

Improved seed viruses

Cross-reference to related applications

The present application claims priority from United States Provisional Patent Application No. 63/508,946 filed on 19 June 2023, the contents of which is incorporated herein by reference in its entirety.

Technical field

The present disclosure relates to the field of influenza viruses. More particularly, this disclosure relates to donor or seed influenza viruses for use in preparing reassortant influenza viruses for vaccine production.

Background

Influenza is a major respiratory disease in mammalian species and is responsible for substantial mortality, morbidity and economic losses each year. Three broad types of influenza viruses are recognised, Type A, Type B and Type C, which are defined by the absence of serological cross-reactivity between their internal proteins. Influenza A viruses are further classified into subtypes based on antigenic and genetic differences of their glycoproteins, the haemagglutinin (HA) and neuraminidase (NA) proteins.

More recent vaccine production methods may include the generation and culturing of reassortant influenza viruses in cell culture, such as in Madin Darby Canine Kidney (MDCK) cells. However, certain influenza viruses appear to be less amenable than others to growth and replication when cultured as a reassortant virus containing the backbone viral segments of a donor influenza virus. This can lead to challenges in viral protein yield and vaccine manufacture. Accordingly, there remains a need for the development of donor influenza viruses whose backbone viral segments confer efficient growth and replication to reassortant viruses expressing the HA and/or NA proteins of vaccine virus candidates in cell culture.

Summary

The present disclosure is based on the surprising discovery of particular influenza viruses that can be utilised as donor or seed viruses in reassortment methods to improve the growth and/or yield of influenza viruses cultured on cells, such as in Madin Darby Canine Kidney (MDCK) cells.

In a first aspect, the present disclosure provides an isolated influenza virus comprising one or more of PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.

Suitably, the present disclosure provides an isolated influenza virus comprising one or more of PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from SEQ ID NOs: 9-17, or a fragment, variant or derivative thereof. In certain examples, the isolated influenza virus comprises PA, PB2, NP, M and NS viral segments that encode the viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from SEQ ID NOs: 9 and 12-17, or a fragment, variant or derivative thereof. In such examples, the isolated influenza virus may or may not include the PB1 viral segment that encodes the viral proteins that independently comprise, consist of or consist essentially of amino acid sequences SEQ ID NO: 10 and/or 11 or a fragment, variant or derivative thereof. Accordingly, in some examples, the isolated influenza virus comprises PA, PB1, PB2, NP, M and NS viral segments that encode the viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from SEQ ID NOs: 9-17, or a fragment, variant or derivative thereof.

Suitably, the isolated influenza virus of the present aspect comprises one or more of the PB2, PB1, PA, M, NP and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a fragment, variant or derivative thereof. In one example, the isolated influenza virus of the present aspect comprises one or more of the PB2, PB1, PA, M, NP and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from SEQ ID NOs: 1-6, or a fragment, variant or derivative thereof. In certain examples, the isolated influenza virus comprises the PB2, PA, M, NP and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from SEQ ID NOs: 1 and 3-6, or a fragment, variant or derivative thereof. For such examples, the isolated influenza virus may or may not include the PB1 viral segment that comprises, consists of or consists essentially of a nucleotide sequence set forth in

SEQ ID NO: 2. Accordingly, in some examples, the isolated influenza virus comprises PA, PB1, PB2, NP, M and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from SEQ ID NOs: 1-6, or a fragment, variant or derivative thereof.

In certain examples, the isolated influenza virus is capable of enhanced replication when grown in cells relative to a wild-type influenza virus isolate that does not comprise the one or more PA, PB1, PB2, NP, M and NS viral segments.

Suitably, the isolated influenza virus is capable of enhanced yield of a viral protein, such as a haemagglutinin (HA) protein, when grown in cells relative to a wild-type influenza virus isolate that does not comprise the one or more PA, PB1, PB2, NP, M and NS viral segments.

In particular examples, the cells are MDCK cells.

Suitably, the isolated influenza virus of the present aspect comprises five or six of the PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from those set forth in:

- (a) SEQ ID NOs: 9-17 or a fragment, variant or derivative thereof;
- (b) SEQ ID NOs: 28-36 or a fragment, variant or derivative thereof;
- (c) SEQ ID NOs: 47-55 or a fragment, variant or derivative thereof;
- (d) SEQ ID NOs: 66-74, or a fragment, variant or derivative thereof;
- (e) SEQ ID NOs: 85-93, or a fragment, variant or derivative thereof; or
- (f) SEQ ID NOs: 104-112, or a fragment, variant or derivative thereof.

Thus, in one example, the isolated influenza virus of the present aspect comprises five or six of the PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 9-17, or a fragment, variant or derivative thereof.

In various examples, the isolated influenza virus of the present aspect comprises five or six of the PA, PB1, PB2, NP, M and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from those set forth in:

- (a) SEQ ID NOs: 1-6 or a fragment, variant or derivative thereof;
- (b) SEQ ID NOs: 20-25 or a fragment, variant or derivative thereof;
- (c) SEQ ID NOs: 39-44 or a fragment, variant or derivative thereof;
- (d) SEQ ID NOs: 58-63 or a fragment, variant or derivative thereof
- (e) SEQ ID NOs: 77-82, or a fragment, variant or derivative thereof; or
- (f) SEQ ID NOs: 96-101, or a fragment, variant or derivative thereof.

Thus, in one example, the isolated influenza virus of the present aspect comprises five or six of the PA, PB1, PB2, NP, M and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from those set forth in SEQ ID NOs: 1-6 or a fragment, variant or derivative thereof.

In some examples, the isolated influenza virus of the present aspect further comprises a heterologous or chimeric HA viral segment and/or a heterologous or chimeric NA viral segment.

Suitably, the isolated influenza virus is of a N1, N2, N3, N7, or N9 subtype. More particularly, the isolated influenza virus can be of an N1 or N2 subtype.

Suitably, the isolated influenza virus is of a H1, H2, H3, H5, H7, or H9 subtype. More particularly, the isolated influenza virus may be of a H1 or H3 subtype.

For certain examples, the isolated influenza virus is of a H1N1 subtype or a H3N2 subtype.

In particular examples, the isolated influenza virus is a recombinant influenza virus.

In other examples, the isolated influenza virus is a reassortant influenza virus.

In a second aspect, the present disclosure provides a method of preparing an influenza virus, said method including the step of contacting a cell with one or more genetic constructs that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.

Suitably, the one or more genetic constructs comprise or encode one or more of PB2, PB1, PA, M, NP and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a nucleotide sequence complementary thereto, or a fragment, variant or derivative thereof.

In a third aspect, the present disclosure provides a method of preparing an influenza virus, said method including the step of contacting a cell with an isolated influenza virus comprising one or more of PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.

For the method of the present aspect, the isolated influenza virus is suitably that of the first aspect.

The method of the second or third aspects may further include the step of isolating or harvesting the influenza virus and/or one or more of the viral proteins from the cells.

In a fourth aspect, the present disclosure provides an isolated influenza virus prepared by the method of the second or third aspects.

In a fifth aspect, the present disclosure provides an isolated cell infected with the isolated influenza virus of the first or fourth aspects.

Suitably, the cell is an MDCK cell.

In a sixth aspect, the present disclosure provides a genetic construct that encodes one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74,, 85-93 and 104-112, or a fragment, variant or derivative thereof.

In a seventh aspect, the present disclosure provides a plurality of genetic constructs that encode one or more viral proteins that independently comprise, consist of or consist essentially of an

amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.

Referring to the sixth and seventh aspects, the genetic construct or the plurality of genetic constructs suitably comprise or encode one or more of PB2, PB1, PA, M, NP and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a nucleotide sequence complementary thereto, or a fragment, variant or derivative thereof.

In an eighth aspect, the present disclosure provides a method of making an immunogenic composition, including the steps of:

- (a) providing the isolated influenza virus of the first or fourth aspects and/or a viral protein derived therefrom; and
- (b) combining the isolated influenza virus and/or the viral protein with an adjuvant and/or treating the isolated influenza virus with an agent that inactivates the virus.

In some examples, the adjuvant comprises an immunostimulatory DNA sequence, a bacterium-derived component, an aluminium salt (alum) or a squalene oil-in-water emulsion system.

In a ninth aspect, the present disclosure provides an immunogenic composition produced according to the method of the eighth aspect.

In a tenth aspect, the present disclosure provides an immunogenic composition comprising the isolated influenza virus of the first or fourth aspects and/or a viral protein derived therefrom and a pharmaceutically acceptable carrier, diluent or excipient.

Suitably, the isolated influenza virus of the first or fourth aspects and/or a viral protein derived therefrom or the immunogenic composition of the ninth or tenth aspects is for use in therapy.

Suitably, the isolated influenza virus of the first or fourth aspects and/or a viral protein derived therefrom or the immunogenic composition of the ninth or tenth aspects is for use in a method of eliciting an immune response in a subject.

Suitably, the isolated influenza virus of the first or fourth aspects and/or a viral protein derived therefrom or the immunogenic composition of the ninth or tenth aspects is for use in a method of preventing and/or treating an influenza-associated disease, disorder or condition in a subject.

In an eleventh aspect, the present disclosure provides a method of eliciting an immune response in a subject, said method including the step of administering a therapeutically effective amount of the isolated influenza virus of the first or fourth aspects and/or a viral protein derived therefrom or the immunogenic composition of the ninth or tenth aspects to the subject to thereby elicit the immune response in the subject.

In a twelfth aspect, the present disclosure provides a method of preventing and/or treating an influenza-associated disease, disorder or condition in a subject, said method including the step of administering a therapeutically effective amount of the isolated influenza virus of the first or fourth aspects and/or a viral protein derived therefrom or the immunogenic composition of the ninth or tenth aspects to the subject to thereby prevent and/or treat the influenza-associated disease, disorder or condition.

In a thirteenth aspect, the present disclosure provides for the use of the isolated influenza virus of the first or fourth aspects and/or a viral protein derived therefrom or the immunogenic composition of the ninth or tenth aspects in the manufacture of a medicament for eliciting an immune response in a subject.

In a fourteenth aspect, the present disclosure provides for the use of the isolated influenza virus of the first or fourth aspects and/or a viral protein derived therefrom or the immunogenic composition of the ninth or tenth aspects in the manufacture of a medicament for preventing and/or treating an influenza-associated disease, disorder or condition in a subject.

Brief description of the drawings

The following figures form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to one or more of these figures in combination with the detailed description of specific embodiments presented herein. It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the above-described embodiments,

without departing from the broad general scope of the present disclosure. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Figure 1. Schematic diagram of candidate vaccine virus (CVV) transfection and rescue.

Figure 2. Individual HA yield results for A/Idaho/07/2018 wild-type and reassortant strains.

Figure 3. Individual HA yield results for A/Nebraska/14/2019 wild-type and reassortant strains.

Figure 4. Individual HA yield results for A/Iowa/56/2019 wild-type and reassortant strains.

Figure 5. Individual HA yield results for A/Delaware/55/2019 wild-type and reassortant strains.

Figure 6. Individual HA yield results for A/Illinois/02/2020 wild-type and reassortant strains.

Figure 7. Individual HA yield results for A/Canberra/407/2019 wild-type and reassortant strains.

Figure 8. Individual HA yield results for A/Tasmania/503/2020 wild-type and reassortant strains.

Figure 9. Individual HA yield results for A/Bangladesh/1002/2020 wild-type and reassortant strains.

Figure 10. Individual HA yield results for A/Darwin/94/2019 wild-type and reassortant strains.

Figure 11. Individual HA yield results for A/Delaware/39/2019 wild-type and reassortant strains.

Figure 12. Individual HA yield results for A/Virginia/03/2020 wild-type and reassortant strains.

Figure 13. Summary of the HA yield results for the A/Ohio/02/2019 donor strain.

Figure 14. Percentage increase in HA yield for reassortant viruses derived from backbone or donor virus strains relative to manufacturing or vaccine virus controls.

Key to the Sequence Listing

SEQ ID NO: 1	Nucleotide sequence of PB2 viral segment of A/Ohio/02/2019
SEQ ID NO: 2	Nucleotide sequence of PB1 viral segment of A/Ohio/02/2019
SEQ ID NO: 3	Nucleotide sequence of PA viral segment of A/Ohio/02/2019
SEQ ID NO: 4	Nucleotide sequence of NP viral segment of A/Ohio/02/2019
SEQ ID NO: 5	Nucleotide sequence of NS viral segment of A/Ohio/02/2019
SEQ ID NO: 6	Nucleotide sequence of M viral segment of A/Ohio/02/2019
SEQ ID NO: 7	Nucleotide sequence of HA viral segment of A/Ohio/02/2019
SEQ ID NO: 8	Nucleotide sequence of NA viral segment of A/Ohio/02/2019
SEQ ID NO: 9	Amino acid sequence of PB2 protein of A/Ohio/02/2019
SEQ ID NO: 10	Amino acid sequence of PB1 protein of A/Ohio/02/2019
SEQ ID NO: 11	Amino acid sequence of PB1-F2 protein of A/Ohio/02/2019
SEQ ID NO: 12	Amino acid sequence of PA protein of A/Ohio/02/2019
SEQ ID NO: 13	Amino acid sequence of NP protein of A/Ohio/02/2019
SEQ ID NO: 14	Amino acid sequence of NS1 protein of A/Ohio/02/2019
SEQ ID NO: 15	Amino acid sequence of NEP protein of A/Ohio/02/2019
SEQ ID NO: 16	Amino acid sequence of M1 protein of A/Ohio/02/2019
SEQ ID NO: 17	Amino acid sequence of M2 protein of A/Ohio/02/2019
SEQ ID NO: 18	Amino acid sequence of HA protein of A/Ohio/02/2019
SEQ ID NO: 19	Amino acid sequence of NA protein of A/Ohio/02/2019
SEQ ID NO: 20	Nucleotide sequence of PB2 viral segment of A/Singapore/TT1384/2016
SEQ ID NO: 21	Nucleotide sequence of PB1 viral segment of A/Singapore/TT1384/2016
SEQ ID NO: 22	Nucleotide sequence of PA viral segment of A/Singapore/TT1384/2016
SEQ ID NO: 23	Nucleotide sequence of NP viral segment of A/Singapore/TT1384/2016
SEQ ID NO: 24	Nucleotide sequence of NS viral segment of A/Singapore/TT1384/2016
SEQ ID NO: 25	Nucleotide sequence of M viral segment of A/Singapore/TT1384/2016

SEQ ID NO: 26	Nucleotide sequence of HA viral segment of A/Singapore/TT1384/2016
SEQ ID NO: 27	Nucleotide sequence of NA viral segment of A/Singapore/TT1384/2016
SEQ ID NO: 28	Amino acid sequence of PB2 protein of A/Singapore/TT1384/2016
SEQ ID NO: 29	Amino acid sequence of PB1 protein of A/Singapore/TT1384/2016
SEQ ID NO: 30	Amino acid sequence of PB1-F2 protein of A/Singapore/TT1384/2016
SEQ ID NO: 31	Amino acid sequence of PA protein of A/Singapore/TT1384/2016
SEQ ID NO: 32	Amino acid sequence of NP protein of A/Singapore/TT1384/2016
SEQ ID NO: 33	Amino acid sequence of NS1 protein of A/Singapore/TT1384/2016
SEQ ID NO: 34	Amino acid sequence of NEP protein of A/Singapore/TT1384/2016
SEQ ID NO: 35	Amino acid sequence of M1 protein of A/Singapore/TT1384/2016
SEQ ID NO: 36	Amino acid sequence of M2 protein of A/Singapore/TT1384/2016
SEQ ID NO: 37	Amino acid sequence of HA protein of A/Singapore/TT1384/2016
SEQ ID NO: 38	Amino acid sequence of NA protein of A/Singapore/TT1384/2016
SEQ ID NO: 39	Nucleotide sequence of PB2 viral segment of A/South Carolina/04/2017
SEQ ID NO: 40	Nucleotide sequence of PB1 viral segment of A/South Carolina/04/2017
SEQ ID NO: 41	Nucleotide sequence of PA viral segment of A/South Carolina/04/2017
SEQ ID NO: 42	Nucleotide sequence of NP viral segment of A/South Carolina/04/2017
SEQ ID NO: 43	Nucleotide sequence of NS viral segment of A/South Carolina/04/2017
SEQ ID NO: 44	Nucleotide sequence of M viral segment of A/South Carolina/04/2017
SEQ ID NO: 45	Nucleotide sequence of HA viral segment of A/South Carolina/04/2017
SEQ ID NO: 46	Nucleotide sequence of NA viral segment of A/South Carolina/04/2017
SEQ ID NO: 47	Amino acid sequence of PB2 protein of A/South Carolina/04/2017
SEQ ID NO: 48	Amino acid sequence of PB1 protein of A/South Carolina/04/2017

