPFAM  900
QMAYRFNGIG VTQNVLYENQ KLIANQFNSA IGKIQDSLSS TASALGKLQD VVNQNAQALN  960
TLVKQLSSNF GAISSVLNDI LSRLDKVEAE VQIDRLITGR LQSLQTYVTQ QLIRAAEIRA  1020
SANLAATKMS ECVLGQSKRV DFCGKGYHLM SFPQSAPHGV VFLHVTYVPA QEKNFTTAPA  1080
ICHDGKAHFP REGVFVSNGT HWFVTQRNFY EPQIITTDNT FVSGNCDVVI GIVNNTVYDP  1140
LQPELDSFKE ELDKYFKNHT SPDVDLGDIS GINASVVNIQ KEIDRLNEVA KNLNESLIDL  1200
QELGKYEQYI KWPWYIWLGF IAGLIAIVMV TIMLCCMTSC CSCLKGCCSC GSCCKFDEDD  1260
SEPVLKGVKL HYT                                                     1273

SEQ ID NO: 37          moltype = AA  length = 275
FEATURE                Location/Qualifiers
source                 1..275
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 37
MDLFMRIFTI GTVTLKQGEI KDATPSDFVR ATATIPIQAS LPFGWLIVGV ALLAVFQSAS  60
KIITLKKRWQ LALSKGVHFV CNLLLLFVTV YSHLLLVAAG LEAPFLYLYA LVYFLQSINF  120
VRIIMRLWLC WKCRSKNPLL YDANYFLCWH TNCYDYCIPY NSVTSSIVIT SGDGTTSPIS  180
EHDYQIGGYT EKWESGVKDC VVLHSYFTSD YYQLYSTQLS TDTGVEHVTF FIYNKIVDEP  240
EEHVQIHTID GSSGVVNPVM EPIYDEPTTT TSVPL                             275

SEQ ID NO: 38          moltype = AA  length = 75
FEATURE                Location/Qualifiers
source                 1..75
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 38
MYSFVSEETG TLIVNSVLLF LAFVVFLLVT LAILTALRLC AYCCNIVNVS LVKPSFYVYS  60
RVKNLNSSRV PDLLV                                                   75

SEQ ID NO: 39          moltype = AA  length = 222
FEATURE                Location/Qualifiers
source                 1..222
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 39
MADSNGTITV EELKKLLEQW NLVIGFLFLT WICLLQFAYA NRNRFLYIIK LIFLWLLWPV  60
TLACFVLAAV YRINWITGGI AIAMACLVGL MWLSYFIASF RLFARTRSMW SFNPETNILL  120
NVPLHGTILT RPLLESELVI GAVILRGHLR IAGHHLGRCD IKDLPKEITV ATSRTLSYYK  180
LGASQRVAGD SGFAAYSRYR IGNYKLNTDH SSSSDNIALL VQ                     222

SEQ ID NO: 40          moltype = AA  length = 61
FEATURE                Location/Qualifiers
source                 1..61
                       mol_type = protein
                       organism = Coronavirus 2
SEQUENCE: 40
MFHLVDFQVT IAEILLIIMR TFKVSIWNLD YIINLIIKNL SKSLTENKYS QLDEEQPMEI  60
D                                                                  61

SEQ ID NO: 41          moltype = AA  length = 121
FEATURE                Location/Qualifiers
source                 1..121
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                     mol_type = protein
                     organism = Coronavirus 2
SEQUENCE: 41
MKIILFLALI TLATCELYHY QECVRGTTVL LKEPCSSGTY EGNSPFHPLA DNKFALTCFS  60
TQFAFACPDG VKHVYQLRAR SVSPKLFIRQ EEVQELYSPI FLIVAAIVFI TLCFTLKRKT  120
E                                                                  121

SEQ ID NO: 42        moltype = AA  length = 43
FEATURE              Location/Qualifiers
source               1..43
                     mol_type = protein
                     organism = Coronavirus 2
SEQUENCE: 42
MIELSLIDFY LCFLAFLLFL VLIMLIIFWF SLELQDHNET CHA                    43

SEQ ID NO: 43        moltype = AA  length = 121
FEATURE              Location/Qualifiers
source               1..121
                     mol_type = protein
                     organism = Coronavirus 2
SEQUENCE: 43
MKFLVFLGII TTVAAFHQEC SLQSCTQHQP YVVDDPCPIH FYSKWYIRVG ARKSAPLIEL  60
CVDEAGSKSP IQYIDIGNYT VSCLPFTINC QEPKLGSLVV RCSFYEDFLE YHDVRVVLDF  120
I                                                                  121

SEQ ID NO: 44        moltype = AA  length = 419
FEATURE              Location/Qualifiers
source               1..419
                     mol_type = protein
                     organism = Coronavirus 2
SEQUENCE: 44
MSDNGPQNQR NAPRITFGGP SDSTGSNQNG ERSGARSKQR RPQGLPNNTA SWFTALTQHG  60
KEDLKFPRGQ GVPINTNSSP DDQIGYYRRA TRRIRGGDGK MKDLSPRWYF YYLGTGPEAG  120
LPYGANKDGI IWVATEGALN TPKDHIGTRN LANNAAIVLQ LPQGTTLPKG FYAEGSRGGS  180
QASSRSSSRS RNSSRNSTPG SSKRTSPARM AGNGGDAALA LLLLDRLNQL ESKMSGKGQQ  240
QQGQTVTKKS AAEASKKPRQ KRTATKAYNV TQAFGRRGPE QTQGNFGDQE LIRQGTDYKH  300
WPQIAQFAPS ASAFFGMSRI GMEVTPSGTW LTYTGAIKLD DKDPNFKDQV ILLNKHIDAY  360
KTFPPTEPKK DKKKKADETQ ALPQRQKKQQ TVTLLPAADL DDFSKQLQQS MSSADSTQA   419

SEQ ID NO: 45        moltype = AA  length = 38
FEATURE              Location/Qualifiers
source               1..38
                     mol_type = protein
                     organism = Coronavirus 2
SEQUENCE: 45
MGYINVFAFP FTIYSLLLCR MNSRNYIAQV DVVNFNLT                          38