SEQ ID NO: 49	Amino acid sequence of PB1-F2 protein of A/South Carolina/04/2017
SEQ ID NO: 50	Amino acid sequence of PA protein of A/South Carolina/04/2017
SEQ ID NO: 51	Amino acid sequence of NP protein of A/South Carolina/04/2017
SEQ ID NO: 52	Amino acid sequence of NS1 protein of A/South Carolina/04/2017
SEQ ID NO: 53	Amino acid sequence of NEP protein of A/South Carolina/04/2017
SEQ ID NO: 54	Amino acid sequence of M1 protein of A/South Carolina/04/2017
SEQ ID NO: 55	Amino acid sequence of M2 protein of A/South Carolina/04/2017
SEQ ID NO: 56	Amino acid sequence of HA protein of A/South Carolina/04/2017
SEQ ID NO: 57	Amino acid sequence of NA protein of A/South Carolina/04/2017
SEQ ID NO: 58	Nucleotide sequence of PB2 viral segment of A/Alaska/06/2019
SEQ ID NO: 59	Nucleotide sequence of PB1 viral segment of A/Alaska/06/2019
SEQ ID NO: 60	Nucleotide sequence of PA viral segment of A/Alaska/06/2019
SEQ ID NO: 61	Nucleotide sequence of NP viral segment of A/Alaska/06/2019
SEQ ID NO: 62	Nucleotide sequence of NS viral segment of A/Alaska/06/2019
SEQ ID NO: 63	Nucleotide sequence of M viral segment of A/Alaska/06/2019
SEQ ID NO: 64	Nucleotide sequence of HA viral segment of A/Alaska/06/2019
SEQ ID NO: 65	Nucleotide sequence of NA viral segment of A/Alaska/06/2019
SEQ ID NO: 66	Amino acid sequence of PB2 protein of A/Alaska/06/2019
SEQ ID NO: 67	Amino acid sequence of PB1 protein of A/Alaska/06/2019
SEQ ID NO: 68	Amino acid sequence of PB1-F2 protein of A/Alaska/06/2019
SEQ ID NO: 69	Amino acid sequence of PA protein of A/Alaska/06/2019
SEQ ID NO: 70	Amino acid sequence of NP protein of A/Alaska/06/2019
SEQ ID NO: 71	Amino acid sequence of NS1 protein of A/Alaska/06/2019
SEQ ID NO: 72	Amino acid sequence of NEP protein of A/Alaska/06/2019
SEQ ID NO: 73	Amino acid sequence of M1 protein of A/Alaska/06/2019
SEQ ID NO: 74	Amino acid sequence of M2 protein of A/Alaska/06/2019
SEQ ID NO: 75	Amino acid sequence of HA protein of A/Alaska/06/2019
SEQ ID NO: 76	Amino acid sequence of NA protein of A/Alaska/06/2019
SEQ ID NO: 77	Nucleotide sequence of PB2 viral segment of A/Darwin/11/2021
SEQ ID NO: 78	Nucleotide sequence of PB1 viral segment of A/Darwin/11/2021
SEQ ID NO: 79	Nucleotide sequence of PA viral segment of A/Darwin/11/2021
SEQ ID NO: 80	Nucleotide sequence of NP viral segment of A/Darwin/11/2021
SEQ ID NO: 81	Nucleotide sequence of NS viral segment of A/Darwin/11/2021

SEQ ID NO: 82	Nucleotide sequence of M viral segment of A/Darwin/11/2021
SEQ ID NO: 83	Nucleotide sequence of HA viral segment of A/Darwin/11/2021
SEQ ID NO: 84	Nucleotide sequence of NA viral segment of A/Darwin/11/2021
SEQ ID NO: 85	Amino acid sequence of PB2 protein of A/Darwin/11/2021
SEQ ID NO: 86	Amino acid sequence of PB1 protein of A/Darwin/11/2021
SEQ ID NO: 87	Amino acid sequence of PB1-F2 protein of A/Darwin/11/2021
SEQ ID NO: 88	Amino acid sequence of PA protein of A/Darwin/11/2021
SEQ ID NO: 89	Amino acid sequence of NP protein of A/Darwin/11/2021
SEQ ID NO: 90	Amino acid sequence of NS1 protein of A/Darwin/11/2021
SEQ ID NO: 91	Amino acid sequence of NEP protein of A/Darwin/11/2021
SEQ ID NO: 92	Amino acid sequence of M1 protein of A/Darwin/11/2021
SEQ ID NO: 93	Amino acid sequence of M2 protein of A/Darwin/11/2021
SEQ ID NO: 94	Amino acid sequence of HA protein of A/Darwin/11/2021
SEQ ID NO: 95	Amino acid sequence of NA protein of A/Darwin/11/2021
SEQ ID NO: 96	Nucleotide sequence of PB2 viral segment of A/Tasmania/503/2020
SEQ ID NO: 97	Nucleotide sequence of PB1 viral segment of A/Tasmania/503/2020
SEQ ID NO: 98	Nucleotide sequence of PA viral segment of A/Tasmania/503/2020
SEQ ID NO: 99	Nucleotide sequence of NP viral segment of A/Tasmania/503/2020
SEQ ID NO: 100	Nucleotide sequence of NS viral segment of A/Tasmania/503/2020
SEQ ID NO: 101	Nucleotide sequence of M viral segment of A/Tasmania/503/2020
SEQ ID NO: 102	Nucleotide sequence of HA viral segment of A/Tasmania/503/2020
SEQ ID NO: 103	Nucleotide sequence of NA viral segment of A/Tasmania/503/2020
SEQ ID NO: 104	Amino acid sequence of PB2 protein of A/Tasmania/503/2020
SEQ ID NO: 105	Amino acid sequence of PB1 protein of A/Tasmania/503/2020
SEQ ID NO: 106	Amino acid sequence of PB1-F2 protein of A/Tasmania/503/2020
SEQ ID NO: 107	Amino acid sequence of PA protein of A/Tasmania/503/2020
SEQ ID NO: 108	Amino acid sequence of NP protein of A/Tasmania/503/2020
SEQ ID NO: 109	Amino acid sequence of NS1 protein of A/Tasmania/503/2020
SEQ ID NO: 110	Amino acid sequence of NEP protein of A/Tasmania/503/2020
SEQ ID NO: 111	Amino acid sequence of M1 protein of A/Tasmania/503/2020
SEQ ID NO: 112	Amino acid sequence of M2 protein of A/Tasmania/503/2020
SEQ ID NO: 113	Amino acid sequence of HA protein of A/Tasmania/503/2020
SEQ ID NO: 114	Amino acid sequence of NA protein of A/Tasmania/503/2020

SEQ ID NO: 115 Nucleotide sequence of 5' non-coding region

SEQ ID NO: 116 Nucleotide sequence of 3' non-coding region

Detailed description

General Techniques and Definitions

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in genomics, immunology, molecular biology, immunohistochemistry, biochemistry, oncology, and pharmacology).

The present disclosure is performed without undue experimentation using, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology and immunology. Such procedures are described, for example in Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, New York, Fourth Edition (2012), whole of Vols I, II, and III; *DNA Cloning: A Practical Approach*, Vols. I and II (D. N. Glover, Second Edition., 1995), IRL Press, Oxford, whole of text; *Oligonucleotide Synthesis: A Practical Approach* (M. J. Gait, ed, 1984) IRL Press, Oxford, whole of text, and particularly the papers therein by Gait, pp1-22; Atkinson *et al*, pp35-81; Sproat *et al*, pp 83-115; and Wu *et al*, pp 135-151; 4. *Nucleic Acid Hybridization: A Practical Approach* (B. D. Hames & S. J. Higgins, eds., 1985) IRL Press, Oxford, whole of text; *Immobilized Cells and Enzymes: A Practical Approach* (1986) IRL Press, Oxford, whole of text; Perbal, B., *A Practical Guide to Molecular Cloning* (1984) and *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.), whole of series.

Those skilled in the art will appreciate that the present disclosure is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

The present disclosure is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally equivalent products, compositions and methods are clearly within the scope of the disclosure, as described

herein.

Each feature of any particular aspect or embodiment or embodiment of the present disclosure may be applied *mutatis mutandis* to any other aspect or embodiment or embodiment of the present disclosure.

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e., one or more) of those steps, compositions of matter, groups of steps or group of compositions of matter.

As used herein, the singular forms of “a”, “and” and “the” include plural forms of these words, unless the context clearly dictates otherwise. For example, a reference to “a bacterium” includes a plurality of such bacteria, and a reference to “an allergen” is a reference to one or more allergens.

The term “and/or”, e.g., “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning.

Throughout this specification, the word “comprise” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

By “consisting essentially of”, in the context of: (a) an amino acid sequence, is meant the recited amino acid sequence together with an additional one, two or three amino acids at the N- or C-terminus thereof; and (b) a nucleotide sequence, is meant the recited nucleotide sequence together with an additional one, two, three, four, five or six nucleic acid bases at the 5' or 3' end thereof.

The term “substantially” does not exclude “completely” (e.g., a composition which is “substantially free” from Y may be completely free from Y).

The term “about” in relation to a numerical value x is optional and means, for example, any

number within 0.5%, 1%, 5% or 10% of the referenced number or value. In certain examples, the term “about” encompasses the exact number recited.

All computer programs, algorithms, patent and scientific literature referred to herein is incorporated herein by reference.

For the present disclosure, the database accession number or unique identifier provided herein for a gene, protein or virus strain, as well as the gene and/or protein sequence or sequences associated therewith, are incorporated by reference herein.

Isolated influenza viruses

The inventors have surprisingly discovered a number of influenza viruses whose backbone viral segments (e.g., PA, PB1, PB2, NP, M and NS viral segments) can be utilised in reassortment techniques to improve the growth and yield of reassortant viruses comprising said backbone viral segments in cell culture, which may be advantageous for vaccine production.

Accordingly, in one form, the present disclosure provides an isolated influenza virus comprising one or more of PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins (e.g., one or more of the backbone viral proteins of PA, PB1, PB1-F2, PB2, NP, M1, M2, NEP and NS1) provided herein.

Suitably, the viral proteins comprise, consist of or consist essentially of an amino acid sequence selected from the group set forth in SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112 or a fragment, variant or derivative thereof. To this end, the PB2 viral protein suitably comprises, consists of or consists essentially of an amino acid sequence selected from the group set forth in SEQ ID NOs: 9, 28, 47, 66, 85 and 104 or a fragment, variant or derivative thereof, the PB1 viral protein suitably comprises, consists of or consists essentially of an amino acid sequence selected from the group set forth in SEQ ID NOs: 10, 29, 48, 67, 86 and 105 or a fragment, variant or derivative thereof, the PB1-F2 viral protein suitably comprises, consists of or consists essentially of an amino acid sequence selected from the group set forth in SEQ ID NOs: 11, 30, 49, 68, 87 and 106 or a fragment, variant or derivative thereof, the PA viral protein suitably comprises, consists of or consists essentially of an amino acid sequence selected from the group set forth in SEQ ID NOs: 12, 31, 50, 69, 88 and 107 or a fragment, variant or derivative thereof, the NP viral protein suitably comprises, consists of or consists

essentially of an amino acid sequence selected from the group set forth in SEQ ID NOs: 13, 32, 51, 70, 89 and 108 or a fragment, variant or derivative thereof, the NS1 viral protein suitably comprises, consists of or consists essentially of an amino acid sequence selected from the group set forth in SEQ ID NOs: 14, 33, 52, 71, 90 and 109 or a fragment, variant or derivative thereof, the NEP viral protein suitably comprises, consists of or consists essentially of an amino acid sequence selected from the group set forth in SEQ ID NOs: 15, 34, 53, 72, 91 and 110 or a fragment, variant or derivative thereof, the M1 viral protein suitably comprises, consists of or consists essentially of an amino acid sequence selected from the group set forth in SEQ ID NOs: 16, 35, 54, 73, 92 and 111 or a fragment, variant or derivative thereof, and/or the M2 viral protein suitably comprises, consists of or consists essentially of an amino acid sequence selected from the group set forth in SEQ ID NOs: 17, 36, 55, 74, 93 and 112 or a fragment, variant or derivative thereof.

In a related form, the present disclosure provides an isolated influenza virus comprising one or more of PB2, PB1, PA, M, NP and NS viral segments (i.e., backbone viral segments) provided herein. Suitably, the PB2, PB1, PA, M, NP and NS viral segments independently comprise, consist of or consist essentially of a nucleotide sequence selected from those set forth in SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101 or a fragment, variant or derivative thereof. In this regard, the PB2 viral segment suitably comprises, consists of or consists essentially of a nucleotide sequence selected from the group set forth in SEQ ID NOs: 1, 20, 39, 58, 77 and 96 or a fragment, variant or derivative thereof, the PB1 viral segment suitably comprises, consists of or consists essentially of a nucleotide sequence selected from the group set forth in SEQ ID NOs: 2, 21, 40, 59, 78 and 97 or a fragment, variant or derivative thereof, the PA viral segment suitably comprises, consists of or consists essentially of a nucleotide sequence selected from the group set forth in SEQ ID NOs: 3, 22, 41, 60, 79 and 98 or a fragment, variant or derivative thereof, the NP viral segment suitably comprises, consists of or consists essentially of a nucleotide sequence selected from the group set forth in SEQ ID NOs: 4, 23, 42, 61, 80 and 99 or a fragment, variant or derivative thereof, the NS viral segment suitably comprises, consists of or consists essentially of a nucleotide sequence selected from the group set forth in SEQ ID NOs: 5, 24, 43, 62, 81 and 100 or a fragment, variant or derivative thereof, and/or the M viral segment suitably comprises, consists of or consists essentially of a nucleotide sequence selected from the group set forth in SEQ ID NOs: 6, 25, 44, 63, 82 and 101 or a fragment, variant or derivative thereof.

Influenza viruses are enveloped RNA viruses that belong to the family of Orthomyxoviridae (Palese and Shaw (2007) Orthomyxoviridae: The Viruses and Their Replication, 5th ed. Fields' Virology, edited by B. N. Fields, D. M. Knipe and P. M. Howley. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, USA, p1647-1689). Influenza A and B viruses are major human pathogens, causing a respiratory disease that ranges in severity from sub-clinical infection to primary viral pneumonia, which can result in death. The clinical effects of infection vary with the virulence of the influenza strain and the exposure, history, age, and immune status of the host. The natural hosts of influenza viruses are predominantly avian, but influenza viruses, particularly influenza A viruses (including those of avian origin), can also infect and cause illness in humans and other animal hosts (e.g., bats, canines, pigs, horses, sea mammals and mustelids).

The isolated influenza virus of the present disclosure can be an influenza A virus or an influenza B virus. According to some examples, the isolated influenza virus is an influenza A virus. In alternative examples, the isolated influenza virus is an influenza B virus. By way of example, the influenza A virus provided herein may include a HA subtype selected from H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16. In some examples, the influenza A virus is of a H1, H2, H3, H5, H7, or H9 subtype. In various examples, the influenza A virus is of a H1 or H3 subtype. Moreover, such viruses may contain the influenza A virus NA subtypes N1, N2, N3, N4, N5, N6, N7, N8 or N9. For some examples, the influenza A virus is of a N1, N2, N3, N7, or N9 subtype. In other examples, the influenza A virus is of an N1 or N2 subtype. In particular, the influenza A virus can be a strain selected from the group consisting of H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H7N1, H7N2, H7N3, H7N7, H9N2, and H10N7. According to some examples, the influenza A virus is a H3N2 strain. In alternative examples, the influenza A virus is a H1N1 strain.

In view of the above, the isolated influenza virus referred to herein suitably comprises a HA viral segment and/or a NA viral segment that is at least partly derived from a seasonal influenza virus strain. As used herein, the term "seasonal influenza virus strain" refers to a strain of influenza virus to which a subject population is exposed to on a seasonal basis. The isolated influenza virus described herein may therefore be suitable for use in a vaccine or immunogenic composition for protecting against seasonal virus strains that are presently being spread or are endemic within a human population. In certain examples, the term seasonal influenza virus strain refers to a strain of influenza A virus. In other examples, the term seasonal influenza

virus strain refers to a strain of influenza A virus that belongs to the H1 or the H3 subtype (i.e., the two subtypes that presently persist or are endemic in the human population). In some examples, the term seasonal influenza virus strain refers to a strain of influenza B virus.

According to other examples, the isolated influenza virus referred to herein comprises a HA viral segment and/or a NA viral segment that is at least partly derived from a pandemic influenza virus strain. As used herein, the term “pandemic influenza virus strain” refers to a strain of influenza virus being associated or susceptible to be associated with an outbreak of influenza disease. Generally, the characteristics of an influenza strain that give it the potential to cause a pandemic outbreak are: (a) it contains a new haemagglutinin compared to the haemagglutinins in currently-circulating human strains, i.e., one that has not been evident in the human population for over a decade (e.g., H2), or has not previously been seen at all in the human population (e.g., H5, H6 or H9, that have generally been found only in bird populations), such that the human population will be immunologically naïve to the strain’s haemagglutinin; (b) it is capable of being transmitted horizontally in the human population; and (c) it is pathogenic to humans. As such, the isolated influenza virus may be suitable for use in a vaccine or immunogenic composition for protecting against potential pandemic virus strains that can or have spread from a non-human animal population to humans. As such, in certain examples, the term pandemic influenza virus strain refers to a strain of influenza A virus. Suitable pandemic strains include, but are not limited to: H5N1, H9N2, H7N7, H2N2, H2N3, H7N1 and H1N1. Others suitable pandemic strains found in humans are H7N3, H10N7 and H5N2.

It is further contemplated that the isolated influenza virus may comprise a HA viral segment that encodes a chimeric HA protein (i.e., a HA protein that contains amino acid sequences from more than one influenza strain). For instance, a chimeric HA may contain the cytoplasmic, or cytoplasmic and transmembrane portions of the HA from one influenza strain and at least the extracellular antigenic portion of the HA from a different influenza strain. This approach has been described previously as a technique for producing influenza viruses containing the antigenic portion of the HA protein in circumstances where the unmodified HA segment may be produced at low yields.

In other examples, the isolated influenza virus expresses a non-chimeric HA protein. In other words, the HA protein sequence comprises the cytoplasmic, transmembrane and extracellular domain from the same influenza strain.

The influenza viruses referred to herein encompass any virus type, subtype or strain including, but not limited to, naturally occurring strains, variants or mutants, mutagenized or modified viruses, reassortant viruses and/or genetically modified viruses.

The influenza viruses, nucleic acids, proteins, genetic constructs or cells described herein may be considered to be isolated. For the purposes of the present disclosure, by “isolated” is meant material that has been removed from its natural state or otherwise been subjected to human manipulation. Isolated material may be substantially or essentially free from components that normally accompany it in its natural state, or may be manipulated so as to be in an artificial state together with components that normally accompany it in its natural state. Isolated material may be in native, chemical synthetic or recombinant form.

Isolated recombinant influenza viruses are contemplated for the present disclosure. A “recombinant” virus is one which has been manipulated *in vitro*, such as by using recombinant DNA techniques, to introduce changes to the viral genome.

Suitably, the isolated influenza virus is a reassortant virus, such as a recombinant reassortant virus. The term “reassortant virus” denotes a virus which contains genetic material that results from the combination of genetic material of at least two donor viruses (e.g., one or more gene segments from a first parent influenza virus strain (the donor, backbone or seed strain), and one or more gene segments from a second parent influenza virus strain (the vaccine strain)). When the reassortant virus is used for preparing a vaccine or immunogenic composition, its genetic material usually contains at least the HA viral segment and optionally the NA viral segment from a seasonal or pandemic influenza virus, whereas the other viral segments (i.e., backbone viral segments) are from one or several other donor or seed viruses which have been selected for their ability to grow easily on the substrate of production used for manufacturing the flu vaccine (e.g., the allantoic cavity of embryonated hen's eggs or a permissive cell line) and/or to be less or non-pathogenic to humans. Non-limiting examples of donor or seed viruses that contribute as donors of backbone viral segments include A/Puerto Rico/8/1934 (PR8), A/Texas/1/1977, A/New York/55/2004, A/Ann Arbor/6/60, A/Leningrad/134/17/57, B/Ann Arbor/1/66, B/Florida/4/2006, B/Panama/45/1990 and B/Lee/1940. The reassortant virus may be produced by any method known in the art, inclusive of reverse genetics, classical reassortment and hybrid versions thereof.

Based on the improvements in viral replication and yield observed in cell culture outlined in the Examples below, the isolated influenza viruses described herein (e.g., A/Ohio/02/2019, A/Singapore/TT1384/2016, A/South Carolina/04/2017, A/Alaska/06/2019, A/Darwin/11/2021 and A/Tasmania/503/2020) may function as donor virus strains with respect to providing backbone viral segments in the generation of a reassortant virus. Influenza donor strains are strains which typically provide one or more of the backbone viral segments in a reassortant influenza virus, even though they may sometimes also provide the NA segment of the virus. The vaccine strain is typically the influenza strain that provides the HA and/or NA segment. Generally, both the HA and the NA segment in a reassortant influenza virus can be from the vaccine strain. The vaccine strain is typically a circulating strain, such as a seasonal or pandemic influenza virus strain. Suitably, the vaccine strain is different or heterologous from the donor strain. The viral segments that are present in a reassortant virus can be described using a gene constellation ratio, which indicates the number of segments that are provided by each parent influenza virus strain. For instance, when a reassortant virus contains genome segments from two parent influenza virus strains (such as a donor strain and a vaccine strain), it may have a gene constellation ratio of 1:7, 2:6, 3:5, 4:4, 5:3, 6:2, or 7:1.

Generally, the majority of gene segments of the reassortant viruses are from the donor strain, because it is desirable to harness the properties of the donor strain (e.g., improved replication and/or yield in cell culture) through reassortment of the donor strains segments with segments from the vaccine strain. In particular examples, the reassortant influenza virus produced by the methods provided herein has a gene constellation ratio of 5:3, 6:2, or 7:1, wherein the first number of the ratio indicates the number of segments from the donor strain and the second number of the ratio indicates the number of segments from the vaccine strain.

In certain examples, the reassortant influenza virus has a gene constellation ratio of 6:2. For these examples, the reassortant influenza virus suitably comprises six backbone segments (i.e., PB1, PB2, PA, NP, M, and NS) from the donor strain and two segments (i.e., HA and NA) from the vaccine strain.

In other examples, the reassortant influenza virus has a gene constellation ratio of 7:1. In such examples, the reassortant influenza virus can comprise the six backbone segments from the donor strain, the HA segment from the vaccine strain, and the NA segment from the donor

strain. In other words, the reassortant influenza virus comprises the HA segment from the vaccine strain and the remaining seven segments are from the donor strain. In alternative examples, the reassortant influenza virus comprises the six backbone segments and the HA segment from the donor strain, and the NA segment from the vaccine strain. In other words, the reassortant influenza virus comprises the NA segment from the vaccine strain and the remaining seven segments from the donor strain.

In further examples, the reassortant influenza virus has a gene constellation ratio of 5:3. In these examples, the reassortant virus may comprise five backbone segments (i.e. five viral segments selected from the group consisting of: PB1, PB2, PA, NP, M, and NS viral segments) from the donor strain and three segments from the vaccine strain. In such examples, the three segments from the vaccine strain are typically HA, NA and one backbone segment (i.e. one segment selected from the group consisting of: PB1, PB2, PA, NP, M and NS). In particular examples, the three segments from the vaccine strain are HA, NA and PB1 and the remaining five backbone segments (i.e., PB2, PA, NP, M and NS viral segments) are from the donor strain.

According to particular examples, the isolated reassortant influenza virus comprises:

- (a) one or more PA, PB1, PB2, NP, NS, and M viral segments derived from a first influenza virus isolate (e.g., one or more of PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins that comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112);
- (b) a HA viral segment, inclusive of chimeric versions thereof, derived from a second influenza virus isolate (e.g., a vaccine virus or strain); and
- (c) optionally an NA viral segment, inclusive of chimeric versions thereof, derived from the first influenza virus isolate, the second influenza virus isolate or a third influenza virus isolate.

To this end, the NA viral segment and the HA viral segment can be from the same influenza virus isolate, whilst the backbone viral segments can be derived from a different or heterologous influenza virus isolate to that of the NA and HA viral segments. Alternatively, the NA viral segment can be derived from the same influenza virus isolate (e.g., a donor virus) as the backbone viral segments (e.g., the NA viral segment comprises, consists of or consists

essentially of a nucleotide sequence set forth in SEQ ID NOs: 8, 27, 46, 65, 84 or 103 and/or the NA viral segment encodes a NA viral protein that comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NOs: 19, 38, 57, 76, 95, 114). Moreover, the NA viral segment can be from an influenza virus isolate that is different or heterologous to that from which the backbone viral segments and the HA viral segment were derived. In this regard, the HA viral segment can be derived from the same influenza virus isolate (e.g., a donor virus) as the backbone viral segments (e.g., the HA viral segment comprises, consists of or consists essentially of a nucleotide sequence set forth in SEQ ID NOs: 7, 26, 45, 64, 83 or 102 and/or the HA viral segment encodes a HA viral protein that comprises, consists of or consists essentially of an amino acid sequence set forth in SEQ ID NOs: 18, 37, 56, 75, 94 or 113).

As used herein, a “heterologous” influenza virus gene or viral segment is from an influenza virus source or isolate that is different than a majority of the other influenza viral genes or gene segments in a reassortant influenza virus or a recombinant influenza virus, inclusive of recombinant reassortant influenza viruses.

For certain examples, the isolated influenza virus comprises viral segments (i.e., the PB2, PB1, PA, M, NP and NS viral segments) that encode two or more (e.g., 2, 3, 4, 5, 6, 7, 8 or 9) of the backbone viral proteins (i.e., the PB2, PB1, PB1-F2, PA, M1, M2, NP, NS1 and NEP viral proteins) provided herein (e.g., SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112 or a fragment, variant or derivative thereof). In particular examples, the isolated influenza virus comprises viral segments that encode two or more, three or more, more particularly four or more, even more particularly five or more, yet even more particularly 6 or more and still even more particularly 7 or more (i.e., 7, 8 or 9), of the backbone viral proteins provided herein.

According to related examples, the isolated influenza virus comprises two or more (e.g., 2, 3, 4, 5 or 6) of the backbone viral segments (i.e., the PB2, PB1, PA, M, NP and NS viral segments) provided herein (e.g., a nucleotide sequence comprising, consisting of or consisting essentially of that set forth in SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101 or a fragment, variant or derivative thereof). In particular examples, the isolated influenza virus comprises three or more, more particularly four or more, or even more particularly five or more (i.e., 5 or 6), of the backbone viral segments provided herein.

Suitably, the isolated influenza virus comprises viral segments (i.e., each of the PB2, PB1, PA, M, NP and NS viral segments) that encode nine (9) of the backbone viral proteins provided herein. In these examples, the isolated influenza virus suitably comprises viral segments that encode PB2, PB1, PB1-F2, PA, M1, M2, NP, NS1 and NEP viral proteins independently comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof. To this end, it is contemplated that the isolated influenza virus may contain any combination of nine (9) of those backbone viral proteins provided herein.

Accordingly, in certain examples, the isolated influenza virus comprises:

(a) a PB2 viral segment that encodes a PB2 viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9, 28, 47, 66, 85 and 104, or a fragment, variant or derivative thereof;

(b) a PB1 viral segment that encodes: a PB1 viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 29, 48, 67, 86 and 105, or a fragment, variant or derivative thereof; and/or a PB1-F2 viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 11, 30, 49, 68, 87 and 106, or a fragment, variant or derivative thereof;

(b) a PA viral segment that encodes a PA viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 31, 50, 69, 88 and 107, or a fragment, variant or derivative thereof;

(c) a NP viral segment that encodes a NP viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 32, 51, 70, 89 and 108, or a fragment, variant or derivative thereof;

(d) a NS viral segment that encodes: a NS1 viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 33, 52, 71, 90 and 109, or a fragment, variant or derivative thereof; and/or a NEP viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 34, 53, 72, 91 and 110, or a fragment, variant or derivative thereof; and

(e) a M viral segment that encodes: a M1 viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 35, 54, 73, 92 and 111, or a fragment, variant or derivative thereof; and/or a M2

viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 36, 55, 74, 93 and 112, or a fragment, variant or derivative thereof.

Suitably, the isolated influenza virus comprises six (6) of the backbone viral segments provided herein. In these examples, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments, each independently comprising, consisting of or consisting essentially of a nucleotide sequence selected from those set forth in SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101 or a fragment, variant or derivative thereof. To this end, it is contemplated that the isolated influenza virus may contain any combination of six of those backbone viral segments provided herein.

Furthermore, in related examples, the isolated influenza virus comprises:

(a) a PB2 viral segment that comprises, consists of or consists essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 20, 39, 58, 77 and 96, or a fragment, variant or derivative thereof;

(a) a PB1 viral segment that comprises, consists of or consists essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2, 21, 40, 59, 78 and 97, or a fragment, variant or derivative thereof;

(b) a PA viral segment that comprises, consists of or consists essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 3, 22, 41, 60, 79 and 98, or a fragment, variant or derivative thereof;

(c) a NP viral segment that comprises, consists of or consists essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 4, 23, 42, 61, 80 and 99, or a fragment, variant or derivative thereof;

(d) a NS viral segment that comprises, consists of or consists essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5, 24, 43, 62, 81 and 100, or a fragment, variant or derivative thereof; and

(e) a M viral segment that comprises, consists of or consists essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 6, 25, 44, 63, 82 and 101, or a fragment, variant or derivative thereof.

For some examples, the isolated influenza virus comprises viral segments that encode seven (7) of the backbone viral proteins provided herein. According to other examples, the isolated

influenza virus comprises viral segments that encode eight (8) of the backbone viral proteins provided herein. In such examples, the isolated influenza virus suitably comprises viral segments that encode seven or eight backbone viral proteins (i.e., 7 or 8 of the PB2, PB1, PB1-F2, PA, M1, M2, NP, NS1 and NEP viral proteins) that independently comprise, consist of or consist essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof. Again, it is envisaged that the isolated influenza virus may express any combination of seven or eight of those backbone viral proteins (i.e., seven or eight viral proteins selected from PB2, PB1, PB1-F2, PA, M1, M2, NP, NS1 and NEP viral proteins) provided herein.

Suitably, the isolated influenza virus comprises viral segments that encode the PB2, PA, M1, M2, NP, NS1 and NEP viral proteins provided herein and optionally the PB1 and/or PB1-F2 viral proteins provided herein. As such, in particular examples, the isolated influenza virus comprises:

(a) a PB2 viral segment that encodes a PB2 viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9, 28, 47, 66, 85 and 104, or a fragment, variant or derivative thereof;

(b) a PA viral segment that encodes a PA viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 31, 50, 69, 88 and 107, or a fragment, variant or derivative thereof;

(c) a NP viral segment that encodes a NP viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 32, 51, 70, 89 and 108, or a fragment, variant or derivative thereof;

(d) a NS viral segment that encodes a NS1 viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 33, 52, 71, 90 and 109, or a fragment, variant or derivative thereof and/or a NEP viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 34, 53, 72, 91 and 110, or a fragment, variant or derivative thereof; and

(e) a M viral segment that encodes a M1 viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 35, 54, 73, 92 and 111, or a fragment, variant or derivative thereof and/or a M2 viral protein that comprises, consists of or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 36, 55, 74, 93 and 112, or a fragment,

variant or derivative thereof.

For other examples, the isolated influenza virus comprises five (5) of the backbone viral segments provided herein. In such examples, the isolated influenza virus comprises five viral segments independently comprising, consisting of or consisting essentially of a nucleotide sequence selected from those set forth in SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a fragment, variant or derivative thereof. Again, it is envisaged that the isolated influenza virus may contain any combination of five of those backbone viral segments (i.e., five viral segments selected from PB2, PB1, PA, M, NP and NS viral segments) provided herein.

Suitably, the isolated influenza virus comprises viral segments that encode the PB2, PA, M, NP and NS viral segment provided herein and optionally the PB1 viral segment provided herein. Moreover, in related examples, the isolated influenza virus comprises:

(a) a PB2 viral segment that comprises, consists of or consists essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 20, 39, 58, 77 and 96, or a fragment, variant or derivative thereof;

(b) a PA viral segment that comprises, consists of or consists essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 3, 22, 41, 60, 79 and 98, or a fragment, variant or derivative thereof;

(c) a NP viral segment that comprises, consists of or consists essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 4, 23, 42, 61, 80 and 99, or a fragment, variant or derivative thereof;

(d) a NS viral segment that comprises, consists of or consists essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5, 24, 43, 62, 81 and 100, or a fragment, variant or derivative thereof; and

(e) a M viral segment that comprises, consists of or consists essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 6, 25, 44, 63, 82 and 101, or a fragment, variant or derivative thereof.

In particular examples, the isolated influenza virus comprises one or more of PB2, PB1, PA, M, NP and NS viral segments that encode one or more viral proteins comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 9-17, or a fragment, variant or derivative thereof. More particularly, the isolated influenza

virus can comprise PB2, PB1, PA, M, NP and NS viral segments that encode seven or more viral proteins comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 9-17, or a fragment, variant or derivative thereof. In particular examples, the isolated influenza virus comprises PB2, PA, M, NP and NS viral segments that encode PB2, PA, M1, M2, NP, NS1 and NEP viral proteins that comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NOs: 9 and 12-17 respectively or a fragment, variant or derivative thereof. In such examples, the isolated influenza virus may optionally further comprise a PB1 viral segment that encodes PB1 and/or PB1-F2 viral proteins independently comprising, consisting of or consisting essentially of an amino acid sequence set forth in SEQ ID NOs: 10 and/or 11 or a fragment, variant or derivative thereof. Suitably, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments that encode PB2, PB1, PB1-F2, PA, M1, M2, NP, NS1 and NEP viral proteins that each independently comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NOs: 9-17 respectively or a fragment, variant or derivative thereof. With respect to the above examples, the isolated influenza virus, such as a reassortant influenza virus, is suitably of a H1N1 or a H3N2 subtype.

According to related examples, the isolated influenza virus comprises one or more of PB2, PB1, PA, M, NP and NS viral segments comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 1-6, or a fragment, variant or derivative thereof. More particularly, the isolated influenza virus can comprise five or more of the PB2, PB1, PA, M, NP and NS viral segments comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 1-6, or a fragment, variant or derivative thereof. By way of example, the isolated influenza virus can comprise PB2, PA, M, NP and NS viral segments that comprise, consist of or consist essentially of a nucleotide sequence set forth in SEQ ID NOs: 1 and 3-6 respectively or a fragment, variant or derivative thereof. In such examples, the isolated influenza virus may optionally further comprise a PB1 viral segment comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NO: 2 or a fragment, variant or derivative thereof. Suitably, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments each independently comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 1-6 respectively, or a fragment, variant or derivative thereof. With respect to the above examples, the isolated influenza virus, such as a reassortant influenza virus, is suitably of a H1N1 or a H3N2 subtype.