SEQ ID NO: 46        moltype =   length =
SEQUENCE: 46
000

SEQ ID NO: 47        moltype =   length =
SEQUENCE: 47
000

SEQ ID NO: 48        moltype =   length =
SEQUENCE: 48
000

SEQ ID NO: 49        moltype =   length =
SEQUENCE: 49
000

SEQ ID NO: 50        moltype =   length =
SEQUENCE: 50
000

SEQ ID NO: 51        moltype = AA  length = 7096
FEATURE              Location/Qualifiers
source               1..7096
                     mol_type = protein
                     organism = synthetic construct
SEQUENCE: 51
MESLVPGFNE KTHVQLSLPV LQVRDVLVRG FGDSVEEVLS EARQHLKDGT CGLVEVEKGV  60
LPQLEQPYVF IKRSDARTAP HGHVMVELVA ELEGIQYGRS GETLGVLVPH VGEIPVAYRK  120
VLLRKNGNKG AGGHSYGADL KSFDLGDELG TDPYEDFQEN WNTKHSSGVT RELMRELNGG  180
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AYTRYVDNNF CGPDGYPLEC IKDLLARAGK ASCTLSEQLD FIDTKRGVYC CREHEHEIAW  240
YTERSEKSYE LQTPFEIKLA KKFDTFNGEC PNFVFPLNSI IKTIQPRVEK KKLDGFMGRI  300
RSVYPVASPN ECNQMCLSTL MKCDHCGETS WQTGDFVKAT CEFCGTENLT KEGATTCGYL  360
PQNAVVKIYC PACHNSEVGP EHSLAEYHNE SGLKTILRKG GRTIAFGGCV FSYVGCHNKC  420
AYWVPRASAN IGCNHTGVVG EGSEGLNDNL LEILQKEKVN INIVGDFKLN EEIAIILASF  480
SASTSAFVET VKGLDYKAFK QIVESCGNFK VTKGKAKKGA WNIGEQKSIL SPLYAFASEA  540
ARVVRSIFSR TLETAQNSVR VLQKAAITIL DGISQYSLRL IDAMMFTSDL ATNNLVVMAY  600
ITGGVVQLTS QWLTNIFGTV YEKLKPVLDW LEEKFKEGVE FLRDGWEIVK FISTCACEIV  660
GGQIVTCAKE IKESVQTFFK LVNKFLALCA DSIIIGGAKL KALNLGETFV THSKGLYRKC  720
VKSREETGLL MPLKAPKEII FLEGETLPTE VLTEEVVLKT GDLQPLEQPT SEAVEAPLVG  780
TPVCINGLML LEIKDTEKYC ALAPNMMVTN NTFTLKGGAP TKVTFGDDTV IEVQGYKSVN  840
ITFELDERID KVLNEKCSAY TVELGTEVNE FACVVADAVI KTLQPVSELL TPLGIDLDEW  900
SMATYYLFDE SGEFKLASHM YCSFYPPDED EEEGDCEEEE FEPSTQYEYG TEDDYQGKPL  960
EFGATSAALQ PEEEQEEDWL DDDSQQTVGQ QDGSEDNQTT TIQTIVEVQP QLEMELTPVV 1020
QTIEVNSFSG YLKLTDNVYI KNADIVEEAK KVKPTVVVNA ANVYLKHGGG VAGALNKATN 1080
NAMQVESDDY IATNGPLKVG GSCVLSGHNL AKHCLHVVGP NVNKGEDIQL LKSAYENFNQ 1140
HEVLLAPLLS AGIFGADPIH SLRVCVDTVR TNVYLAVFDK NLYDKLVSSF LEMKSEKQVE 1200
QKIAEIPKEE VKPFITESKP SVEQRKQDDK KIKACVEEVT TTLEETKFLT ENLLLYIDIN 1260
GNLHPDSATL VSDIDITFLK KDAPYIVGDV VQEGVLTAVV IPTKKAGGTT EMLAKALRKV 1320
PTDNYITTYP GQGLNGYTVE EAKTVLKKCK SAFYILPSII SNEKQEILGT VSWNLREMLA 1380
HAEETRKLMP VCVETKAIVS TIQRKYKGIK IQEGVVDYGA RFYFYTSKTT VASLINTLND 1440
LNETLVTMPL GYVTHGLNLE EAARYMRSLK VPATVSVSSP DAVTAYNGYL TSSSKTPEEH 1500
FIETISLAGS YKDWSYSGQS TQLGIEFLKR GDKSVYYTSN PTTFHLDGEV ITFDNLKTLL 1560
SLREVRTIKV FTTVDNINLH TQVVDMSMTY GQQFGPTYLD GADVTKIKPH NSHEGKTFYV 1620
LPNDDTLRVE AFEYYHTTDP SFLGRYMSAL NHTKKWKYPQ VNGLTSIKWA DNNCYLATAL 1680
LTLQQIELKF NPPALQDAYY RARAGEAANF CALILAYCNK TVGELGDVRE TMSYLFQHAN 1740
LDSCKRVLNV VCKTCGQQQT TLKGVEAVMY MGTLSYEQFK KGVQIPCTCG KQATKYLVQQ 1800
ESPFVMMSAP PAQYELKHGT FTCASEYTGN YQCGHYKHIT SKETLYCIDG ALLTKSSEYK 1860
GPITDVFYKE NSYTTTIKPV TYKLDGVVCT EIDPKLDNYY KKDNSYFTEQ PIDLVPNQPY 1920
PNASFDNFKF VCDNIKFADD LNQLTGYKKP ASRELKVTFF PDLNGDVVAI DYKHYTPSFK 1980
KGAKLLHKPI VWHVNNATNK ATYKPNTWCI RCLWSTKPVE TSNSFDVLKS EDAQGMDNLA 2040
CEDLKPVSEE VVENPTIQKD VLECNVKTTE VVGDIILKPA NNSLKITEEV GHTDLMAAYV 2100
DNSSLTIKKP NELSRVLGLK TLATHGLAAV NSVPWDTIAN YAKPFLNKVV STTTNIVTRC 2160
LNRVCTNYMP YFFTLLLQLC TFTRSTNSRI KASMPTTIAK NTVKSVGKFC LEASFNYLKS 2220
PNFSKLINII IWFLLLSVCL GSLIYSTAAL GVLMSNLGMP SYCTGYREGY LNSTNVTIAT 2280
YCTGSIPCSV CLSGLDSLDT YPSLETIQIT ISSFKWDLTA FGLVAEWFLA YILFTRFFYV 2340
LGLAAIMQLF FSYFAVHFIS NSWLMWLIIN LVQMAPISAM VRMYIFFASF YYVWKSYVHV 2400
VDGCNSSTCM MCYKRNRATR VECTTIVNGV RRSFYVYANG GKGFCKLHNW NCVNCDTFCA 2460
GSTFISDEVA RDLSLQFKRP INPTDQSSYI VDSVTVKNGS IHLYFDKAGQ KTYERHSLSH 2520
FVNLDNLRAN NTKGSLPINV IVFDGKSKCE ESSAKSASVY YSQLMCQPIL LLDQALVSDV 2580
GDSAEVAVKM FDAYVNTFSS TFNVPMEKLK TLVATAEAEL AKNVSLDNVL STFISAARQG 2640
FVDSDVETKD VVECLKLSHQ SDIEVTGDSC NNYMLTYNKV ENMTPRDLGA CIDCSARHIN 2700
AQVAKSHNIA LIWNVKDFMS LSEQLRKQIR SAAKKNNLPF KLTCATTRQV VNVVTTKIAL 2760
KGGKIVNNWL KQLIKVTLVF LFVAAIFYLI TPVHVMSKHT DFSSEIIGYK AIDGGVTRDI 2820
ASTDTCFANK HADFDTWFSQ RGGSYTNDKA CPLIAAVITR EVGFVVPGLP GTILRTTNGD 2880
FLHFLPRVFS AVGNICYTPS KLIEYTDFAT SACVLAAECT IFKDASGKPV PYCYDTNVLE 2940
GSVAYESLRP DTRYVLMDGS IIQFPNTYLE GSVRVVTTFD SEYCRHGTCE RSEAGVCVST 3000
SGRWVLNNDY YRSLPGVFCG VDAVNLLTNM FTPLIQPIGA LDISASIVAG GIVAIVVTCL 3060
AYYFMRFRRA FGEYSHVVAF NTLLFLMSFT VLCLTPVYSF LPGVYSVIYL YLTFYLTNDV 3120
SFLAHIQWMV MFTPLVPFWI TIAYIICIST KHFYWFFSNY LKRRVVFNGV SFSTFEEAAL 3180
CTFLLNKEMY LKLRSDVLLP LTQYNRYLAL YNKYKYFSGA MDTTSYREAA CCHLAKALND 3240
FSNSGSDVLY QPPQTSITSA VLQSGFRKMA FPSGKVEGCM VQVTCGTTTL NGLWLDDVVY 3300
CPRHVICTSE DMLNPNYEDL LIRKSNHNFL VQAGNVQLRV IGHSMQNCVL KLKVDTANPK 3360