In certain examples, the isolated influenza virus comprises one or more of PB2, PB1, PA, M, NP and NS viral segments that encode one or more viral proteins comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 28-36, or a fragment, variant or derivative thereof. More particularly, the isolated influenza virus can comprise PB2, PB1, PA, M, NP and NS viral segments that encode seven or more viral proteins comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 28-36, or a fragment, variant or derivative thereof. In particular examples, the isolated influenza virus comprises PB2, PA, M, NP and NS viral segments that encode PB2, PA, M1, M2, NP, NS1 and NEP viral proteins that comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NOs: 28 and 31-36 respectively or a fragment, variant or derivative thereof. In such examples, the isolated influenza virus may optionally further comprise a PB1 viral segment that encodes PB1 and/or PB1-F2 viral proteins comprising, consisting of or consisting essentially of an amino acid sequence set forth in SEQ ID NO: 29 and/or 30 respectively or a fragment, variant or derivative thereof. Suitably, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments that encode PB2, PB1, PB1-F2, PA, M1, M2, NP, NS1 and NEP viral proteins that each independently comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NOs: 28-36 respectively or a fragment, variant or derivative thereof. With respect to the above examples, the isolated influenza virus, such as a reassortant influenza virus, is suitably of a H1N1 subtype.

For other examples, the isolated influenza virus comprises one or more of PB2, PB1, PA, M, NP and NS viral segments comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 20-25, or a fragment, variant or derivative thereof. More particularly, the isolated influenza virus can comprise five or more of the PB2, PB1, PA, M, NP and NS viral segments comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 20-25, or a fragment, variant or derivative thereof. For instance, the isolated influenza virus can comprise PB2, PA, M, NP and NS viral segments that comprise, consist of or consist essentially of a nucleotide sequence set forth in SEQ ID NOs: 20 and 22-25 respectively or a fragment, variant or derivative thereof. In such examples, the isolated influenza virus may optionally further comprise a PB1 viral segment comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NO: 21 or a fragment, variant or derivative thereof. Suitably, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments each independently comprising, consisting of or

consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 20-25 respectively, or a fragment, variant or derivative thereof. With respect to the above examples, the isolated influenza virus, such as a reassortant influenza virus, is suitably of a H1N1 subtype.

In various examples, the isolated influenza virus comprises one or more of PB2, PB1, PA, M, NP and NS viral segments that encode one or more viral proteins comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 47-55, or a fragment, variant or derivative thereof. More particularly, the isolated influenza virus can comprise PB2, PB1, PA, M, NP and NS viral segments that encode seven or more viral proteins comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 47-55, or a fragment, variant or derivative thereof. In particular examples, the isolated influenza virus comprises PB2, PA, M, NP and NS viral segments that encode PB2, PA, M1, M2, NP, NS1 and NEP viral proteins that comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NOs: 47 and 50-55 respectively or a fragment, variant or derivative thereof. In such examples, the isolated influenza virus may optionally further comprise a PB1 viral segment that encodes PB1 and/or PB1-F2 viral proteins comprising, consisting of or consisting essentially of an amino acid sequence set forth in SEQ ID NO: 48 and/or 49 or a fragment, variant or derivative thereof. Suitably, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments that encode PB2, PB1, PB1-F2, PA, M1, M2, NP, NS1 and NEP viral proteins that each independently comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NOs: 47-55 respectively or a fragment, variant or derivative thereof. With respect to the above examples, the isolated influenza virus, such as a reassortant influenza virus, is suitably of a H1N1 or a H3N2 subtype.

According to related examples, the isolated influenza virus comprises one or more of PB2, PB1, PA, M, NP and NS viral segments comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 39-44, or a fragment, variant or derivative thereof. More particularly, the isolated influenza virus can comprise five or more of the PB2, PB1, PA, M, NP and NS viral segments comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 39-44, or a fragment, variant or derivative thereof. For instance, the isolated influenza virus can comprise PB2, PA, M, NP and NS viral segments that comprise, consist of or consist essentially of a nucleotide sequence set forth in SEQ ID NOs: 39 and 41-44 respectively or a fragment, variant or derivative thereof. In such

examples, the isolated influenza virus may optionally further comprise a PB1 viral segment comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NO: 40 or a fragment, variant or derivative thereof. Suitably, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments each independently comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 39-44 respectively, or a fragment, variant or derivative thereof. With respect to the above examples, the isolated influenza virus, such as a reassortant influenza virus, is suitably of a H1N1 or a H3N2 subtype.

For particular examples, the isolated influenza virus comprises one or more of PB2, PB1, PA, M, NP and NS viral segments that encode one or more viral proteins comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 66-74, or a fragment, variant or derivative thereof. More particularly, the isolated influenza virus can comprise PB2, PB1, PA, M, NP and NS viral segments that encode seven or more viral proteins comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 66-74, or a fragment, variant or derivative thereof. In particular examples, the isolated influenza virus comprises PB2, PA, M, NP and NS viral segments that encode PB2, PA, M1, M2, NP, NS1 and NEP viral proteins that comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NOs: 66 and 69-74 respectively or a fragment, variant or derivative thereof. In such examples, the isolated influenza virus may optionally further comprise a PB1 viral segment that encodes PB1 and/or PB1-F2 viral proteins comprising, consisting of or consisting essentially of an amino acid sequence set forth in SEQ ID NO: 67 and/or 68 or a fragment, variant or derivative thereof. Suitably, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments that encode PB2, PB1, PB1-F2, PA, M1, M2, NP, NS1 and NEP viral proteins that each independently comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NOs: 66-74 respectively or a fragment, variant or derivative thereof. With respect to the above examples, the isolated influenza virus, such as a reassortant influenza virus, is suitably of a H1N1 subtype.

In various examples, the isolated influenza virus comprises one or more of PB2, PB1, PA, M, NP and NS viral segments comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 58-63, or a fragment, variant or derivative thereof. More particularly, the isolated influenza virus can comprise five or more of the PB2, PB1, PA, M,

NP and NS viral segments comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 58-63, or a fragment, variant or derivative thereof. For instance, the isolated influenza virus can comprise PB2, PA, M, NP and NS viral segments that comprise, consist of or consist essentially of a nucleotide sequence set forth in SEQ ID NOs: 58 and 60-63 respectively or a fragment, variant or derivative thereof. In such examples, the isolated influenza virus may optionally further comprise a PB1 viral segment comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NO: 59 or a fragment, variant or derivative thereof. Suitably, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments each independently comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 58-63 respectively, or a fragment, variant or derivative thereof. With respect to the above examples, the isolated influenza virus, such as a reassortant influenza virus, is suitably of a H1N1 subtype.

For particular examples, the isolated influenza virus comprises one or more of PB2, PB1, PA, M, NP and NS viral segments that encode one or more viral proteins comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 85-93, or a fragment, variant or derivative thereof. More particularly, the isolated influenza virus can comprise PB2, PB1, PA, M, NP and NS viral segments that encode seven or more viral proteins comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 85-93, or a fragment, variant or derivative thereof. In particular examples, the isolated influenza virus comprises PB2, PA, M, NP and NS viral segments that encode PB2, PA, M1, M2, NP, NS1 and NEP viral proteins that comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NOs: 85 and 88-93 respectively or a fragment, variant or derivative thereof. In such examples, the isolated influenza virus may optionally further comprise a PB1 viral segment that encodes PB1 and/or PB1-F2 viral proteins comprising, consisting of or consisting essentially of an amino acid sequence set forth in SEQ ID NO: 86 and/or 87 or a fragment, variant or derivative thereof. Suitably, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments that encode PB2, PB1, PB1-F2, PA, M1, M2, NP, NS1 and NEP viral proteins that each independently comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NOs: 85-93 respectively or a fragment, variant or derivative thereof. With respect to the above examples, the isolated influenza virus, such as a reassortant influenza virus, is suitably of a H1N1 or a H3N2 subtype.

In various examples, the isolated influenza virus comprises one or more of PB2, PB1, PA, M, NP and NS viral segments comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 77-82, or a fragment, variant or derivative thereof. More particularly, the isolated influenza virus can comprise five or more of the PB2, PB1, PA, M, NP and NS viral segments comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 77-82, or a fragment, variant or derivative thereof. For instance, the isolated influenza virus can comprise PB2, PA, M, NP and NS viral segments that comprise, consist of or consist essentially of a nucleotide sequence set forth in SEQ ID NOs: 77 and 79-82 respectively or a fragment, variant or derivative thereof. In such examples, the isolated influenza virus may optionally further comprise a PB1 viral segment comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NO: 78 or a fragment, variant or derivative thereof. Suitably, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments each independently comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 77-82 respectively, or a fragment, variant or derivative thereof. With respect to the above examples, the isolated influenza virus, such as a reassortant influenza virus, is suitably of a H1N1 or a H3N2 subtype.

For particular examples, the isolated influenza virus comprises one or more of PB2, PB1, PA, M, NP and NS viral segments that encode one or more viral proteins comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 104-112, or a fragment, variant or derivative thereof. More particularly, the isolated influenza virus can comprise PB2, PB1, PA, M, NP and NS viral segments that encode seven or more viral proteins comprising, consisting of or consisting essentially of an amino acid sequence selected from those set forth in SEQ ID NOs: 104-112, or a fragment, variant or derivative thereof. In particular examples, the isolated influenza virus comprises PB2, PA, M, NP and NS viral segments that encode PB2, PA, M1, M2, NP, NS1 and NEP viral proteins that comprise, consist of or consist essentially of an amino acid sequence set forth in SEQ ID NOs: 104 and 107-112 respectively or a fragment, variant or derivative thereof. In such examples, the isolated influenza virus may optionally further comprise a PB1 viral segment that encodes PB1 and/or PB1-F2 viral proteins comprising, consisting of or consisting essentially of an amino acid sequence set forth in SEQ ID NO: 105 and/or 106 or a fragment, variant or derivative thereof. Suitably, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments that encode PB2, PB1, PB1-F2, PA, M1, M2, NP, NS1 and NEP viral proteins that each independently comprise, consist of or consist essentially of an amino acid

sequence set forth in SEQ ID NOs: 104-112 respectively or a fragment, variant or derivative thereof. With respect to the above examples, the isolated influenza virus, such as a reassortant influenza virus, is suitably of a H1N1 or a H3N2 subtype.

In various examples, the isolated influenza virus comprises one or more of PB2, PB1, PA, M, NP and NS viral segments comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 96-101, or a fragment, variant or derivative thereof. More particularly, the isolated influenza virus can comprise five or more of the PB2, PB1, PA, M, NP and NS viral segments comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 96-101, or a fragment, variant or derivative thereof. For instance, the isolated influenza virus can comprise PB2, PA, M, NP and NS viral segments that comprise, consist of or consist essentially of a nucleotide sequence set forth in SEQ ID NOs: 96 and 98-101 respectively or a fragment, variant or derivative thereof. In such examples, the isolated influenza virus may optionally further comprise a PB1 viral segment comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NO: 97 or a fragment, variant or derivative thereof. Suitably, the isolated influenza virus comprises PB2, PB1, PA, M, NP and NS viral segments each independently comprising, consisting of or consisting essentially of a nucleotide sequence set forth in SEQ ID NOs: 96-101 respectively, or a fragment, variant or derivative thereof. With respect to the above examples, the isolated influenza virus, such as a reassortant influenza virus, is suitably of a H1N1 or a H3N2 subtype.

Suitably, the isolated influenza virus provided herein is capable of growth or replication in cells and, more particularly, mammalian cells, such as MDCK cells. To this end, the isolated influenza virus is suitably capable of forming virions when cultured in cells. More particularly, the isolated influenza virus comprising the backbone viral segments described herein is suitably capable of improved growth or replication in cells, such as when cultured under standard culture conditions, inclusive of those described herein. In this regard, the influenza virus when expressing an unmodified, wild-type or reference form of the one or more backbone viral segments provided herein suitably has a limited or reduced capability for growth or replication in cells. More particularly, for reassortant influenza viruses, an isolated reassortant influenza virus derived from a candidate vaccine virus strain that comprises one or more of the backbone viral segments described herein is suitably capable of improved growth or replication in cells (e.g., MDCK cells) when compared to a wild-type or reference influenza virus (e.g., a wild-type version of the corresponding candidate vaccine virus strain) that does not comprise the

one or more backbone viral segments described herein (e.g., comprises wild-type versions of the backbone viral segments). The isolated influenza virus and the wild-type influenza virus, however, may comprise or express the same HA and/or NA viral segments. In other examples, the isolated influenza virus provided herein is capable of improved growth or replication in cells, and more particularly mammalian cells (e.g., MDCK cells), when compared to a donor influenza virus, as are known in the art and inclusive of those described herein (e.g., A/Puerto Rico/8/1934 (PR8), A/Texas/1/1977, A/New York/55/2004, A/Ann Arbor/6/60, A/Leningrad/134/17/57, B/Ann Arbor/1/66, B/Florida/4/2006, B/Panama/45/1990 and B/Lee/1940). More particularly, the isolated influenza virus provided herein is suitably capable of improved growth or replication in cells, and more particularly MDCK cells, when compared to an A/Puerto Rico/8/1934 strain, inclusive of cell-adapted versions thereof (e.g., PR8x). Accordingly, the one or more backbone viral segments described herein suitably provide the isolated influenza virus comprising such viral segments with an improved capability of growth and replication in cells, such as MDCK cells.

A level of growth or replication of the isolated influenza virus may be assessed by any means in the art, and may be assessed indirectly, such as by virus yield or viral protein yield (e.g., viral titres, HA and/or NA protein yield at end of infection) or directly, such as by rescue capability, growth capability and/or replication capability thereof when grown in cell culture, such as in mammalian cells like MDCK cells. As such, the phrases “increasing the growth capability of the isolated influenza virus”, “enhancing the growth capability of the isolated influenza virus” or the like can mean that a virus having one or more of the backbone viral segments in accordance with the present disclosure has an improved growth capability and/or replication capability when grown in cell culture (such as in mammalian cells like MDCK cells), compared to a virus having wild-type versions of the backbone viral segments (e.g., a wild-type virus that has not been modified to express one or more of the backbone viral segments of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a fragment, variant or derivative thereof). Suitably, the isolated influenza virus in question comprises one or both of the HA and NA viral segments (or a chimeric version thereof) of the reference or wild-type influenza virus (e.g., a vaccine virus strain).

By virtue of this increased or enhanced growth capability or otherwise, the isolated influenza virus may demonstrate enhanced yield of a viral protein (e.g., one or more of HA, NA, PA, PB1, PB1-F2, PB2, NP, M1, M2, NEP and NS1) produced thereby when grown in cells. Yield

of an influenza virus protein, such as HA or NA, may be measured by any means of protein quantification known in the art, such as by gel electrophoresis (e.g., Western Blot); by ELISA, or by chromatographic methods, such as HPLC (high performance liquid chromatography), HPLC-UV or mass spectrometry methods, such as LC-MS (Liquid Chromatography-Mass Spectrometry). In particular examples, the isolated influenza virus demonstrates enhanced yield of HA protein when grown in cells.

Terms such as “higher”, “enhanced”, “increased” and “greater” as used herein refer to an elevated level of growth or yield of the isolated influenza virus and/or an elevated level of yield of a viral protein produced by the isolated influenza virus, such as when compared to a control or reference level or amount thereof. The level of growth or yield of the isolated influenza virus or the viral protein may be relative or absolute (*i.e.*, relatively or absolutely higher, increased or greater). In particular examples, an enhanced or increased growth capability or yield of the isolated influenza virus (or a viral protein produced thereby), refers to the increase in a level of growth, replication or yield when compared to that of a control, wild-type or reference influenza virus (e.g., an influenza virus strain comprising wild-type or unmodified versions of PA, PB1, PB2, NP, M and NS viral segments typical for that viral strain rather than those viral segments provided in SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101).

It is contemplated that the virus and/or the viral protein may be contained in a cell culture supernatant. As such, a yield of the isolated influenza virus and/or a viral protein thereof may be assessed following harvesting, isolation, purification or separation thereof from the cells and/or the cell culture media. To do this, for example, the cells or cell residues may be separated from the culture media by methods known to the person skilled in the art, such as centrifugation, separators or filters. The influenza virus, inclusive of viral proteins thereof, present in the test culture media may then be isolated, concentrated or harvested by methods known to the person skilled in the art, such as those described herein (e.g., gradient centrifugation, filtration, precipitation and the like). In particular examples, the influenza virus and/or the viral protein produced thereby are isolated or harvested from the culture media at least in part by gradient ultracentrifugation. Accordingly, a level of yield can be assessed in relation to the isolated influenza virus or a viral protein thereof that has been at least partially purified or separated from the test culture media, such as by gradient ultracentrifugation.

In order to assess yield of the isolated influenza virus and/or a viral protein thereof, the obtained

or measured levels of yield may be compared to one or more threshold levels thereof. The nature and numerical value of the threshold level of yield will typically vary based on the influenza virus and/or the influenza virus protein in question and/or the method or means chosen to determine the level of yield for that virus or virus protein. According to various examples, the improved growth capability and/or replication capability of the isolated influenza virus may be determined by achieving a threshold level of virus yield or viral protein yield (e.g., HA protein yield) when the isolated influenza virus is grown on cells (e.g., MDCK cells). Suitably, the threshold level of virus yield or viral protein yield is one that allows for the production of a quantity of virus and/or viral protein suitable for use in the preparation of a vaccine or immunogenic composition at a commercial scale.

As noted above, it is envisaged that the assessment with respect to yield of the isolated influenza virus can be based at least partly on a particular influenza virus protein, such as a HA protein. Referring to HA, suitable threshold levels of yield for this viral protein can be at least about 10 mg/L (e.g., about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 mg/L or any range therein), at least about 15 mg/L, at least about 20 mg/L or at least about 25 mg/L in the culture media at the end of the infection or suitable incubation period. Accordingly, an isolated influenza virus that produces a yield of HA of at least about 10 mg/L (e.g., about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 mg/L or any range therein), at least about 15 mg/L, at least about 20 mg/L or at least about 25 mg/L in the test culture media at the end of the infection or incubation period (e.g., about 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180, 192, 204, 216, 228, 240 hours or any range therein after infection or incubation of the cells with the isolated influenza virus) may be considered as demonstrating an improved or enhanced growth or replicative capability.