TPKYKFVRIQ PGQTFSVLAC YNGSPSGVYQ CAMRPNFTIK GSFLNGSCGS VGFNIDYDCV 3420
SFCYMHHMEL PTGVHAGTDL EGNFYGPFVD RQTAQAAGTD TTITVNVLAW LYAAVINGDR 3480
WFLNRFTTTL NDFNLVAMKY NYEPLTQDHV DILGPLSAQT GIAVLDMCAS LKELLQNGMN 3540
GRTILGSALL EDEFTPFDVV RQCSGVTFQS AVKRTIKGTH HWLLLTILTS LLVLVQSTQW 3600
SLFFFLYENA FLPFAMGIIA MSAFAMMFVK HKHAFLCLFL LPSLATVAYF NMVYMPASWV 3660
MRIMTWLDMV DTSLSGFKLK DCVMYASAVV LLILMTARTV YDDGARRVWT LMNVLTLVYK 3720
VYYGNALDQA ISMWALIISV TSNYSGVVTT VMFLARGIVF MCVEYCPIFF ITGNTLQCIM 3780
LVYCFLGYFC TCYFGLFCLL NRYFRLTLGV YDYLVSTQEF RYMNSQGLLP PKNSIDAFKL 3840
NIKLLGVGGK PCIKVATVQS KMSDVKCTSV VLLSVLQQLR VESSSKLWAQ CVQLHNDILL 3900
AKDTTEAFEK MVSLLSVLLS MQGAVDINKL CEEMLDNRAT LQAIASEFSS LPSYAAFATA 3960
QEAYEQAVAN GDSEVVLKKL KKSLNVAKSE FDRDAAMQRK LEKMADQAMT QMYKQARSED 4020
KRAKVTSAMQ TMLFTMLRKL DNDALNNIIN NARDGCVPLN IIPLTTAAKL MVVIPDYNTY 4080
KNTCDGTTFT YASALWEIQQ VVDADSKIVQ LSEISMDNSP NLAWPLIVTA LRANSAVKLQ 4140
NNELSPVALR QMSCAAGTTQ TACTDDNALA YYNTTKGGRF VLALLSDLQD LKWARFPKSD 4200
GTGTIYTELE PPCRFVTDTP KGPKVKYLYF IKGLNNLNRG MVLGSLAATV RLQAGNATEV 4260
PANSTVLSFC AFAVDAAKAY KDYLASGGQP ITNCVKMLCT HTGTGQAITV TPEANMDQES 4320
FGGASCCLYC RCHIDHPNPK GFCDLKGKYV QIPTTCANDP VGFTLKNTVC TVCGMWKGYG 4380
CSCDQLREPM LQSADAQSFL NRVCGVSAAR LTPCGTGTST DVVYRAFDIY NDKVAGFAKF 4440
LKTNCCRFQE KDEDDNLIDS YFVVKRHTFS NYQHEETIYN LLKDCPAVAK HDFFKFRIDG 4500
DMVPHISRQR LTKYTMADLV YALRHFDEGN CDTLKEILVT YNCCDDDYFN KKDWYDFVEN 4560
PDILRVYANL GERVRQALLK TVQFCDAMRN AGIVGVLTLD NQDLNGNWYD FGDFIQTTPG 4620
SGVPVVDSYY SLLMPILTLT RALTAESHVD TDLTKPYIKW DLLKYDFTEE RLKLFDRYFK 4680
YWDQTYHPNC VNCLDDRCIL HCANFNVLFS TVFPPTSFGP LVRKIFVDGV PFVVSTGYHF 4740
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RELGVVHNQD VNLHSSRLSF KELLVYAADP AMHAASGNLL LDKRTTCFSV AALTNNVAFQ  4800
TVKPGNFNKD FYDFAVSKGF FKEGSSVELK HFFFAQDGNA AISDYDYYRY NLPTMCDIRQ  4860
LLFVVEVVDK YFDCYDGGCI NANQVIVNNL DKSAGFPFNK WGKARLYYDS MSYEDQDALF  4920
AYTKRNVIPT ITQMNLKYAI SAKNRARTVA GVSICSTMTN RQFHQKLLKS IAATRGATVV  4980
IGTSKFYGGW HNMLKTVYSD VENPHLMGWD YPKCDRAMPN MLRIMASLVL ARKHTTCCSL  5040
SHRFYRLANE CAQVLSEMVM CGGSLYVKPG GTSSGDATTA YANSVFNICQ AVTANVNALL  5100
STDGNKIADK YVRNLQHRLY ECLYRNRDVD TDFVNEFYAY LRKHFSMMIL SDDAVVCFNS  5160
TYASQGLVAS IKNFKSVLYY QNNVFMSEAK CWTETDLTKG PHEFCSQHTM LVKQGDDYVY  5220
LPYPDPSRIL GAGCFVDDIV KTDGTLMIER FVSLAIDAYP LTKHPNQEYA DVFHLYLQYI  5280
RKLHDELTGH MLDMYSVMLT NDNTSRYWEP EFYEAMYTPH TVLQAVGACV LCNSQTSLRC  5340
GACIRRPFLC CKCCYDHVIS TSHKLVLSVN PYVCNAPGCD VTDVTQLYLG GMSYYCKSHK  5400
PPISFPLCAN GQVFGLYKNT CVGSDNVTDF NAIATCDWTN AGDYILANTC TERLKLFAAE  5460
TLKATEETFK LSYGIATVRE VLSDRELHLS WEVGKPRPPL NRNYVFTGYR VTKNSKVQIG  5520
EYTFEKGDYG DAVVYRGTTT YKLNVGDYFV LTSHTVMPLS APTLVPQEHY VRITGLYPTL  5580
NISDEFSSNV ANYQKVGMQK YSTLQGPPGT GKSHFAIGLA LYYPSARIVY TACSHAAVDA  5640
LCEKALKYLP IDKCSRIIPA RARVECFDKF KVNSTLEQYV FCTVNALPET TADIVVFDEI  5700
SMATNYDLSV VNARLRAKHY VYIGDPAQLP APRTLLTKGT LEPEYFNSVC RLMKTIGPDM  5760
FLGTCRRCPA EIVDTVSALV YDNKLKAHKD KSAQCFKMFY KGVITHDVSS AINRPQIGVV  5820
REFLTRNPAW RKAVFISPYN SQNAVASKIL GLPTQTVDSS QGSEYDYVIF TQTTETAHSC  5880
NVNRFNVAIT RAKVGILCIM SDRDLYDKLQ FTSLEIPRRN VATLQAENVT GLFKDCSKVI  5940
TGLHPTQAPT HLSVDTKFKT EGLCVDIPGI PKDMTYRRLI SMMGFKMNYQ VNGYPNMFIT  6000
REEAIRHVRA WIGFDVEGCH ATREAVGTNL PLQLGFSTGV NLVAVPTGYV DTPNNTDFSR  6060
VSAKPPPGDQ FKHLIPLMYK GLPWNVVRIK IVQMLSDTLK NLSDRVVFVL WAHGFELTSM  6120
KYFVKIGPER TCCLCDRRAT CFSTASDTYA CWHHSIGFDY VYNPFMIDVQ QWGFTGNLQS  6180
NHDLYCQVHG NAHVASCDAI MTRCLAVHEC FVKRVDWTIE YPIIGDELKI NAACRKVQHM  6240
VVKAALLADK FPVLHDIGNP KAIKCVPQAD VEWKFYDAQP CSDKAYKIEE LFYSYATHSD  6300
KFTDGVCLFW NCNVDRYPAN SIVCRFDTRV LSNLNLPGCD GGSLYVNKHA FHTPAFDKSA  6360
FVNLKQLPFF YYSDSPCESH GKQVVSDIDY VPLKSATCIT RCNLGGAVCR HHANEYRLYL  6420
DAYNMMISAG FSLWVYKQFD TYNLWNTFTR LQSLENVAFN VVNKGHFDGQ QGEVPVSIIN  6480
NTVYTKVDGV DVELFENKTT LPVNVAFELW AKRNIKPVPE VKILNNLGVD IAANTVIWDY  6540
KRDAPAHIST IGVCSMTDIA KKPTETICAP LTVFFDGRVD GQVDLFRNAR NGVLITEGSV  6600
KGLQPSVGPK QASLNGVTLI GEAVKTQFNY YKKVDGVVQQ LPETYFTQSR NLQEFKPRSQ  6660
MEIDFLELAM DEFIERYKLE GYAFEHIVYG DFSHSQLGGL HLLIGLAKRF KESPFELEDF  6720
IPMDSTVKNY FITDAQTGSS KCVCSVIDLL LDDFVEIIKS QDLSVVSKVV KVTIDYTEIS  6780
FMLWCKDGHV ETFYPKLQSS QAWQPGVAMP NLYKMQRMLL EKCDLQNYGD SATLPKGIMM  6840
NVAKYTQLCQ YLNTLTLAVP YNMRVIHFGA GSDKGVAPGT AVLRQWLPTG TLLVDSDLND  6900
FVSDADSTLI GDCATVHTAN KWDLIISDMY DPKTKNVTKE NDSKEGFFTY ICGFIQQKLA  6960
LGGSVAIKIT EHSWNADLYK LMGHFAWWTA FVTNVNASSS EAFLIGCNYL GKPREQIDGY  7020
VMHANYIFWR NTNPIQLSSY SLFDMSKFPL KLRGTAVMSL KEGQINDMIL SLLSKGRLII  7080
RENNRVVISS DVLVNN                                                  7096

SEQ ID NO: 52          moltype = AA  length = 180
FEATURE                Location/Qualifiers
source                 1..180
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 52
MESLVPGFNE KTHVQLSLPV LQVRDVLVRG FGDSVEEVLS EARQHLKDGT CGLVEVEKGV  60
LPQLEQPYVF IKRSDARTAP HGHVMVELVA ELEGIQYGRS GETLGVLVPH VGEIPVAYRK  120
VLLRKNGNKG AGGHSYGADL KSFDLGDELG TDPYEDFQEN WNTKHSSGVT RELMRELNGG  180

SEQ ID NO: 53          moltype = AA  length = 638
FEATURE                Location/Qualifiers
source                 1..638
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 53
AYTRYVDNNF CGPDGYPLEC IKDLLARAGK ASCTLSEQLD FIDTKRGVYC CREHEHEIAW  60
YTERSEKSYE LQTPFEIKLA KKFDTFNGEC PNFVFPLNSI IKTIQPRVEK KKLDGFMGRI  120
RSVYPVASPN ECNQMCLSTL MKCDHCGETS WQTGDFVKAT CEFCGTENLT KEGATTCGYL  180
PQNAVVKIYC PACHNSEVGP EHSLAEYHNE SGLKTILRKG GRTIAFGGCV FSYVGCHNKC  240
AYWVPRASAN IGCNHTGVVG EGSEGLNDNL LEILQKEKVN INIVGDFKLN EEIAIILASF  300
SASTSAFVET VKGLDYKAFK QIVESCGNFK VTKGKAKKGA WNIGEQKSIL SPLYAFASEA  360
ARVVRSIFSR TLETAQNSVR VLQKAAITIL DGISQYSLRL IDAMMFTSDL ATNNLVVMAY  420
ITGGVVQLTS QWLTNIFGTV YEKLKPVLDW LEEKFKEGVE FLRDGWEIVK FISTCACEIV  480
GGQIVTCAKE IKESVQTFFK LVNKFLALCA DSIIIGGAKL KALNLGETFV THSKGLYRKC  540
VKSREETGLL MPLKAPKEII FLEGETLPTE VLTEEVVLKT GDLQPLEQPT SEAVEAPLVG  600
TPVCINGLML LEIKDTEKYC ALAPNMMVTN NTFTLKGG                          638

SEQ ID NO: 54          moltype = AA  length = 1945
FEATURE                Location/Qualifiers
source                 1..1945
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 54
APTKVTFGDD TVIEVQGYKS VNITFELDER IDKVLNEKCS AYTVELGTEV NEFACVVADA  60
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VIKTLQPVSE LLTPLGIDLD EWSMATYYLF DESGEFKLAS HMYCSFYPPD EDEEEGDCEE  120
EEFEPSTQYE YGTEDDYQGK PLEFGATSAA LQPEEEQEED WLDDDSQQTV GQQDGSEDNQ  180
TTTIQTIVEV QPQLEMELTP VVQTIEVNSF SGYLKLTDNV YIKNADIVEE AKKVKPTVVV  240
NAANVYLKHG GGVAGALNKA TNNAMQVESD DYIATNGPLK VGGSCVLSGH NLAKHCLHVV  300
GPNVNKGEDI QLLKSAYENF NQHEVLLAPL LSAGIFGADP IHSLRVCVDT VRTNVYLAVF  360
DKNLYDKLVS SFLEMKSEKQ VEQKIAEIPK EEVKPFITES KPSVEQRKQD DKKIKACVEE  420
VTTTLEETKF LTENLLLYID INGNLHPDSA TLVSDIDITF LKKDAPYIVG DVVQEGVLTA  480
VVIPTKKAGG TTEMLAKALR KVPTDNYITT YPGQGLNGYT VEEAKTVLKK CKSAFYILPS  540
IISNEKQEIL GTVSWNLREM LAHAEETRKL MPVCVETKAI VSTIQRKYKG IKIQEGVVDY  600
GARFYFYTSK TTVASLINTL NDLNETLVTM PLGYVTHGLN LEEAARYMRS LKVPATVSVS  660
SPDAVTAYNG YLTSSSKTPE EHFIETISLA GSYKDWSYSG QSTQLGIEFL KRGDKSVYYT  720
SNPTTFHLDG EVITFDNLKT LLSLREVRTI KVFTTVDNIN LHTQVVDMSM TYGQQFGPTY  780
LDGADVTKIK PHNSHEGKTF YVLPNDDTLR VEAFEYYHTT DPSFLGRYMS ALNHTKKWKY  840
PQVNGLTSIK WADNNCYLAT ALLTLQQIEL KFNPPALQDA YYRARAGEAA NFCALILAYC  900
NKTVGELGDV RETMSYLFQH ANLDSCKRVL NVVCKTCGQQ QTTLKGVEAV MYMGTLSYEQ  960
FKKGVQIPCT CGKQATKYLV QQESPFVMMS APPAQYELKH GTFTCASEYT GNYQCGHYKH 1020
ITSKETLYCI DGALLTKSSE YKGPITDVFY KENSYTTTIK PVTYKLDGVV CTEIDPKLDN 1080
YYKKDNSYFT EQPIDLVPNQ PYPNASFDNF KFVCDNIKFA DDLNQLTGYK KPASRELKVT 1140
FFPDLNGDVV AIDYKHYTPS FKKGAKLLHK PIVWHVNNAT NKATYKPNTW CIRCLWSTKP 1200
VETSNSFDVL KSEDAQGMDN LACEDLKPVS EEVVENPTIQ KDVLECNVKT TEVVGDIILK 1260
PANNSLKITE EVGHTDLMAA YVDNSSLTIK KPNELSRVLG LKTLATHGLA AVNSVPWDTI 1320
ANYAKPFLNK VVSTTTNIVT RCLNRVCTNY MPYFFTLLLQ LCTFTRSTNS RIKASMPTTI 1380
AKNTVKSVGK FCLEASFNYL KSPNFSKLIN IIIWFLLLSV CLGSLIYSTA ALGVLMSNLG 1440
MPSYCTGYRE GYLNSTNVTI ATYCTGSIPC SVCLSGLDSL DTYPSLETIQ ITISSFKWDL 1500
TAFGLVAEWF LAYILFTRFF YVLGLAAIMQ LFFSYFAVHF ISNSWLMWLI INLVQMAPIS 1560
AMVRMYIFFA SFYYVWKSYV HVVDGCNSST CMMCYKRNRA TRVECTTIVN GVRRSFYVYA 1620
NGGKGFCKLH NWNCVNCDTF CAGSTFISDE VARDLSLQFK RPINPTDQSS YIVDSVTVKN 1680
GSIHLYFDKA GQKTYERHSL SHFVNLDNLR ANNTKGSLPI NVIVFDGKSK CEESSAKSAS 1740
VYYSQLMCQP ILLLDQALVS DVGDSAEVAV KMFDAYVNTF SSTFNVPMEK LKTLVATAEA 1800
ELAKNVSLDN VLSTFISAAR QGFVDSDVET KDVVECLKLS HQSDIEVTGD SCNNYMLTYN 1860
KVENMTPRDL GACIDCSARH INAQVAKSHN IALIWNVKDF MSLSEQLRKQ IRSAAKKNNL 1920
PFKLTCATTR QVVNVVTTKI ALKGG                                       1945