The extent of the improvement in yield and/or growth capability of the isolated influenza virus may vary. For example, the extent of the improvement may be more than about 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400% or at least about 500% (or any range therein) higher than that level observed in a control, reference or wild-type influenza virus. For example, the extent of the improvement in growth or replicative ability or yield of the isolated influenza virus may be more than about 50% higher compared to a virus not having one or

more of the backbone viral segments provided herein, such as when cultured under standard culture conditions known in the art, inclusive of those described herein.

Based on the foregoing, in another form the present disclosure provides a method of improving the growth and/or yield of an influenza virus in cells, said method including the step of modifying the influenza virus to comprise one or more of PA, PB1, PB2, NP, M and NS viral segments provided herein (e.g., SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a fragment, variant or derivative thereof) and/or those that encode one or more backbone viral proteins described herein (e.g., one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof). Such influenza viruses may be prepared or modified by those methods described herein, inclusive of classical reassortment, reverse genetics or a combination thereof.

By “protein” is meant an amino acid polymer. The amino acids may be natural or non-natural amino acids, D- or L-amino acids as are well understood in the art.

The term “protein” includes and encompasses “peptide”, which is typically used to describe a protein having no more than fifty (50) amino acids (e.g., no more than 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 amino acids and any range therein) and “polypeptide”, which is typically used to describe a protein having more than fifty (50) amino acids.

Fragments, variants and derivatives of the backbone viral proteins (i.e., PB2, PB1, PB1-F2, PA, NP, NS1, NEP, M1 and M2) encoded by the backbone viral segments described herein are envisaged by the present disclosure.

As used herein, a protein, polypeptide or peptide “variant” shares a definable amino acid sequence relationship with a reference amino acid sequence. The reference amino acid sequence may be the amino acid sequence of any one of SEQ ID NOs: 9-19, 28-38, 47-57, 66-76, 85-95 and 104-114, for example. The “variant” protein, polypeptide or peptide may have one or a plurality of amino acids of the reference or wild-type amino acid sequence deleted or substituted by different amino acids. Such protein variants may include, for example, amino acid residues and/or amino acid sequences of naturally occurring variants and orthologs (e.g.,

from a different influenza strain or a different host animal) of a viral protein, inclusive of a consensus sequence thereof. Thus, variant backbone viral HA proteins may have at least about 2, 3, 4, 5, 6, 7 or more different residues in other positions as compared to a respective wild-type or reference backbone viral protein. As will be appreciated by those of skill in the art, the number of additional positions that may have amino acid substitutions will depend on the wild-type viral protein or encoding nucleic acid used to generate the variants. To this end, the modified viral proteins provided herein may be derived from any of the known backbone protein sequences or encoding nucleotide sequences from influenza isolates known in the art. For instance, the National Center for Biotechnology information (NCBI) maintains a database (<https://www.ncbi.nlm.nih.gov/genomes/FLU/Database/>) of known backbone viral protein sequences and encoding nucleic acids thereof.

It is further contemplated that some amino acid residues of a viral protein may be substituted or deleted without changing the activity of the variant protein (i.e., conservative substitutions). Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu, and Ile; interchange of the hydroxyl residues Ser and Thr; exchange of the acidic residues Asp and Glu; substitution between the amide residues Asn and Gln; exchange of the basic residues Lys and Arg; and replacements among the aromatic residues Phe and Tyr. Guidance concerning which amino acid changes are likely to be phenotypically silent can be found in, for example, Bowie et al., *Science* 247:1306-1310 (1990). Accordingly, one or more of the residues of a viral backbone protein described herein (e.g., a PB2, PB1, PB1-F2, PA, NP, NS1, NEP, M1 or M2 protein), such as those defined by SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, may be conservatively modified (e.g., by amino acid substitution or deletion) so as to substantially retain the functionality and/or immunogenicity of the backbone viral protein. Furthermore, the variant backbone proteins may include additional amino acid variations that are known in the art, for example, to further increase or enhance a growth capability or yield of the isolated influenza virus. Such additional amino acid variations are described in WO2015009743, WO2015196150, WO2017007839, WO2017143236 and WO2020223699, which are incorporated herein in their entirety.

It is envisaged that protein or peptide variants share at least about 70% or 75%, more particularly at least about 80% or 85% or even more particularly at least about 90%, 90.5%, 91%, 91.5%, 92%, 92.5%, 93%, 93.5%, 94%, 94.5%, 95%, 95.5%, 96%, 96.5%, 97%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9%, 98%, 98.1%, 98.2%, 98.3%,

98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%, 99.3%, 99.4, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity with a reference amino acid sequence, such as any of those set forth in SEQ ID NOs: 9-19, 28-38, 47-57, 66-76, 85-95 and 104-114 or more particularly any of those set forth in SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85 to 93 and 104-112.

Terms used generally herein to describe sequence relationships between respective proteins and nucleic acids include "comparison window", "sequence identity", "percentage of sequence identity" and "substantial identity". Because respective nucleic acids/proteins may each comprise (1) only one or more portions of a complete nucleic acid/protein sequence that are shared by the nucleic acids/proteins, and (2) one or more portions which are divergent between the nucleic acids/proteins, sequence comparisons are typically performed by comparing sequences over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of, for example, 6, 9, 12 or 20 contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or deletions (i.e., gaps) of about 20% or less as compared to the reference sequence for optimal alignment of the respective sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerised implementations of algorithms (Geneworks program by IntelliGenetics; GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA, incorporated herein by reference) or by inspection and the best alignment (i.e. resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul et al., 1991, Nucl. Acids Res. 25 3389, which is incorporated herein by reference. A detailed discussion of sequence analysis can be found in Unit 19.3 of CURRENT PROTOCOLS IN MOLECULAR BIOLOGY Eds. Ausubel et al. (John Wiley & Sons Inc NY, 1995-1999).

The term "sequence identity" is used herein in its broadest sense to include the number of exact nucleotide or amino acid matches having regard to an appropriate alignment using a standard algorithm, having regard to the extent that sequences are identical over a window of comparison. Thus, a "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 amino acid residue

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. For example, "sequence identity" may be understood to mean the "match percentage" calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA).

As used herein, a "fragment" is a segment, domain, portion or region of a protein or peptide (such as those set forth in SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112) which constitutes less than 100% of the amino acid sequence of the protein or peptide. It will be appreciated that the fragment may be a single fragment or may be repeated alone or with other fragments. In general, fragments may comprise, consist essentially of or consist of up to about 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 520, 540, 560, 580, 600, 620, 640, 660, 680, 700, 710, 720, 730, 740, 750 or 755 contiguous amino acids (such as of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112). Suitably, the fragment is a functional fragment that at least partly retains the function of the corresponding full length peptide or protein.

As used herein, "derivatives" are molecules such as proteins, fragments or variants thereof that have been altered, for example by conjugation or complexing with other chemical moieties, by post-translational modification (*e.g.*, phosphorylation, acetylation and the like), modification of glycosylation (*e.g.*, adding, removing or altering glycosylation), lipidation and/or inclusion of additional amino acid sequences as would be understood in the art.

In particular examples, a derivative or variant protein or peptide may comprise one or a plurality of amino acid residues at an N and/or C-terminus thereof. Additional amino acid sequences may also include fusion partner amino acid sequences, which create a fusion protein. Other derivatives disclosed herein include, but are not limited to, modification to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide, or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the isolated proteins of the present disclosure. In this regard, the skilled person is referred to Chapter 15 of CURRENT PROTOCOLS IN PROTEIN SCIENCE, Eds. Coligan et al. (John Wiley & Sons NY 1995-2008) for more extensive methodology relating to chemical modification of proteins.

Also contemplated herein are fragments, variants and derivatives of the nucleic acid molecules, inclusive of the backbone viral segments, described herein. Variants may comprise a nucleotide sequence at least about 70%, at least about 75%, preferably at least about 80%, at least about 85%, more preferably at least about 90%, 90.5%, 91%, 91.5%, 93%, 93.5%, 94%, 94.5%, 95%, 95.5%, 96%, 96.5%, 97%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%, 99.3%, 99.4, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% nucleotide sequence identity with any nucleotide sequence encoding the viral proteins of the present disclosure (e.g., SEQ ID NOs: 1-8, 20-27, 39-46, 58-65, 77-84 or 96-103) or more particularly, the backbone viral proteins of the present disclosure (e.g., SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 or 96-101). Nucleic acid derivatives may include chemically modified nucleic acids, modified internucleotide linkages, nucleic acid analogues, artificial nucleic acids and combinations thereof, as are known in the art.

Fragments of the isolated nucleic acid may comprise or consist of up to 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95-99% of the contiguous nucleotides present in any nucleotide sequence encoding the viral proteins, inclusive of the backbone viral proteins, of the present disclosure, such that they encode at least a portion of the viral protein. In general, fragments may comprise, consist essentially of or consist of up to 150, 165, 180, 195, 210, 225, 240, 255, 270, 285, 300, 315, 330, 345, 360, 375, 390, 405, 420, 435, 450, 465, 480, 495, 510, 525, 540, 555, 570, 585, 600, 615, 630, 645, 660, 675, 690, 705, 720, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 1950, 2000, 2050, 2100, 2150, 2200, 2250 contiguous nucleic acids that encode a portion of a viral protein described herein (e.g., SEQ ID NOs: 1-8, 20-27, 39-46, 58-65, 77-84 or 96-103 or a nucleotide sequence complementary thereto).

In particular examples, the isolated nucleic acid described herein may be modified to include, or alternatively modified to not include, a 5' non-coding region (e.g., AGC[A/G]AAAGCAGG (SEQ ID NO: 115)), wherein [A/G] indicates a nucleotide variation of A or G at this position) and/or a 3' non-coding region (e.g., CCTTGTTTCTACT (SEQ ID NO: 116)) of an influenza virus, as are known in the art.

The present disclosure also provides nucleic acids, such as backbone viral segments, that have been modified such as by taking advantage of codon sequence redundancy. In a more particular example, codon usage may be modified to optimize expression of a nucleic acid in a particular organism or cell type.

The variant backbone viral segments and viral proteins may be produced by any means known in the art, including but not limited to, chemical synthesis, recombinant DNA technology, including site-specific mutagenesis, or replacing a portion of the coding sequence with a portion that includes the characteristic residue(s), and proteolytic cleavage to produce peptide fragments.

Chemical synthesis is inclusive of solid phase and solution phase synthesis. Such methods are well known in the art, although reference is made to examples of chemical synthesis techniques as provided in Chapter 9 of SYNTHETIC VACCINES Ed. Nicholson (Blackwell Scientific Publications) and Chapter 15 of CURRENT PROTOCOLS IN PROTEIN SCIENCE Eds. Coligan et al, (John Wiley & Sons, Inc. NY USA 1995-2008). In this regard, reference is also made to International Publication WO 99/02550 and International Publication WO 97/45444.

Recombinant nucleic acids and proteins may be conveniently prepared by a person skilled in the art using standard protocols as for example described in Sambrook et al, MOLECULAR CLONING. A Laboratory Manual (Cold Spring Harbor Press, 1989), in particular Sections 16 and 17; CURRENT PROTOCOLS IN MOLECULAR BIOLOGY Eds. Ausubel et al, (John Wiley & Sons, Inc. NY USA 1995-2008), in particular Chapters 10 and 16; and CURRENT PROTOCOLS IN PROTEIN SCIENCE Eds. Coligan et al, (John Wiley & Sons, Inc. NY USA 1995-2008), in particular Chapters 1, 5 and 6. Typically, recombinant protein preparation includes expression of a nucleic acid encoding the protein in a suitable host cell. Modified proteins can, for example, be obtained by mutating the gene or genes (i.e., viral gene segments) encoding the protein of interest by site-directed or random mutagenesis. Such mutations may include point mutations, deletion mutations and insertional mutations. For example, one or more point mutations (e.g., substitution of one or more amino acids with one or more different amino acids) may be used to construct the modified proteins described herein.

Encoding nucleic acids

The present disclosure also provides an isolated nucleic acid encoding the backbone viral proteins described herein.

The term “nucleic acid” as used herein designates single- or double- stranded DNA and RNA. DNA includes genomic DNA and cDNA. RNA includes mRNA, RNA, RNAi, siRNA, cRNA and autocatalytic RNA. Nucleic acids may also be DNA-RNA hybrids. A nucleic acid comprises a nucleotide sequence which typically includes nucleotides that comprise an A, G, C, T or U base. However, nucleotide sequences may include other bases such as modified purines (for example inosine, methylinosine and methyladenosine) and modified pyrimidines (for example thiouridine and methylcytosine).

In particular examples, the isolated nucleic acid is or comprises a viral segment (i.e., an influenza RNA segment) that encodes the viral proteins, inclusive of backbone viral proteins, provided herein. Suitably, the viral segment comprises, consists of or consists essentially of a nucleotide sequence set forth in any one of SEQ ID NOs: 1-8, 20-27, 39-46, 58-65, 77-84 and 96-103, or a fragment, derivative or variant thereof. More particularly, the viral segment may comprise, consist of or consist essentially of a nucleotide sequence set forth in any one of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a nucleotide sequence complementary thereto, or a fragment, derivative or variant thereof. In some examples, the isolated nucleic acid is or comprises a nucleotide sequence complementary to a viral segment that encodes a viral protein, and more particularly a backbone viral protein, provided herein. Accordingly, the present disclosure further contemplates that the isolated nucleic acid suitably is or comprises a nucleotide sequence that is complementary to that set forth in any one of SEQ ID NOs: 1-8, 20-27, 39-46, 58-65, 77-84 and 96-103, or a fragment, derivative or variant thereof. In alternative examples, the isolated nucleic acid is or comprises a DNA or cDNA sequence that encodes a viral segment (i.e., viral RNA) that encodes the viral protein, and more particularly a backbone viral protein, provided herein. In various examples, the isolated nucleic acid is or comprises a viral mRNA sequence that encodes the viral protein, and more particularly a backbone viral protein, provided herein.

As used herein, a “*polynucleotide*” is a nucleic acid generally having eighty (80) or more contiguous nucleotides, while an “*oligonucleotide*” generally has less than eighty (80) contiguous nucleotides. A “*primer*” is usually a single-stranded oligonucleotide, suitably having 15-50 contiguous nucleotides, which is capable of annealing to a complementary

nucleic acid “template” and being extended in a template-dependent fashion by the action of a DNA polymerase such as *Taq* polymerase, RNA-dependent DNA polymerase or Sequenase™. A “probe” may be a single or double-stranded oligonucleotide or polynucleotide, suitably labelled for the purpose of detecting complementary sequences in Northern or Southern blotting, for example.

The isolated nucleic acids disclosed herein can be conveniently prepared using standard protocols such as those described in Chapter 2 and Chapter 3 of CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Eds. Ausubel *et al.* John Wiley & Sons NY, 1995-2008).

Nucleic acids of the present disclosure may be produced, isolated, detected and/or subjected to recombinant DNA technology using nucleic acid sequence amplification techniques.

Suitable nucleic acid amplification techniques covering both thermal and isothermal methods are well known to the skilled addressee, and include polymerase chain reaction (PCR); strand displacement amplification (SDA); rolling circle replication (RCR); nucleic acid sequence-based amplification (NASBA), Q- β replicase amplification, recombinase polymerase amplification (RPA) and helicase-dependent amplification, although without limitation thereto.

Genetic constructs

The present disclosure also provides a genetic construct comprising and/or encoding the isolated nucleic acid hereinbefore described. The genetic construct may be a vector.

In particular examples, the genetic construct comprises the isolated nucleic acid operably linked or connected to one or more other genetic components. A genetic construct may be suitable for therapeutic delivery of the isolated nucleic acid (e.g., a DNA or RNA vaccine) or for recombinant production of the viral proteins of the disclosure in a host cell. Additionally, the genetic construct may be used for the production or generation of influenza viruses (e.g., a variant or modified strain of the influenza virus isolate or a reassortant influenza virus isolate) that expresses the viral proteins, and more particularly the viral backbone proteins, provided herein.

Broadly, the genetic construct can be in the form of, or comprises genetic components of, a plasmid, bacteriophage, a cosmid, a yeast or bacterial artificial chromosome as are well

understood in the art. Genetic constructs may be suitable for maintenance and propagation of the isolated nucleic acid in bacteria or other host cells, for manipulation by recombinant DNA technology and/or expression of the nucleic acid or an encoded protein of the present disclosure. A vector can also be a naked RNA polynucleotide, a naked DNA polynucleotide, a polynucleotide composed of both DNA and RNA within the same strand, a poly-lysine-conjugated DNA or RNA, a peptide-conjugated DNA or RNA, a liposome-conjugated DNA, or the like. Such vectors may be or may not be autonomously replicating.

For the purposes of host cell expression, the genetic construct is an expression construct. Suitably, the expression construct comprises the nucleic acid of the present disclosure operably linked to one or more additional sequences in an expression vector. An “*expression vector*” may be either a self-replicating extra-chromosomal vector such as a plasmid, or a vector that integrates into a host genome. An expression construct may alternatively be a linear expression construct. Such linear expression constructs will typically not contain any amplification and/or selection sequences. However, linear constructs comprising such amplification and/or selection sequences are also within the scope of the present disclosure. A linear expression construct may, for example, include individual linear expression constructs for each viral segment. It is also possible to include more than one, such as two, three four, five or six, viral segments on the same linear expression construct.