SEQ ID NO: 55          moltype = AA  length = 500
FEATURE                Location/Qualifiers
source                 1..500
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 55
KIVNNWLKQL IKVTLVFLFV AAIFYLITPV HVMSKHTDFS SEIIGYKAID GGVTRDIAST  60
DTCFANKHAD FDTWFSQRGG SYTNDKACPL IAAVITREVG FVVPGLPGTI LRTTNGDFLH  120
FLPRVFSAVG NICYTPSKLI EYTDFATSAC VLAAECTIFK DASGKPVPYC YDTNVLEGSV  180
AYESLRPDTR YVLMDGSIIQ FPNTYLEGSV RVVTTFDSEY CRHGTCERSE AGVCVSTSGR  240
WVLNNDYYRS LPGVFCGVDA VNLLTNMFTP LIQPIGALDI SASIVAGGIV AIVVTCLAYY  300
FMRFRRAFGE YSHVVAFNTL LFLMSFTVLC LTPVYSFLPG VYSVIYLYLT FYLTNDVSFL  360
AHIQWMVMFT PLVPFWITIA YIICISTKHF YWFFSNYLKR RVVFNGVSFS TFEEAALCTF  420
LLNKEMYLKL RSDVLLPLTQ YNRYLALYNK YKYFSGAMDT TSYREAACCH LAKALNDFSN  480
SGSDVLYQPP QTSITSAVLQ                                             500

SEQ ID NO: 56          moltype = AA  length = 306
FEATURE                Location/Qualifiers
source                 1..306
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 56
SGFRKMAFPS GKVEGCMVQV TCGTTTLNGL WLDDVVYCPR HVICTSEDML NPNYEDLLIR  60
KSNHNFLVQA GNVQLRVIGH SMQNCVLKLK VDTANPKTPK YKFVRIQPGQ TFSVLACYNG  120
SPSGVYQCAM RPNFTIKGSF LNGSCGSVGF NIDYDCVSFC YMHHMELPTG VHAGTDLEGN  180
FYGPFVDRQT AQAAGTDTTI TVNVLAWLYA AVINGDRWFL NRFTTTLNDF NLVAMKYNYE  240
PLTQDHVDIL GPLSAQTGIA VLDMCASLKE LLQNGMNGRT ILGSALLEDE FTPFDVVRQC  300
SGVTFQ                                                            306

SEQ ID NO: 57          moltype = AA  length = 290
FEATURE                Location/Qualifiers
source                 1..290
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 57
SAVKRTIKGT HHWLLLTILT SLLVLVQSTQ WSLFFFLYEN AFLPFAMGII AMSAFAMMFV  60
KHKHAFLCLF LLPSLATVAY FNMVYMPASW VMRIMTWLDM VDTSLSGFKL KDCVMYASAV  120
VLLILMTART VYDDGARRVW TLMNVLTLVY KVYYGNALDQ AISMWALIIS VTSNYSGVVT  180
TVMFLARGIV FMCVEYCPIF FITGNTLQCI MLVYCFLGYF CTCYFGLFCL LNRYFRLTLG  240
VYDYLVSTQE FRYMNSQGLL PPKNSIDAFK LNIKLLGVGG KPCIKVATVQ             290

SEQ ID NO: 58          moltype = AA  length = 83
FEATURE                Location/Qualifiers
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source                  1..83
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 58
SKMSDVKCTS VVLLSVLQQL RVESSSKLWA QCVQLHNDIL LAKDTTEAFE KMVSLLSVLL  60
SMQGAVDINK LCEEMLDNRA TLQ                                          83

SEQ ID NO: 59           moltype = AA  length = 198
FEATURE                 Location/Qualifiers
source                  1..198
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 59
AIASEFSSLP SYAAFATAQE AYEQAVANGD SEVVLKKLKK SLNVAKSEFD RDAAMQRKLE  60
KMADQAMTQM YKQARSEDKR AKVTSAMQTM LFTMLRKLDN DALNNIINNA RDGCVPLNII  120
PLTTAAKLMV VIPDYNTYKN TCDGTTFTYA SALWEIQQVV DADSKIVQLS EISMDNSPNL  180
AWPLIVTALR ANSAVKLQ                                                198

SEQ ID NO: 60           moltype = AA  length = 113
FEATURE                 Location/Qualifiers
source                  1..113
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 60
NNELSPVALR QMSCAAGTTQ TACTDDNALA YYNTTKGGRF VLALLSDLQD LKWARFPKSD  60
GTGTIYTELE PPCRFVTDTP KGPKVKYLYF IKGLNNLNRG MVLGSLAATV RLQ         113

SEQ ID NO: 61           moltype = AA  length = 139
FEATURE                 Location/Qualifiers
source                  1..139
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 61
AGNATEVPAN STVLSFCAFA VDAAKAYKDY LASGGQPITN CVKMLCTHTG TGQAITVTPE  60
ANMDQESFGG ASCCLYCRCH IDHPNPKGFC DLKGKYVQIP TTCANDPVGF TLKNTVCTVC  120
GMWKGYGCSC DQLREPMLQ                                               139

SEQ ID NO: 62           moltype = AA  length = 13
FEATURE                 Location/Qualifiers
source                  1..13
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 62
SADAQSFLNG FAV                                                     13

SEQ ID NO: 63           moltype = AA  length = 932
FEATURE                 Location/Qualifiers
source                  1..932
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 63
SADAQSFLNR VCGVSAARLT PCGTGTSTDV VYRAFDIYND KVAGFAKFLK TNCCRFQEKD  60
EDDNLIDSYF VVKRHTFSNY QHEETIYNLL KDCPAVAKHD FFKFRIDGDM VPHISRQRLT  120
KYTMADLVYA LRHFDEGNCD TLKEILVTYN CCDDDYFNKK DWYDFVENPD ILRVYANLGE  180
RVRQALLKTV QFCDAMRNAG IVGVLTLDNQ DLNGNWYDFG DFIQTTPGSG VPVVDSYYSL  240
LMPILTLTRA LTAESHVDTD LTKPYIKWDL LKYDFTEERL KLFDRYFKYW DQTYHPNCVN  300
CLDDRCILHC ANFNVLFSTV FPPTSFGPLV RKIFVDGVPF VVSTGYHFRE LGVVHNQDVN  360
LHSSRLSFKE LLVYAADPAM HAASGNLLLD KRTTCFSVAA LTNNVAFQTV KPGNFNKDFY  420
DFAVSKGFFK EGSSVELKHF FFAQDGNAAI SDYDYYRYNL PTMCDIRQLL FVVEVVDKYF  480
DCYDGGCINA NQVIVNNLDK SAGFPFNKWG KARLYYDSMS YEDQDALFAY TKRNVIPTIT  540
QMNLKYAISA KNRARTVAGV SICSTMTNRQ FHQKLLKSIA ATRGATVVIG TSKFYGGWHN  600
MLKTVYSDVE NPHLMGWDYP KCDRAMPNML RIMASLVLAR KHTTCCSLSH RFYRLANECA  660
QVLSEMVMCG GSLYVKPGGT SSGDATTAYA NSVFNICQAV TANVNALLST DGNKIADKYV  720
RNLQHRLYEC LYRNRDVDTD FVNEFYAYLR KHFSMMILSD DAVVCFNSTY ASQGLVASIK  780
NFKSVLYYQN NVFMSEAKCW TETDLTKGPH EFCSQHTMLV KQGDDYVYLP YPDPSRILGA  840
GCFVDDIVKT DGTLMIERFV SLAIDAYPLT KHPNQEYADV FHLYLQYIRK LHDELTGHML  900
DMYSVMLTND NTSRYWEPEF YEAMYTPHTV LQ                                932

SEQ ID NO: 64           moltype = AA  length = 601
FEATURE                 Location/Qualifiers
source                  1..601
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 64
AVGACVLCNS QTSLRCGACI RRPFLCCKCC YDHVISTSHK LVLSVNPYVC NAPGCDVTDV  60
TQLYLGGMSY YCKSHKPPIS FPLCANGQVF GLYKNTCVGS DNVTDFNAIA TCDWTNAGDY  120
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ILANTCTERL KLFAAETLKA TEETFKLSYG IATVREVLSD RELHLSWEVG KPRPPLNRNY  180
VFTGYRVTKN SKVQIGEYTF EKGDYGDAVV YRGTTTYKLN VGDYFVLTSH TVMPLSAPTL  240
VPQEHYVRIT GLYPTLNISD EFSSNVANYQ KVGMQKYSTL QGPPGTGKSH FAIGLALYYP  300
SARIVYTACS HAAVDALCEK ALKYLPIDKC SRIIPARARV ECFDKFKVNS TLEQYVFCTV  360
NALPETTADI VVFDEISMAT NYDLSVVNAR LRAKHYVYIG DPAQLPAPRT LLTKGTLEPE  420
YFNSVCRLMK TIGPDMFLGT CRRCPAEIVD TVSALVYDNK LKAHKDKSAQ CFKMFYKGVI  480
THDVSSAINR PQIGVVREFL TRNPAWRKAV FISPYNSQNA VASKILGLPT QTVDSSQGSE  540
YDYVIFTQTT ETAHSCNVNR FNVAITRAKV GILCIMSDRD LYDKLQFTSL EIPRRNVATL  600
Q                                                                  601

SEQ ID NO: 65          moltype = AA  length = 527
FEATURE                Location/Qualifiers
source                 1..527
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 65
AENVTGLFKD CSKVITGLHP TQAPTHLSVD TKFKTEGLCV DIPGIPKDMT YRRLISMMGF  60
KMNYQVNGYP NMFITREEAI RHVRAWIGFD VEGCHATREA VGTNLPLQLG FSTGVNLVAV  120
PTGYVDTPNN TDFSRVSAKP PPGDQFKHLI PLMYKGLPWN VVRIKIVQML SDTLKNLSDR  180
VVFVLWAHGF ELTSMKYFVK IGPERTCCLC DRRATCFSTA SDTYACWHHS IGFDYVYNPF  240
MIDVQQWGFT GNLQSNHDLY CQVHGNAHVA SCDAIMTRCL AVHECFVKRV DWTIEYPIIG  300
DELKINAACR KVQHMVVKAA LLADKFPVLH DIGNPKAIKC VPQADVEWKF YDAQPCSDKA  360
YKIEELFYSY ATHSDKFTDG VCLFWNCNVD RYPANSIVCR FDTRVLSNLN LPGCDGGSLY  420
VNKHAFHTPA FDKSAFVNLK QLPFFYYSDS PCESHGKQVV SDIDYVPLKS ATCITRCNLG  480
GAVCRHHANE YRLYLDAYNM MISAGFSLWV YKQFDTYNLW NTFTRLQ                527

SEQ ID NO: 66          moltype = AA  length = 346
FEATURE                Location/Qualifiers
source                 1..346
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 66
SLENVAFNVV NKGHFDGQQG EVPVSIINNT VYTKVDGVDV ELFENKTTLP VNVAFELWAK  60
RNIKPVPEVK ILNNLGVDIA ANTVIWDYKR DAPAHISTIG VCSMTDIAKK PTETICAPLT  120
VFFDGRVDGQ VDLFRNARNG VLITEGSVKG LQPSVGPKQA SLNGVTLIGE AVKTQFNYYK  180
KVDGVVQQLP ETYFTQSRNL QEFKPRSQME IDFLELAMDE FIERYKLEGY AFEHIVYGDF  240
SHSQLGGLHL LIGLAKRFKE SPFELEDFIP MDSTVKNYFI TDAQTGSSKC VCSVIDLLLD  300
DFVEIIKSQD LSVVSKVVKV TIDYTEISFM LWCKDGHVET FYPKLQ                 346

SEQ ID NO: 67          moltype = AA  length = 298
FEATURE                Location/Qualifiers
source                 1..298
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 67
SSQAWQPGVA MPNLYKMQRM LLEKCDLQNY GDSATLPKGI MMNVAKYTQL CQYLNTLTLA  60
VPYNMRVIHF GAGSDKGVAP GTAVLRQWLP TGTLLVDSDL NDFVSDADST LIGDCATVHT  120
ANKWDLIISD MYDPKTKNVT KENDSKEGFF TYICGFIQQK LALGGSVAIK ITEHSWNADL  180
YKLMGHFAWW TAFVTNVNAS SSEAFLIGCN YLGKPREQID GYVMHANYIF WRNTNPIQLS  240
SYSLFDMSKF PLKLRGTAVM SLKEGQINDM ILSLLSKGRL IIRENNRVVI SSDVLVNN    298

SEQ ID NO: 68          moltype = AA  length = 1273
FEATURE                Location/Qualifiers
source                 1..1273
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 68
MFVFLVLLPL VSSQCVNLTT RTQLPPAYTN SFTRGVYYPD KVFRSSVLHS TQDLFLPFFS  60
NVTWFHAIHV SGTNGTKRFD NPVLPFNDGV YFASTEKSNI IRGWIFGTTL DSKTQSLLIV  120
NNATNVVIKV CEFQFCNDPF LGVYYHKNNK SWMESEFRVY SSANNCTFEY VSQPFLMDLE  180
GKQGNFKNLR EFVFKNIDGY FKIYSKHTPI NLVRDLPQGF SALEPLVDLP IGINITRFQT  240
LLALHRSYLT PGDSSSGWTA GAAAYYVGYL QPRTFLLKYN ENGTITDAVD CALDPLSETK  300
CTLKSFTVEK GIYQTSNFRV QPTESIVRFP NITNLCPFGE VFNATRFASV YAWNRKRISN  360
CVADYSVLYN SASFSTFKCY GVSPTKLNDL CFTNVYADSF VIRGDEVRQI APGQTGKIAD  420
YNYKLPDDFT GCVIAWNSNN LDSKVGGNYN YLYRLFRKSN LKPFERDIST EIYQAGSTPC  480
NGVEGFNCYF PLQSYGFQPT NGVGYQPYRV VVLSFELLHA PATVCGPKKS TNLVKNKCVN  540
FNFNGLTGTG VLTESNKKFL PFQQFGRDIA DTTDAVRDPQ TLEILDITPC SFGGVSVITP  600
GTNTSNQVAV LYQDVNCTEV PVAIHADQLT PTWRVYSTGS NVFQTRAGCL IGAEHVNNSY  660
ECDIPIGAGI CASYQTQTNS PRRARSVASQ SIIAYTMSLG AENSVAYSNN SIAIPTNFTI  720
SVTTEILPVS MTKTSVDCTM YICGDSTECS NLLLQYGSFC TQLNRALTGI AVEQDKNTQE  780
VFAQVKQIYK TPPIKDFGGF NFSQILPDPS KPSKRSFIED LLFNKVTLAD AGFIKQYGDC  840
LGDIAARDLI CAQKFNGLTV LPPLLTDEMI AQYTSALLAG TITSGWTFGA GAALQIPFAM  900
QMAYRFNGIG VTQNVLYENQ KLIANQFNSA IGKIQDSLSS TASALGKLQD VVNQNAQALN  960
TLVKQLSSNF GAISSVLNDI LSRLDKVEAE VQIDRLITGR LQSLQTYVTQ QLIRAAEIRA  1020
SANLAATKMS ECVLGQSKRV DFCGKGYHLM SFPQSAPHGV VFLHVTYVPA QEKNFTTAPA  1080
ICHDGKAHFP REGVFVSNGT HWFVTQRNFY EPQIITTDNT FVSGNCDVVI GIVNNTVYDP  1140
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LQPELDSFKE ELDKYFKNHT SPDVDLGDIS GINASVVNIQ KEIDRLNEVA KNLNESLIDL  1200
QELGKYEQYI KWPWYIWLGF IAGLIAIVMV TIMLCCMTSC CSCLKGCCSC GSCCKFDEDD  1260
SEPVLKGVKL HYT                                                     1273