Expression constructs suitable for use in the methods of the present disclosure may be uni-directional or bi-directional expression constructs. As influenza viruses require a protein for infectivity, it is generally preferred to use bi-directional expression constructs as this reduces the total number of expression constructs required by the host cell. Bi-directional expression constructs contain at least two promoters which drive expression in different directions (i.e. both 5' to 3' and 3' to 5') from the same construct. The two promoters can be operably linked to different strands of the same double stranded DNA. Suitably, one of the promoters is a pol I promoter and at least one of the other promoters is a pol II promoter. Thus, the methods of the present disclosure may utilise at least one bi-directional expression construct wherein at least one gene or cDNA is located between an upstream pol II promoter and a downstream non-endogenous pol I promoter. Transcription of the gene or cDNA from the pol II promoter produces capped positive-sense viral mRNA, which can be translated into a protein, while transcription from the non-endogenous pol I promoter produces negative-sense viral RNA (vRNA).

By “*operably linked*” is meant that said additional nucleotide sequence(s) is/are positioned relative to the nucleic acid of the present disclosure preferably to initiate, regulate or otherwise control transcription thereof.

Regulatory nucleotide sequences will generally be appropriate for the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells, as described herein. Expression vectors can be designed for expression of the viral proteins described herein using prokaryotic (e.g., *E. coli*) or eukaryotic cells (e.g., insect cells (using baculovirus expression vectors, see, e.g., Treanor et al., 2007, JAMA, 297(14):1577-1582 incorporated by reference herein in its entirety), yeast cells, plant cells, algae or mammalian cells).

Typically, said one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, polyadenylation sequences, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive, repressible or inducible promoters as known in the art are contemplated by the present disclosure.

In some examples, the genetic construct includes one or more untranslated 5' and/or 3' regions that are operably linked or connected to a viral segment that encodes the corresponding viral protein. To this end, the UTRs may be derived from the same virus isolate or a different influenza virus isolate from which the viral segment and/or the viral protein is derived.

The expression construct may also include an additional nucleotide sequence encoding a fusion partner (typically provided by the expression vector) so that the recombinant protein is expressed as a fusion protein.

The expression construct may also include an additional nucleotide sequence encoding a selection marker, such as amp^R , neo^R or kan^R , although without limitation thereto.

Suitably, the genetic construct encoding a viral protein provided herein is suitable or adapted for use in the production or generation of reassortant influenza viruses that express said viral protein by a reverse genetics method or a hybrid reverse genetics-classical reassortment

method. Accordingly, the one or more genetic construct(s) provided herein may be introduced into a host cell using any method for introducing expression construct(s) from known reverse genetics techniques. For example, genetic constructs can be introduced into host cells by employing electroporation, DEAE-dextran, calcium phosphate precipitation, liposomes, microinjection, or microparticle-bombardment. In some examples, a genetic construct may be in the form of naked nucleic acid. The naked nucleic acid may have been purified from an influenza virus. In another example, a genetic construct may be in the form of transcribed RNA (e.g., viral mRNA). In other examples, a genetic construct may be in the form of one or more shuttle vectors. Examples of shuttle vectors include non-influenza viruses and replicons, for instance alphavirus based replicons.

The genetic construct provided herein may include an RNA transcription termination sequence. The termination sequence may be an endogenous termination sequence or a termination sequence, which is not endogenous to the host cell. Suitable termination sequences will be evident to those of skill in the art and include, but are not limited to, RNA polymerase I transcription termination sequence, RNA polymerase II transcription termination sequence, and ribozymes. Furthermore, the expression constructs may contain one or more polyadenylation signals for mRNAs, particularly at the end of a gene whose expression is controlled by a pol II promoter.

In another form, the disclosure also provides a plurality of genetic constructs, including a genetic construct that includes a nucleic acid that: (a) encodes one or more of the viral proteins, or more particularly backbone viral proteins, described herein; and/or (b) comprises and/or encodes one or more of the viral segments, or more particularly backbone viral segments, described herein, inclusive of a nucleotide sequence complementary thereto. The plurality of genetic constructs may further include one or more further genetic constructs that may be utilised in preparing reassortant viruses, including 6:1:1 reassortants, 6:2 reassortants, 5:3 reassortants and 7:1 reassortants. The further genetic constructs may comprise and/or encode one or both of HA and NA viral segments (i.e., encode one or more of NA and HA viral proteins).

In a related form, the present disclosure also relates to a method of producing the viral proteins provided herein, said method including the steps of: (i) culturing the previously transformed

host cell described herein; and (ii) isolating said viral protein from said host cell cultured in step (i).

Cells

The present disclosure also provides a host cell that is: (a) transformed with the isolated nucleic acid and/or the genetic construct described herein; or (b) infected with the isolated influenza virus provided herein.

The host cells may be any as are known in the art. One well known method for influenza virus growth uses specific pathogen-free (SPF) embryonated hen eggs, with virus being inoculated into, grown and purified from the egg contents (i.e., allantoic fluid). Influenza viruses may also be grown in animal cell culture and, for reasons of replication accuracy, speed and patient allergies, this culture method is preferred.

Referring to the cells described herein, the present methods will typically use a cell line, although primary cells may be used as an alternative. Such cells or cell lines may be bacterial, insect cells, yeast cells, plant cells, algae or mammalian cells. Examples of yeast host cells include, but are not limited to *S. pombe* and *S. cerevisiae*. Examples of mammalian host cells include, but are not limited to, Crucell Per.C6 cells, Vero cells, CHO cells, VERY cells, BHK cells, HeLa cells, COS cells, MDCK cells, 293 cells, 3T3 cells or WI-38 cells. In certain examples, the hosts cells are myeloma cells, such as NSO cells, 45.6 TG1.7 cells, AF-2 clone 9B5 cells, AF-2 clone 9B5 cells, J558L cells, MOPC 315 cells, MPC-11 cells, NCI-H929 cells, NP cells, NSO/1 cells, P3 NS1 Ag4 cells, P3/NS1/1-Ag4-1 cells, P3U1 cells, P3X63Ag8 cells, P3X63Ag8.653 cells, P3X63Ag8U.1 cells, RPMI 8226 cells, Sp20-Ag14 cells, U266B1 cells, X63AG8.653 cells, Y3.Ag.1.2.3 cells, and YO cells. Non-limiting examples of insect cells include SJ9, SJ21, *Trichoplusia ni*, *Spodoptera fugiperda* and *Bombyx mori*. Exemplary plant cell systems for the expression of viral proteins are provided in U.S. Pat. Nos. 7,504,560; 6,770,799; 6,551,820; 6,136,320; 6,034,298; 5,914,935; 5,612,487; and 5,484,719, and U.S. patent application publication Nos. 2009/0208477, 2009/0082548, 2009/0053762, 2008/0038232, 2007/0275014 and 2006/0204487

In particular examples, the host cell is mammalian. Suitable mammalian cells include, but are not limited to, hamster, cattle, primate (including humans and monkeys) and dog cells. Various cell types may be used, such as kidney cells, fibroblasts, retinal cells and lung cells, as are

known in the art. Examples of suitable hamster cells are the cell lines having the names BHK21 or HKCC. Suitable monkey cells include African green monkey cells, such as kidney cells as in the Vero cell line (Kistner et al. (1998) Vaccine 16:960-8; Kistner et al. (1999) Dev Biol Stand 98:101-110; Bruhl et al. (2000) Vaccine 19:1149-58). Suitable dog cells include canine kidney cells, as in the CLDK and MDCK cell lines (W097/37000; Brands et al. (1999) Dev Biol Stand 98:93-100; Halperin et al. (2002) Vaccine 20:1240-7; Tree et al. (2001) Vaccine 19:3444-50). Thus, suitable cell lines include, but are not limited to: MDCK; CHO; 293T; BHK; Vero; MRC-5; PER.C6 and WI-38 cell lines.

According to particular examples, the cell or cell line described herein is suitable for growing influenza viruses. Such cell lines may include: MDCK cells derived from Madin Darby canine kidney; Vero cells derived from the African green monkey (*Cercopithecus aethiops*) kidney; or PER.C6 cells derived from human embryonic retinoblasts (Pau et al. (2001) Vaccine 19:2716-21). These cell lines are widely available, such as from the American Type Cell Culture (ATCC) collection, the Coriell Cell Repositories and the European Collection of Cell Cultures (ECACC). Alternative cell lines may include avian cell lines (see, e.g., WO2003/076601; WO2005/042728; WO2003/043415), including cell lines derived from ducks (e.g., duck retinal cells) or hens (e.g., chicken embryo fibroblasts (CEF)). Examples include avian embryonic stem cells, including the EBx cell line derived from chicken embryonic stem cells, EB45, EB14, EB14-074 and EB66.

Suitably, the cell or cell line are MDCK cells derived from Madin Darby canine kidney. The original MDCK cells are available from the ATCC as CCL-34. Derivatives of MDCK cells may also be used. For instance, the MDCK cell line may be adapted for growth in suspension culture (e.g., 'MDCK 33016', deposited as DSM ACC 2219). Similarly, WO2001/064846 discloses a MDCK-derived cell line that grows in suspension in serum-free culture ('B-702', deposited as FERM BP-7449). WO2006/071563 discloses non-tumorigenic MDCK cells, including 'MDCK-S' (ATCC PTA-6500), 'MDCK-SF101' (ATCC PTA-6501), 'MDCK-SF102' (ATCC PTA-6502) and 'MDCK-SF103' (PTA-6503). WO2005/113758 discloses MDCK cell lines with high susceptibility to infection, including 'MDCK.5F1' cells (ATCC CRL-12042). Any MDCK cell line, including those provided herein, can be used in the methods of the present disclosure.

For viral growth or propagation on a cell line, such as on MDCK cells, the influenza virus may

be grown on cells in suspension or in adherent culture. Further, the cells described herein can be cultured in various serum-free media or media that is substantially free of serum, as are known to the person skilled in the art (e.g., Iscove's medium, ultra CHO medium (Bio Whittaker), EX-CELL (JRH Biosciences)). Otherwise, the cells for replication can alternatively be cultured in the serum-containing media (e.g., MEM or DMEM medium with about 0.5% to about 10%, more particularly about 1.5% to about 5%, of foetal calf serum) or protein-free media (e.g., PF-CHO (JRH Biosciences)). Suitable culture vessels, which can be employed in the course of the methods described herein can be vessels known to the person skilled in the art, such as, for example, spinner bottles, roller bottles or fermenters.

Where cells are used as a culture host for the influenza viruses described herein, it is known that cell culture conditions (e.g. temperature, moi, cell density, pH value, trypsin, etc.) are variable over a wide range subject to the cell line and the influenza virus strain employed and can be adapted to the requirements of the application. The following information therefore merely represents a guide to standard culture conditions.

In particular examples, the cells are suitably grown in serum-free culture media and/or protein free media, such as for cell proliferation and/or supporting influenza virus replication. A medium is referred to as a serum-free medium in the context of the present disclosure if it contains no additives or substantially no additives (e.g., less than 0.5%, 0.25% or 0.1% by weight thereof) from serum of human or animal origin. Protein-free refers to a culture media in which multiplication of the cells occurs with the exclusion of proteins, growth factors, other protein additives and non-serum proteins, but can optionally include proteins such as trypsin or other proteases that may be necessary for viral growth. The cells growing in such cultures naturally contain proteins themselves.

Multiplication of the cells can be conducted in accordance with methods known to those of skill in the art. For example, the cells can be cultivated in a perfusion system using ordinary support methods like centrifugation or filtration. Moreover, the cells can be multiplied according to the invention in a fed-batch system before infection. In this context, a culture system can be referred to as a fed-batch system in which the cells are initially cultured in a batch system and depletion of nutrients (or part of the nutrients) in the medium is compensated by controlled feeding of concentrated nutrients. The pH value of the medium may be adjusted during multiplication of the cells before infection to a value between pH 6.6 and pH 7.8 and

especially between a value between pH 7.2 and pH 7.3. After infection with the influenza viruses, the cells are suitably cultured at a pH of between about 6.7 to about 7.7.

Cell lines supporting influenza virus replication are suitably cultured at a temperature below 37°C (e.g., about 30°C to about 36°C, or at about 30°C, 31°C, 32°C, 33°C, 34°C, 35°C, 36°C or any range therein) during viral replication. Where virus is grown on a cell line then the culture media, and also the viral inoculum used to start the culture, is suitably free from (e.g., will have been tested for and given a negative result for contamination by) contaminating viruses, such as herpes simplex virus, respiratory syncytial virus, parainfluenza virus 3, SARS coronavirus, adenovirus, rhinovirus, reoviruses, polyomaviruses, bimaviruses, circoviruses, and/or parvoviruses.

The method for propagating influenza virus in cultured cells generally includes the steps of inoculating the cultured cells with the strain to be cultured, cultivating the infected cells for a desired time period for virus propagation, such as determined by virus titer or antigen expression (e.g., between about 24 and about 168 hours after inoculation) and collecting the propagated virus. The cultured cells can be inoculated with a virus (such as measured by PFU or TCID₅₀) to cell ratio of 1:500 to 1:1, more particularly 1:100 to 1:5 or even more particularly 1:50 to 1:10. The virus can then be added to a suspension of the cells or is applied to a monolayer of the cells, and the virus is absorbed on the cells for at least 60 minutes but usually less than 300 minutes, suitably between 90 and 240 minutes at 25°C to 40°C, or more particularly 28° C to 37° C. The infected cell culture (e.g., monolayers) may be removed either by freeze-thawing or by enzymatic action to increase the viral content of the harvested culture supernatants. The harvested fluids may then either be inactivated or stored frozen.

Cultured cells may be infected at a multiplicity of infection ("m.o.i.") of about 10⁻⁸ to about 10 (e.g., a MOI of about 10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵, 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10 and any range therein), more particularly about 0.002 to about 5, even more particularly about 0.001 to about 2. Infected cells may be harvested 30 to 96 hours post infection or more particularly 34 to 48 hours post infection. Determining the optimal harvest time is within the normal capabilities of a person skilled in the art. Proteases, such as trypsin, are generally added during cell culture to allow viral release, and the proteases can be added at any suitable stage during the culture.

Methods of preparing influenza viruses

Also provided herein are methods of preparing or producing an influenza virus in cells.

Such methods suitably include the step of contacting the cells with a genetic construct comprising a nucleic acid that: (a) encodes a backbone viral protein described herein; and/or (b) encodes and/or comprises a backbone viral segment described herein. Accordingly, in one form, the present disclosure provides a method of preparing an influenza virus, said method including the step of contacting a cell, such as an MDCK cell, with one or more genetic constructs that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.

It is also envisaged by the present disclosure that methods of classical reassortment (or portions thereof) may be utilised to prepare or produce an influenza virus in cells, such as MDCK cells. Accordingly, in another form, the present disclosure provides a method of preparing an influenza virus, said method including the step of contacting the cells with an influenza virus that expresses one or more of the backbone viral proteins described herein (e.g., the influenza virus isolate comprises one or more PB2, PB1, PA, M, NP and NS viral segments that encode the one or more backbone viral proteins that comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof).

In view of the foregoing, the present methods may be utilised to prepare or produce a modified or variant strain of an influenza virus, such as those provided herein, that includes one or more backbone viral segments encoding the backbone viral proteins described herein. To this end, the present methods may be utilised to prepare or produce a reassortant influenza virus, such as those provided herein, that includes one or more backbone viral segments encoding the backbone viral proteins described herein. In particular examples, seven (7), eight (8) or all of the nine (9) backbone viral proteins (i.e., PA, PB1, PB1-F2, PB2, NP, NS1, NEP, M1 and M2 viral proteins) can be derived from a single influenza virus isolate (e.g., SEQ ID NOs: 9-17 from A/Ohio/02/2019; SEQ ID NOs: 28-36 from A/Singapore/TT1384/2016; SEQ ID NOs: 47-55 from A/South Carolina/04/2017; SEQ ID NOs: 66-74 from A/Alaska/06/2019; SEQ ID NOs: 85-93 from A/Darwin/11/2021; SEQ ID NOs: 104-112 from A/Tasmania/503/2020;

inclusive of fragments, variants or derivatives thereof). In certain examples, five (5) or all of the six (6) backbone viral segments (i.e., PA, PB1, PB2, NP, NS and M viral segments) can be derived from a single influenza virus isolate (e.g., SEQ ID NOs: 1-6 from A/Ohio/02/2019; SEQ ID NOs: 20-25 from A/Singapore/TT1384/2016; SEQ ID NOs: 39-44 from A/South Carolina/04/2017; SEQ ID NOs: 58-63 from A/Alaska/06/2019; SEQ ID NOs: 77-82 from A/Darwin/11/2021; SEQ ID NOs: 96-101 from A/Tasmania/503/2020; inclusive of fragments, variants or derivatives thereof).

In other examples, all of the eight viral segments (i.e., PA, PB1, PB2, NP, NS, M, NA and HA) can be derived from a single influenza virus isolate. In such examples, the influenza virus isolate is suitably a backbone or donor influenza virus isolate, such as those hereinbefore described. Additionally, such isolated influenza virus strains are suitably not considered to be a reassortant influenza virus.