SEQ ID NO: 69          moltype = AA  length = 275
FEATURE                Location/Qualifiers
source                 1..275
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 69
MDLFMRIFTI GTVTLKQGEI KDATPSDFVR ATATIPIQAS LPFGWLIVGV ALLAVFQSAS  60
KIITLKKRWQ LALSKGVHFV CNLLLLFVTV YSHLLLVAAG LEAPFLYLYA LVYFLQSINF  120
VRIIMRLWLC WKCRSKNPLL YDANYFLCWH TNCYDYCIPY NSVTSSIVIT SGDGTTSPIS  180
EHDYQIGGYT EKWESGVKDC VVLHSYFTSD YYQLYSTQLS TDTGVEHVTF FIYNKIVDEP  240
EEHVQIHTID GSSGVVNPVM EPIYDEPTTT TSVPL                             275

SEQ ID NO: 70          moltype = AA  length = 75
FEATURE                Location/Qualifiers
source                 1..75
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 70
MYSFVSEETG TLIVNSVLLF LAFVVFLLVT LAILTALRLC AYCCNIVNVS LVKPSFYVYS  60
RVKNLNSSRV PDLLV                                                   75

SEQ ID NO: 71          moltype = AA  length = 222
FEATURE                Location/Qualifiers
source                 1..222
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 71
MADSNGTITV EELKKLLEQW NLVIGFLFLT WICLLQFAYA NRNRFLYIIK LIFLWLLWPV  60
TLACFVLAAV YRINWITGGI AIAMACLVGL MWLSYFIASF RLFARTRSMW SFNPETNILL  120
NVPLHGTILT RPLLESELVI GAVILRGHLR IAGHHLGRCD IKDLPKEITV ATSRTLSYYK  180
LGASQRVAGD SGFAAYSRYR IGNYKLNTDH SSSSDNIALL VQ                     222

SEQ ID NO: 72          moltype = AA  length = 61
FEATURE                Location/Qualifiers
source                 1..61
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 72
MFHLVDFQVT IAEILLIIMR TFKVSIWNLD YIINLIIKNL SKSLTENKYS QLDEEQPMEI  60
D                                                                  61

SEQ ID NO: 73          moltype = AA  length = 121
FEATURE                Location/Qualifiers
source                 1..121
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 73
MKIILFLALI TLATCELYHY QECVRGTTVL LKEPCSSGTY EGNSPFHPLA DNKFALTCFS  60
TQFAFACPDG VKHVYQLRAR SVSPKLFIRQ EEVQELYSPI FLIVAAIVFI TLCFTLKRKT  120
E                                                                  121

SEQ ID NO: 74          moltype = AA  length = 43
FEATURE                Location/Qualifiers
source                 1..43
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 74
MIELSLIDFY LCFLAFLLFL VLIMLIIFWF SLELQDHNET CHA                    43

SEQ ID NO: 75          moltype = AA  length = 121
FEATURE                Location/Qualifiers
source                 1..121
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 75
MKFLVFLGII TTVAAFHQEC SLQSCTQHQP YVVDDPCPIH FYSKWYIRVG ARKSAPLIEL  60
CVDEAGSKSP IQYIDIGNYT VSCLPFTINC QEPKLGSLVV RCSFYEDFLE YHDVRVVLDF  120
I                                                                  121

SEQ ID NO: 76          moltype = AA  length = 419
FEATURE                Location/Qualifiers
source                 1..419
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                     mol_type = protein
                     organism = synthetic construct
SEQUENCE: 76
MSDNGPQNQR NAPRITFGGP SDSTGSNQNG ERSGARSKQR RPQGLPNNTA SWFTALTQHG  60
KEDLKFPRGQ GVPINTNSSP DDQIGYYRRA TRRIRGGDGK MKDLSPRWYF YYLGTGPEAG  120
LPYGANKDGI IWVATEGALN TPKDHIGTRN PANNAAIVLQ LPQGTTLPKG FYAEGSRGGS  180
QASSRSSSRS RNSSRNSTPG SSRGTSPARM AGNGGDAALA LLLLDRLNQL ESKMSGKGQQ  240
QQGQTVTKKS AAEASKKPRQ KRTATKAYNV TQAFGRRGPE QTQGNFGDQE LIRQGTDYKH  300
WPQIAQFAPS ASAFFGMSRI GMEVTPSGTW LTYTGAIKLD DKDPNFKDQV ILLNKHIDAY  360
KTFPPTEPKK DKKKKADETQ ALPQRQKKQQ TVTLLPAADL DDFSKQLQQS MSSADSTQA   419

SEQ ID NO: 77          moltype = AA  length = 38
FEATURE                Location/Qualifiers
source                 1..38
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 77
MGYINVFAFP FTIYSLLLCR MNSRNYIAQV DVVNFNLT                          38

SEQ ID NO: 78          moltype = DNA  length = 29903
FEATURE                Location/Qualifiers
source                 1..29903
                       mol_type = genomic DNA
                       organism = Coronavirus 2
SEQUENCE: 78
attaaaggtt tataccttcc caggtaacaa accaaccaac tttcgatctc ttgtagatct  60
gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcatgc ttagtgcact  120
cacgcagtat aattaataac taattactgt cgttgacagg acacgagtaa ctcgtctatc  180
ttctgcaggc tgcttacggt ttcgtccgtg ttgcagccga tcatcagcac atctaggttt  240
tgtccgggtg tgaccgaaag gtaagatgga gagccttgtc cctggtttca acgagaaaac  300
acacgtccaa cttagtttgc ctgttttaca ggttcgcgac gtgctcgtac gtggctttgg  360
agactccgtg gaggaggtct tatcagaggc acgtcaacat cttaaagatg gcacttgtgg  420
cttagtagaa gttgaaaaag gcgttttgcc tcaacttgaa cagccctatg tgttcatcaa  480
acgttcggat gctcgaactg cacctcatgg tcatgttatg gttgagctgg tagcagaact  540
cgaaggcatt cagtacggtc gtagtggtga gacacttggt gtccttgtcc ctcatgtggg  600
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aaaaaaaaaa aaaaaaaaaa aaa                                          29903
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1. (canceled)

2. The method of claim **13** a) wherein one plasmid has eight transcription cassettes for vRNA production.

3. The method of claim **2** wherein one plasmid has one transcription cassette for mRNA production and another plasmid has three transcription cassettes for mRNA production.

4. The method of claim **13** a) wherein one plasmid has six transcription cassettes for vRNA production and another plasmid has two transcription cassettes for vRNA production.