Suitably, the present method may be or at least partly comprise a reverse genetics method of generating reassortant viruses. In reverse genetics, the genetic information required to produce the desired influenza virus is delivered to a cell, which is then able to generate influenza virus. Reverse genetics initially required the *in vitro* assembly and transfection of viral ribonucleoprotein (RNP) into cells infected with a helper virus (Luytjes *et al.* (1989) Cell 59(6): 1107-1113; Enami *et al.* (1990) PNAS 87(10):3802-3805). Subsequent techniques involved the transfection of RNA polymerase I plasmids encoding all of the viral RNAs (vRNAs) together with protein expression constructs for the polymerase and NP genes (Fodor *et al.* (1999) J Virol. 73(11):9679-9682). More recently, reverse genetics methods involve the use of modified RNA polymerase I systems that allow the expression of both negative sense vRNA and positive sense mRNA from the same template (Hoffmann *et al.* (2000) PNAS 97(11):6108-6113). In such a method, each of the desired genes is cloned into the pHW2000 plasmid, which consists of the viral cDNA inserted between the RNA polymerase I promoter and termination sequences, and flanked by the CMV promoter and polyadenylation signal. After transfection of the eight plasmids into cells, synthesis of both vRNA and mRNA occurs, resulting in the production of virus. Further refinement has led to the development of systems in which linear DNA expression constructs are used instead of plasmids (see, e.g., WO2009/000891) and the use of a single expression construct (see, e.g., WO2011/012999).

As such, the present method may further include the step of contacting the cells with one or more further genetic constructs, wherein the one or more further genetic constructs comprise one or more further nucleic acids that encode one or more of a HA protein, a PB1 protein, a PB1-F2 protein and an NA protein (e.g., a 5:3 reassortant virus), more particularly a HA protein and an NA protein (e.g., a 6:2 reassortant virus), even more particularly a HA protein and optionally an NA protein (e.g., a 6:2 or a 7:1 reassortant virus) or yet even more particularly an NA protein and optionally a HA protein (e.g., a 6:2 or a 7:1 reassortant virus).

In particular examples, the present method includes one or more of the following steps:

(a) contacting the cells with one or more expression constructs, such as those hereinbefore described, comprising one or more nucleic acid molecules that comprise and/or encode one or more of a PA viral segment, a PB1 viral segment, a PB2 viral segment, a NP viral segment, a M viral segment and a NS viral segment derived from a first influenza virus (e.g., one or more backbone viral segments that independently: (i) encode one or more viral proteins that comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof; and/or (ii) encode and/or comprise, consist of or consist essentially of a nucleotide sequence selected from those set forth in SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a nucleotide sequence complementary thereto, or a fragment, variant or derivative thereof);

(b) contacting the cells with one or more expression constructs, such as those hereinbefore described, comprising one or more nucleic acid molecules that comprise or encode a HA viral segment and optionally a NA viral segment derived from a second influenza virus (e.g., a vaccine influenza virus);

(c) culturing the cells in order to produce reassortant viruses; and

(d) selecting for reassortant viruses that comprise the HA viral segment and optionally the NA viral segment from the second influenza virus.

In other examples, the present method may involve a hybrid method of classical reassortment and reverse genetics. For instance, a method in which a host cell is infected with a first influenza strain (e.g., a donor strain) and transfected (e.g., before, after or simultaneously) with one or more expression construct(s) encoding at least one viral segment from a second influenza strain (e.g., HA and optionally NA viral segments from a vaccine strain). An example of such a method is outlined in WO2021099419, which is incorporated by reference herein.

Accordingly, in certain examples, the present method includes one or more of the following steps:

- (a) contacting the cells with a donor influenza virus strain comprising:
 - (i) one or more of PA, PB1, PB2, NP, M and NS viral segments that: encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof; and/or independently comprise, consist of or consist essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a nucleotide sequence complementary thereto, or a fragment, variant or derivative thereof; and
 - (ii) a first HA viral segment and a first NA viral segment (e.g., HA and NA viral segments that: respectively encode HA and NA viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 19, 37, 38, 56, 57, 75, 76, 94, 95, 113 and 114, or a fragment, variant or derivative thereof; and/or independently comprise, consist of or consist essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 7, 8, 26, 27, 45, 46, 64, 65, 83, 84, 102 and 103, or a fragment, variant or derivative thereof);
- (b) contacting the cells with one or more expression constructs comprising one or more nucleic acid molecules that comprise or encode a second HA viral segment and optionally a second NA viral segment derived from a vaccine influenza virus strain;
- (c) culturing the cells in order to produce reassortant viruses; and
- (d) selecting for reassortant viruses that comprise the second HA viral segment and optionally the second NA viral segment.

The term “vaccine influenza virus strain” as used herein refers to an influenza virus strain suitable for use in an immunogenic composition or an immunogenic virus (e.g., a reassortant virus). Vaccine influenza virus strains can include, but are not necessarily limited to, pathogenic strains, non-pathogenic or relatively non-pathogenic strains, killed strains and/or attenuated strains. In particular examples, the vaccine influenza virus strain is a pandemic influenza virus strain. In other examples, the vaccine influenza virus strain is a seasonal influenza virus strain.

The vectors or expression constructs utilised in the present method may be as per those known in the art, inclusive of those hereinbefore described. Thus, the present disclosure envisages the use of isolated and purified vectors or plasmids, which express or encode influenza virus proteins, or express or encode influenza vRNA, both native and recombinant vRNA. The vectors may comprise influenza cDNA (see, e.g., Fields *Virology* (Fields et al. (eds.), Lippincott, Williams and Wickens (2013), which is incorporated by reference herein). Any suitable promoter or transcription termination sequence may be employed to express a protein or peptide, e.g., a viral protein, such as a backbone viral protein described herein. By way of the example, one or more of the expression constructs may be adapted for: (a) vRNA production and comprise a promoter operably linked to an influenza virus DNA molecule linked to a transcription termination sequence; and/or (b) mRNA production and comprise a promoter operably linked to a DNA segment encoding an influenza viral segment.

As noted above, an additional selection step to enhance the production of reassortant viruses comprising the second HA viral segment and optionally the second NA viral segment are also contemplated by the present disclosure. The selection step may comprise any method that enhances the selection of reassortant viruses comprising a HA viral segment that encodes a HA protein derived from the vaccine virus strain. Suitably, the selection step is carried out after the host cells have been cultured in order to produce reassortant influenza viruses that may express the HA protein and/or NA protein derived from the vaccine virus strain.

Suitably, the methods provided herein comprise a step of harvesting, isolating or separating reassortant viruses from the host cells prior to the selection step. By way of example, cell culture supernatant comprising the reassortant viruses is separated from the host cells and the selection step is performed on the supernatant that comprises the reassortant viruses.

In some examples, the selection step comprises negative selection against reassortant viruses comprising the HA protein from the donor virus strain. Negative selection can include, for example, contacting the host cells, reassortant viruses that have been separated therefrom and/or cell culture supernatant with one or more antibodies that specifically binds or are raised against the HA protein from the donor virus strain. Negative selection may also comprise exposure of the host cells to inhibitory agents (e.g., short interfering RNAs (siRNA), double-stranded RNAs (dsRNA), micro-RNAs (miRNAs), short hairpin RNAs (shRNA), or small interfering DNAs (siDNAs)) that preferentially or specifically reduce the transcription and/or

translation of the donor strain's HA viral segment or protein relative to the vaccine strain's HA viral segment or protein. In addition to the above, the selection step may include negative selection against reassortant viruses comprising the NA protein from the donor strain, such as by utilising one or more antibodies that specifically binds or are raised against the NA protein from the donor strain.

In other examples, the selection step is or comprises a positive selection step. The positive selection step may include contacting the host cells, reassortant viruses that have been separated therefrom and/or cell culture supernatant with one or more antibodies that are specific for the HA protein derived from the vaccine virus strain. In this way, reassortant viruses that comprise the HA viral segment derived from the vaccine virus strain can be positively selected from the host cells or cell culture supernatant. Suitably, one or more antibodies used for positive selection are labelled (e.g., with a magnetic bead). Labelling aids subsequent isolation, such as by affinity chromatography, of reassortant viruses comprising the HA gene that encodes the HA protein from the vaccine virus strain.

The methods described herein may comprise one or more positive and/or negative selection steps. For example, reassortant virus may be passaged multiple times in the presence of the one or more antibodies described above for positive and/or negative selection. Multiple selection steps may be performed to enhance the selection of reassortant influenza viruses comprising the HA viral segment that encodes the HA protein derived from the vaccine virus strain.

The present methods suitably produce a pool of reassortant viruses from which a particular class of reassortant virus can be isolated. A reassortant virus having, for example, high growth properties and expressing the HA protein and optionally NA protein of a seasonal or pandemic influenza strain can be isolated for use in vaccine manufacture. Accordingly, the above methods may further include the step of isolating a reassortant influenza virus comprising the HA viral segment that encodes the HA protein of a vaccine virus strain. In other examples, the present methods may further include the step of isolating a reassortant influenza virus comprising the NA viral segment that encodes the NA protein of a vaccine virus strain.

Suitably, the present methods include contacting the cells with an influenza virus isolate and/or one or more genetic constructs in an amount sufficient to yield infectious influenza virus. To this end, the present methods may include harvesting or isolating intact or whole virions from

the cell culture media. Alternatively or additionally, the present methods can include harvesting or isolating split virions from the culture media. In such examples, the harvesting or isolating step suitably includes contacting the influenza virus with a splitting agent, such as a detergent. The present methods may alternatively or additionally include harvesting or isolating one or more particular influenza virus proteins, such as HA protein, from the culture media or from the harvested influenza virus, such as by affinity chromatography.

During harvesting or isolation of the influenza virus and/or the influenza virus proteins, the cells may be separated from the culture medium by standard methods like centrifugation, separation, filtration or ultrafiltration. The influenza virus or the viral proteins produced therefrom can then be concentrated and/or purified according to methods known to those skilled in the art, such as gradient centrifugation (e.g., gradient ultracentrifugation (GUC)), filtration, precipitation, chromatography, and any combination thereof. In particular examples, the influenza virus and/or one or more proteins produced thereby are isolated or harvested from the culture media by gradient ultracentrifugation. Suitably, the influenza viruses are inactivated during or after purification. Virus inactivation can occur, for example, by the contacting the influenza virus with an inactivating agent (e.g., addition of b-propionolactone or formaldehyde) at any point within the purification process.

Suitably, the present method may further include the step of selecting the influenza virus, such as a reassortant influenza virus, that is capable of enhanced growth and/or yield in cells and, more particularly, mammalian cells like MDCK cells. In particular examples, the present method includes the step of determining whether the influenza virus is capable of enhanced growth and/or yield when cultured in cells. Accordingly, in one broad form the present disclosure provides a method of preparing an influenza virus capable of enhanced growth and/or yield when grown in cells.

Based on the foregoing, the present disclosure further provides an isolated influenza virus prepared by the methods described herein. Additionally, the present disclosure relates to an isolated viral protein (e.g., HA and/or NA viral proteins) produced by the isolated influenza virus and the methods provided herein.

Immunogenic compositions

The present disclosure envisages that the isolated influenza virus, and more particularly

isolated reassortant influenza viruses, described herein, or one or more viral proteins isolated therefrom, may be utilised in immunogenic compositions, such as vaccine compositions.

Accordingly, in one form, the present disclosure provides a method of making an immunogenic composition, including the steps of:

- (a) providing the isolated influenza virus provided herein; and
- (b) combining the isolated influenza virus with an adjuvant and/or treating the isolated influenza virus with an agent that inactivates or attenuates the virus.

In another form, the present disclosure provides a method of making an immunogenic composition, including the steps of:

- (a) providing one or more viral proteins (e.g., HA and/or NA viral proteins) isolated from the isolated influenza virus provided herein; and
- (b) combining the one or more viral proteins with an adjuvant.

In a related form, the present disclosure relates to an immunogenic composition, wherein the immunogenic composition is produced according to the methods provided herein.

In another related form, the present disclosure relates to an immunogenic composition, wherein the immunogenic composition: comprises the isolated influenza virus described herein and a pharmaceutically acceptable carrier, diluent or excipient.

In a further related form, the present disclosure relates to an immunogenic composition, wherein the immunogenic composition: comprises one or more of the isolated viral proteins (e.g., HA and/or NA viral proteins) described herein and a pharmaceutically acceptable carrier, diluent or excipient.

Influenza vaccines are generally based either on a live attenuated virus or on an inactivated virus. Inactivated vaccines may be based on whole virions, “split” virions, or on purified surface antigens. Viral antigens can also be presented in the form of virosomes. The present methods can be used for manufacturing any of these types of vaccine or immunogenic composition. Where an inactivated influenza virus is used, the immunogenic composition may comprise whole virion, split virion, or purified surface antigens (e.g., haemagglutinin and optionally neuraminidase). Chemical means for inactivating a virus include treatment with an

effective amount of one or more of the following inactivating agents: detergents, formaldehyde, b-propiolactone, methylene blue, psoralen, carboxyfullerene (C60), binary ethylamine, acetyl ethyleneimine, or combinations thereof. Non-chemical methods of viral inactivation are also known in the art, such as UV light or gamma irradiation. Subunit vaccines are also contemplated.

Virions can be harvested from virus-containing fluids, such as cell culture supernatant, by various methods. For example, a purification process may involve zonal centrifugation using a linear sucrose gradient solution (that optionally includes a detergent to disrupt the virions) or affinity chromatography methods. Antigens may then be purified, after optional dilution, by diafiltration.

The immunogenic composition disclosed herein may comprise two or more (e.g., 2, 3, 4, 5 etc) influenza viruses (or viral proteins isolated from two or more influenza viruses) whose HA and/or NA proteins are derived from different influenza strains or subtypes. Thus, the immunogenic composition disclosed herein may be a multivalent composition. For example, the immunogenic composition disclosed herein may be a trivalent or a quadrivalent composition. In one example, the immunogenic composition is a quadrivalent composition comprising at least one virus containing HA and/or NA proteins derived from an H1N1 strain, at least one virus containing HA and/or NA proteins derived from an H3N2 strain, and one or two viruses containing HA and/or NA proteins derived from a B strain. In another example, the immunogenic composition is a quadrivalent composition comprising one or more viral proteins derived from: at least one virus containing HA and/or NA proteins derived from an H1N1 strain; at least one virus containing HA and/or NA proteins derived from an H3N2 strain; and one or two viruses containing HA and/or NA proteins derived from a B strain.

The amounts of each virus or viral protein included in the compositions disclosed herein may vary according to the intended use of those compositions. For example, a standard dose, a low dose, or a high dose of HA and/or NA may be included, depending on the intended use (for example, the intended patient population) for the composition. Typically, "standard dose" influenza compositions contain about 15 µg of HA per strain in each dosage form or dosage unit. Thus, "low dose" influenza compositions may contain less than about 15 µg of HA per strain in each dosage form or dosage unit, for example, about 12 µg, about 9 µg, about 7.5 µg, about 5 µg, or about 3.75 µg. "High dose" influenza compositions may contain more than

about 15 µg of HA per strain in each dosage form or dosage unit, for example, about 30 µg, about 45 µg, or about 60 µg.

The immunogenic composition may contain a pharmaceutically-acceptable carrier, diluent or excipient. By “pharmaceutically-acceptable carrier, diluent or excipient” is meant a solid or liquid filler, diluent or encapsulating substance that may be safely used in systemic administration. Depending upon the particular route of administration, a variety of carriers, diluent and excipients well known in the art may be used. These may be selected from a group including sugars, starches, cellulose and its derivatives, malt, gelatine, talc, calcium sulfate, vegetable oils, synthetic oils, polyols, alginic acid, phosphate buffered solutions, emulsifiers, isotonic saline and salts such as mineral acid salts including hydrochlorides, bromides and sulfates, organic acids such as acetates, propionates and malonates, water and pyrogen-free water.

A useful reference describing acceptable carriers, diluents and excipients is Remington's Pharmaceutical Sciences (Mack Publishing Co. N.J. USA, 1991) which is incorporated herein by reference.

Suitably, for the purposes of eliciting an immune response, certain immunological or immunogenic agents may be used in combination with the isolated influenza virus described herein. The term “immunogenic agent” includes within its scope carriers, delivery agents, immunostimulants and/or adjuvants as are well known in the art. As will be understood in the art, immunostimulants and adjuvants refer to or include one or more substances that enhance the immunogenicity and/or efficacy of a composition. Non-limiting examples of suitable immunostimulants and adjuvants include squalane and squalene (or other oils of plant or animal origin), inclusive of squalene oil-in-water emulsions (e.g., MF59, AS03 and AF03); block copolymers; TLR agonists, such as pathogen-derived compounds, including lipopeptides, glycolipids, nucleotides, small-molecule inhibitors and bacterial-derived components, such as flagellin; detergents such as Tween®-80; Quil® A, mineral oils such as Drakeol or Marcol, vegetable oils such as peanut oil; *Corynebacterium*-derived adjuvants such as *Corynebacterium parvum*; *Propionibacterium*-derived adjuvants such as *Propionibacterium acne*; *Mycobacterium bovis* (Bacille Calmette and Guerin or BCG); *Bordetella pertussis* antigens; tetanus toxoid; diphtheria toxoid; surface active substances such as hexadecylamine, octadecylamine, octadecyl amino acid esters, lysolecithin, dimethyldioctadecylammonium

bromide, *N,N*-dioctadecyl-*N'*, *N'*bis(2-hydroxyethyl-propanediamine), methoxyhexadecylglycerol, and pluronic polyols; polyamines such as pyran, dextran sulfate, poly IC carbopol; peptides such as muramyl dipeptide and derivatives, dimethylglycine, tuftsin; oil emulsions; and mineral gels such as aluminium phosphate, aluminium hydroxide or alum; interleukins such as interleukin 2 and interleukin 12; monokines such as interleukin 1; tumour necrosis factor; interferons such as gamma interferon; immunostimulatory DNA such as CpG DNA, combinations such as saponin-aluminium hydroxide or Quil-A aluminium hydroxide; saponins, such as Matrix-M; liposomes; ISCOM® and ISCOMATRIX® adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as muramyl dipeptides or other derivatives; Avridine; Lipid A derivatives; dextran sulfate; DEAE-Dextran alone or with aluminium phosphate; carboxypolymethylene such as Carbopol' EMA; acrylic copolymer emulsions such as Neocryl A640 (*e.g.* U.S. Pat. No. 5,047,238); water in oil emulsifiers such as Montanide ISA 720; poliovirus, vaccinia or animal poxvirus proteins; or mixtures thereof. Suitably, the adjuvant to be included in the immunogenic compositions of the present disclosure is or comprises squalene. In certain examples, the adjuvant is or comprises a squalene oil-in-water emulsion. In a preferred example, the isolated influenza virus described herein, and/or one or more viral proteins isolated therefrom, is provided together with MF59.