5. The method of claim **4** wherein one plasmid has four transcription cassettes for mRNA production.

6. The method of claim **13** a) wherein the HA is a type A HA.

7. The) The method of claim **13** a) wherein the HA is a type B HA.

8. (canceled)

9. The method of claim **13** b) further comprising four transcription cassettes for mRNA production.

10. The method of claim **9** wherein one plasmid has one transcription cassette for mRNA production and another plasmid has three transcription cassettes for mRNA production.

11. The method of claim **9** wherein one plasmid has the four transcription cassettes for mRNA production.

12. (canceled)

13. A method to prepare influenza virus, comprising:

contacting a cell with a set of plasmids in an amount effective to yield infectious influenza virus, wherein the set of plasmids is

a) a set of three plasmids for influenza virus production together having eight transcription cassettes for influenza virus vRNA production and four transcription cassettes for influenza virus mRNA production, wherein the transcription cassettes for vRNA production include a transcription cassette comprising a promoter operably linked to an influenza virus PA cDNA linked to a transcription termination sequence, a transcription cassette comprising a promoter operably linked to an influenza virus PB1 cDNA linked to a transcription termination sequence, a transcription cassette comprising a promoter operably linked to an influenza virus PB2 cDNA linked to a transcription termination sequence, a transcription cassette comprising a promoter operably linked to an influenza virus HA cDNA linked to a transcription termination sequence, a transcription cassette comprising a promoter operably linked to an influenza virus NP cDNA linked to a transcription termination sequence, a transcription cassette comprising a promoter operably linked to an influenza virus NA cDNA linked to a transcription termination sequence, a transcription cassette comprising a promoter operably linked to an influenza virus M cDNA linked to a transcription termination sequence, and a transcription cassette comprising a promoter operably linked to an influenza virus NS cDNA linked to a transcription termination sequence; wherein the transcription cassettes for mRNA production include a transcription cassette comprising a PolII promoter operably linked to a DNA coding region for influenza virus PA linked to a PolII transcription termination sequence, a transcription cassette comprising a PolII promoter operably linked to a DNA coding region for influenza virus PB1 linked to a PolII transcription termination sequence, a transcription cassette comprising a PolII promoter operably linked to a DNA coding region for influenza virus PB2 linked to a PolII transcription termination sequence, and a transcription cassette comprising a PolII promoter operably linked to a DNA coding region for influenza virus NP linked to a PolII transcription termination sequence, and wherein transfection of a cell with the composition yields titers of at least 1×10^2 $TCID_{50}$/mL;

b) a set of two plasmids for influenza virus vRNA production together comprising eight transcription cassettes for vRNA production, which transcription cassettes comprise a transcription cassette comprising a PolI promoter operably linked to an influenza virus PA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus PB1 cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus PB2 cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus HA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus NP cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus NA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus M cDNA linked to a PolI transcription termination sequence, and/or a transcription cassette comprising a PolI promoter operably linked to an influenza virus NS cDNA linked to a PolI transcription termination sequence, wherein the composition includes at least six transcription cassettes for vRNA, transfection of a cell with the composition yields titers of at least 1×10^2 $TCID_{50}$/mL; or

c) a set of plasmids comprising one or two plasmids having eight transcription cassettes for influenza virus vRNA production and four plasmids each having a transcription cassette for influenza virus mRNA production for a different influenza virus protein, wherein the transcription cassettes for vRNA production include a transcription cassette comprising a PolI promoter operably linked to an influenza virus PA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus PB1 cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus PB2 cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus HA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus NP cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus NA

cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus M cDNA linked to a PolI transcription termination sequence, and a transcription cassette comprising a PolI promoter operably linked to an influenza virus NS cDNA linked to a PolI transcription termination sequence; wherein the transcription cassettes for mRNA production include a transcription cassette comprising a PolII promoter operably linked to a DNA coding region for influenza virus PA linked to a PolII transcription termination sequence, a transcription cassette comprising a PolII promoter operably linked to a DNA coding region for influenza virus PB1 linked to a PolII transcription termination sequence, a transcription cassette comprising a PolII promoter operably linked to a DNA coding region for influenza virus PB2 linked to a PolII transcription termination sequence, and a transcription cassette comprising a PolII promoter operably linked to a DNA coding region for influenza virus NP linked to a PolII transcription termination sequence.

14. (canceled)

15. A method to prepare influenza virus, comprising contacting a cell with at least two plasmids which include transcription cassettes for vRNA production including a transcription cassette comprising a PolI promoter operably linked to an influenza virus PA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus PB1 cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus PB2 cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus HA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus NP cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus NA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus M cDNA linked to a PolI transcription termination sequence, and a transcription cassette comprising a PolI promoter operably linked to an influenza virus NS cDNA linked to a PolI transcription termination sequence, wherein the cell is contacted with eight transcription cassettes for vRNA, wherein the cell expresses influenza virus PA, PB1, PB2 and NP and wherein the cell produces titers of at least 1×10^2 $TCID_{50}$/mL.

16. The method of claim **15** wherein the influenza virus PA, PB1, PB2 and NP are expressed from four plasmids for mRNA production each having a transcription cassette for mRNA production of one of PA, PB1, PB2 or NP.

17. A method to prepare influenza virus, comprising contacting a cell with four plasmids for mRNA production and eight transcription cassettes for influenza virus vRNA production, wherein the eight transcription cassettes for vRNA production include a transcription cassette comprising a PolI promoter operably linked to an influenza virus PA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a Pol promoter operably linked to an influenza virus PB1 cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus PB2 cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus HA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus NP cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus NA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus M cDNA linked to a PolI transcription termination sequence, and a transcription cassette comprising a PolI promoter operably linked to an influenza virus NS cDNA linked to a PolI transcription termination sequence, wherein the four plasmids for mRNA production each having a different transcription cassettes for mRNA production of an influenza virus protein selected from PA, PB1, PB2 and NP, and wherein the cell produces titers of at least 1×10^2 TCID 50/mL

18. The method of claim **15** or **17** further comprising a transcription cassette comprising a promoter linked to 5' influenza virus sequences comprising 5' influenza virus noncoding sequences linked to a DNA of interest linked to 3' influenza virus sequences comprising 3' influenza virus noncoding sequences linked to a transcription termination sequence.

19. The method of claim **15** or **17** further comprising isolating the virus.

20. A plasmid for vRNA production comprising a transcription cassette comprising a PolI promoter operably linked to an influenza virus HA cDNA linked to a PolI transcription termination sequence and a transcription cassette comprising a PolI promoter operably linked to an influenza virus NA cDNA linked to a PolI transcription termination sequence, wherein transfection of a cell with the plasmid and transcription cassettes for vRNA production of PA, PB1, PB2, NP, M and NS yields titers of at least 1×10^2 $TCID_{50}$/mL.

21. A plasmid for influenza virus production comprising a transcription cassette comprising a PolI promoter operably linked to an influenza virus HA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus NA cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus M cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter operably linked to an influenza virus NS cDNA linked to a PolI transcription termination sequence, a transcription cassette comprising a PolI promoter and a PolI transcription termination sequence and a PolII promoter and a PolII transcription termination sequence each operably linked to a DNA segment for influenza virus PA, a transcription cassette comprising a PolI promoter and a PolI transcription termination sequence and a PolII promoter and PolII transcription termination sequence each operably linked to a DNA segment for influenza virus PB1, a transcription cassette comprising a PolI promoter and a PolI transcription termination sequence and a PolII promoter and a PolII transcription termination sequence each operably linked to a DNA segment encoding influenza virus PB2, and a transcription cassette comprising

a PolI promoter and a PolI transcription termination sequence and a PolII promoter and a PolII transcription termination sequence each operably linked to a DNA segment for influenza virus NP.

22. A method to prepare influenza virus, comprising:

contacting a cell with the plasmid of claim **21** in an amount effective to yield infectious influenza virus.

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