Immunogenic agents may include carriers such as thyroglobulin; albumins such as human serum albumin; toxins, toxoids or any mutant cross-reactive material (CRM) of the toxin from tetanus, diphtheria, pertussis, *Pseudomonas*, *E. coli*, *Staphylococcus*, and *Streptococcus*; polyamino acids such as poly(lysine:glutamic acid); influenza; Rotavirus VP6, Parvovirus VP1 and VP2; hepatitis B virus core protein; hepatitis B virus recombinant vaccine and the like. Alternatively, a fragment or epitope of a carrier protein or other immunogenic protein may be used. For example, a T cell epitope of a bacterial toxin, toxoid or CRM may be used. In this regard, reference may be made to U.S. Patent No 5,785,973 which is incorporated herein by reference. It is contemplated that the carrier protein or other immunogenic protein may be directly linked or indirectly linked (such as by way of a linker as are known in the art) to the viral proteins described herein.

Oil-in-water emulsions have been found to be particularly suitable for use in adjuvanting influenza virus vaccines or immunogenic compositions. Various such emulsions are known, and they typically include at least one oil and at least one surfactant, with the oil(s) and

surfactant(s) being biodegradable (metabolisable) and biocompatible. The oil droplets in the emulsion are generally less than 5 μm in diameter, and may even have a sub-micron diameter, with these small sizes being achieved with a microfluidiser to provide stable emulsions. Droplets with an average size less than 220 nm are preferred as they can be subjected to filter sterilization.

In various examples, the oil-in-water emulsion is uniform. A uniform emulsion is characterized in that a majority of droplets (particles) dispersed therein is within a specified size range (e.g., in diameter). A suitable specified size range can be, for example, between about 50-220 nm (e.g., about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220 nm or any range therein), between about 50-180 nm, between about 80-180 nm, between about 100-175 nm, between about 120-185 nm, between about 130-190 nm, between about 135-175 nm, between about 150-175 nm. In some examples, the uniform emulsion contains <10% of the number of droplets (particles) that are outside of the specified range of diameters. In certain examples, the mean particle size of oil droplets in the oil-in-water emulsion preparation is between about 135-175 nm, e.g., about 155 nm $\pm$ 20 nm as measured by dynamic light scattering, and such a preparation contains not more than 1×10^7 large particles per mL of the preparation, as measured by optical particle sensing. "Large particles" as used herein mean those having diameters $\geq 1.2 \mu\text{m}$, typically between about 1.2-400 μm . In particular examples, the uniform emulsion contains less than 10%, less than 5%, or less than 3% of the droplets that fall outside of the preferred size range. In some examples, the mean droplet size of particles in an oil-in-water emulsion preparation is between about 125-185 nm, e.g., about 130 nm, about 140 nm, about 150 nm, about 155 nm, about 160 nm, about 170 nm, or about 180 nm, and the oil-in-water emulsion is uniform in that less than 5% of the number of droplets in the preparation fall outside the 125-185 nm range.

The immunogenic composition described herein can be used with oils, such as those from an animal (such as fish) or vegetable source. Sources for vegetable oils include nuts, seeds and grains. Peanut oil, soybean oil, coconut oil, and olive oil, the most commonly available, exemplify the nut oils. Jojoba oil obtained from the jojoba bean can also be used. Seed oils include safflower oil, cottonseed oil, sunflower seed oil, sesame seed oil and the like. In the grain group, corn oil is the most readily available, but the oil of other cereal grains such as wheat, oats, rye, rice, teff, triticale and the like may also be used. 6-10 carbon fatty acid esters of glycerol and 1,2- propanediol, while not occurring naturally in seed oils, may be prepared

by hydrolysis, separation and esterification of the appropriate materials starting from the nut and seed oils. Fats and oils from mammalian milk are metabolizable and may therefore be used in the immunogenic composition described herein. The procedures for separation, purification, saponification and other means necessary for obtaining pure oils from animal sources are well known in the art. Most fish contain metabolizable oils which may be readily recovered. For example, cod liver oil, shark liver oils, and whale oil, such as spermaceti, exemplify several of the fish oils which may be used herein.

A number of branched chain oils are synthesized biochemically in 5-carbon isoprene units and are generally referred to as terpenoids. Shark liver oil contains a branched, unsaturated terpenoid known as squalene, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene. Squalane, the saturated analog to squalene, may also be utilized in the present immunogenic composition. Fish oils, including squalene and squalane, are readily available from commercial sources or may be obtained by methods known in the art. Other suitable oils are the tocopherols. Mixtures of oils are also envisaged.

Surfactants can be classified by their 'HLB' (hydrophile/lipophile balance). Suitably, surfactants described herein have a HLB of at least 10, more particularly at least 15, and even more particularly at least 16. The immunogenic composition may include one or more surfactants including, but not limited to: the polyoxyethylene sorbitan esters surfactants (commonly referred to as the Tweens), especially polysorbate 20 and polysorbate 80; copolymers of ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO), sold under the DOWFAX™ tradename, such as linear EO/PO block copolymers; octoxynols, which can vary in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups, with octoxynol-9 (Triton X-100, or t-octylphenoxy polyethoxy ethanol); (octylphenoxy) polyethoxy ethanol (IGEPAL CA-630/NP-40); phospholipids such as phosphatidylcholine (lecithin); polyoxyethylene fatty ethers derived from lauryl, cetyl, stearyl and oleyl alcohols (known as Brij surfactants), such as triethyleneglycol monolauryl ether (Brij 30); and sorbitan esters (commonly known as the SPANs), such as sorbitan trioleate (Span 85) and sorbitan monolaurate. Non-ionic surfactants are preferred. Exemplary surfactants for including in the emulsion are Tween 80 (polyoxyethylene sorbitan monooleate), Span 85 (sorbitan trioleate), lecithin and Triton X-100.

Mixtures of surfactants can also be used (e.g., Tween 80/Span 85 mixtures). A combination of a polyoxyethylene sorbitan ester, such as polyoxyethylene sorbitan monooleate (Tween 80) and an octoxynol, such as t-octylphenoxypolyethoxyethanol (Triton X-100) is also suitable. Another envisaged combination comprises laureth 9 plus a polyoxyethylene sorbitan ester and/or an octoxynol.

Exemplary amounts of surfactants (% by weight) are: polyoxyethylene sorbitan esters (such as Tween 80) 0.01% to 1%, in particular about 0.1%; octyl- or nonylphenoxy polyoxyethanols (such as Triton X-100, or other detergents in the Triton series) 0.001% to 0.1 %, in particular 0.005% to 0.02%; polyoxyethylene ethers (such as laureth 9) 0.1% to 20 %, more particularly 0.1% to 10 % and even more particularly 0.1% to 1 % or about 0.5%.

In particular examples, the oil-in-water emulsions are squalene-in-water emulsions, and more particularly, submicron squalene-in-water emulsions.

Any suitable procedure is contemplated for producing immunogenic compositions or vaccine compositions. Exemplary procedures include, for example, those described in *New Generation Vaccines* (1997, Levine *et al.*, Marcel Dekker, Inc. New York, Basel, Hong Kong), which is incorporated herein by reference.

Any safe route of administration may be employed, including oral, rectal, parenteral, sublingual, buccal, intravenous, intra-articular, intra-muscular, intra-dermal, subcutaneous, inhalational, intranasal, intraocular, intraperitoneal, intracerebroventricular, topical, mucosal and transdermal administration, although without limitation thereto.

Dosage forms include tablets, dispersions, suspensions, injections, solutions, syrups, troches, capsules, nasal sprays, suppositories, aerosols, transdermal patches and the like. These dosage forms may also include injecting or implanting controlled releasing devices designed specifically for this purpose or other forms of implants modified to act additionally in this fashion. Controlled release may be effected by coating with hydrophobic polymers including acrylic resins, waxes, higher aliphatic alcohols, polylactic and polyglycolic acids and certain cellulose derivatives such as hydroxypropylmethyl cellulose. In addition, the controlled release may be effected by using other polymer matrices, liposomes and/or microspheres.

Compositions may be presented as discrete units such as capsules, sachets, functional foods/feeds or tablets each containing a pre-determined amount of one or more therapeutic agents of the disclosure, as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion or a water-in-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association one or more agents as described above with the carrier which may constitute one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the agents of the disclosure with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation.

The above compositions may be administered in a manner compatible with the dosage formulation, and in such amount as effective. The dose administered to a subject, in the context of the present disclosure, should be sufficient to effect a beneficial response in a subject over an appropriate period of time (e.g., generate a protective immune response). The quantity of agent(s) to be administered may depend on the subject to be treated inclusive of the age, sex, weight and general health condition thereof, factors that will depend on the judgement of the practitioner.

Also disclosed herein is a container comprising the immunogenic or vaccine compositions disclosed herein. Any suitable container known in the art may be used. For example, the container may be selected from the group consisting of a vial, a syringe, an ampoule, a flask, a fermentor, a bioreactor, a bag, a jar, an ampoule, a cartridge and a disposable pen. In one example, the container is a vial, ampoule or a syringe.

The container may be made of glass, metals (e.g., steel, stainless steel, aluminium, etc.) and/or polymers (e.g., thermoplastics, elastomers, thermoplastic-elastomers). The container may be at least partially siliconized.

The immunogenic compositions disclosed herein may further comprise a buffer. The buffer may be any suitable buffer known in the art. For example, the buffer may be a TRIS, acetate, glutamate, lactate, maleate, tartrate, phosphate, citrate, carbonate, glycinate, histidine, glycine, succinate and triethanolamine bufferphosphate buffer. In one example, the buffer is a phosphate buffer. In another example, the buffer is a succinate buffer. In another example, the

buffer is a histidine buffer. In another example, the buffer is a citrate buffer.

The buffer may be selected from USP compatible buffers for parenteral use, in particular, when the pharmaceutical formulation is for parenteral use. For example the buffer may be selected from the group consisting of monobasic acids such as acetic, benzoic, gluconic, glyceric and lactic; dibasic acids such as aconitic, adipic, ascorbic, carbonic, glutamic, malic, succinic and tartaric, polybasic acids such as citric and phosphoric; and bases such as ammonia, diethanolamine, glycine, triethanolamine, and TRIS.

Methods for eliciting an immune response and treatment

The isolated influenza virus and the immunogenic compositions described herein may be suitable for administration to human or non-human animal subjects, such that the present disclosure provides methods of raising an immune response and/or preventing and/or treating an influenza-associated disease, disorder or condition in a subject. The present disclosure also provides a composition for use as a medicament, and provides the use of a composition of the present disclosure for the manufacture of a medicament for raising an immune response and/or preventing and/or treating an influenza-associated disease, disorder or condition in a subject.

Accordingly, in one form, the present disclosure provides a method of eliciting an immune response in a subject, said method including the step of administering a therapeutically effective amount of the isolated influenza virus or the immunogenic composition provided herein to the subject to thereby elicit the immune response in the subject.

In a related form, the present disclosure provides a method of preventing and/or treating an influenza-associated disease, disorder or condition in a subject, said method including the step of administering a therapeutically effective amount of the isolated influenza virus or the immunogenic composition described herein to the subject to thereby prevent and/or treat the influenza-associated disease, disorder or condition.

In another related form, the present disclosure provides a method of immunizing or vaccinating a subject against an influenza-associated disease, disorder or condition, said method including the step of administering a therapeutically effective amount of the isolated influenza virus or the immunogenic composition described herein to the subject to thereby immunize or vaccinate against the influenza-associated disease, disorder or condition.

In another form, the present disclosure relates to the use of the isolated influenza virus described herein and/or a viral protein derived therefrom or the immunogenic composition described herein in the manufacture of a medicament for eliciting an immune response in a subject.

In a further form, the present disclosure provides for the use of the isolated influenza virus described herein and/or a viral protein derived therefrom or the immunogenic composition described herein in the manufacture of a medicament for preventing and/or treating an influenza-associated disease, disorder or condition in a subject.

In another related form, the present disclosure provides for the use of the isolated influenza virus described herein and/or a viral protein derived therefrom or the immunogenic composition described herein in the manufacture of a medicament for immunizing or vaccinating a subject against an influenza-associated disease, disorder or condition

In one broad form, the present disclosure also relates the isolated influenza virus described herein and/or a viral protein derived therefrom or the immunogenic composition described herein for use in therapy.

In a related form, the present disclosure also provides the isolated influenza virus described herein and/or a viral protein derived therefrom or the immunogenic composition described herein for use in a method of eliciting an immune response in a subject.

In a further related form, the present disclosure provides the isolated influenza virus described herein and/or a viral protein derived therefrom or the immunogenic composition described herein for use in a method of preventing and/or treating an influenza-associated disease, disorder or condition in a subject.

In still another related form, the present disclosure provides the isolated influenza virus described herein and/or a viral protein derived therefrom or the immunogenic composition described herein for use in a method of immunizing or vaccinating a subject against an influenza-associated disease, disorder or condition.

With respect to the aspects described herein, the term “subject”, “patient” and “individual” includes, but is not limited to, mammals, inclusive of humans, performance animals (such as horses, camels, greyhounds), livestock (such as cows, sheep, horses, pigs, chickens, ducks) and companion animals (such as cats and dogs). Suitably, the subject is a human.

By “elicit an immune response” is meant generate or stimulate the production or activity of one or more elements of the immune system inclusive of the cellular immune system, humoral immune system (i.e., antibodies) and/or the native immune system. Suitably, the immune response described herein includes one or more elements of the immune system, such as T lymphocytes, B lymphocytes, antibodies, neutrophils, dendritic cells inclusive of plasmacytoid dendritic cells, cytokines and/or chemokines. Non-limiting examples of cytokines include pro-inflammatory cytokines such as TNF- α , IL-2, IL-6, IL-8, IL-17A and IL-1 (*e.g.*, IL-1 β). A non-limiting example of a chemokine is the neutrophil chemo-attractant IL-8. In certain examples, the immune response that is elicited by the immunogenic compositions described herein is protective.

As generally used herein, the terms “immunize”, “vaccinate” and “vaccine” refer to methods and/or compositions that elicit a protective immune response against an influenza virus, whereby subsequent infection by the influenza virus, or a related serotype, strain or variant, is at least partly prevented or minimized.

By “protective immunity” is meant a level of immunity whereby the responsiveness to an antigen or antigens is sufficient to lead to rapid binding and/or elimination of said antigens and thus at least partially ameliorate or prevent a subsequent influenza virus infection in a subject.

By “protective immune response” is meant a level of immune response that is sufficient to prevent or reduce the severity, symptom, aspect, or characteristic of a current and/or influenza virus infection in a subject.

As used herein, the terms “treating”, “treat” or “treatment” refers to a therapeutic intervention that at least partly ameliorates, eliminates or reduces a symptom or pathological sign of an influenza-associated disease, disorder or condition, such as an influenza infection, after it has begun to develop. Treatment need not be absolute to be beneficial to the subject. The beneficial effect can be determined using any methods or standards known to the ordinarily skilled artisan.

As used herein, “preventing”, “prevent” or “prevention” refers to a course of action initiated prior to infection by, or exposure to, an influenza virus or molecular components thereof and/or before the onset of a symptom or pathological sign of the disease, disorder or condition, so as to prevent infection and/or reduce the symptom or pathological sign. It is to be understood that such preventing need not be absolute to be beneficial to a subject. A “prophylactic” treatment is a treatment administered to a subject who does not exhibit signs of the disease, disorder or condition, or exhibits only early signs for the purpose of decreasing the risk of developing a symptom or pathological sign of the disease, disorder or condition.

The vaccine and immunogenic compositions described herein may be used to treat both children and adults. Influenza vaccines are currently recommended for use in paediatric and adult immunisation, from the age of 6 months. Thus, a human subject may be less than 1 year old, 1-5 years old, 5-15 years old, 15-55 years old, or at least 55 years old. Preferred subjects for receiving the vaccines are the elderly (e.g. ≥ 50 years old, ≥ 60 years old, and preferably ≥ 65 years), the young (e.g. ≤ 5 years old), hospitalised subjects, healthcare workers, armed service and military personnel, pregnant women, the chronically ill, immunodeficient subjects, subjects who have taken an antiviral compound in the 7 days prior to receiving the vaccine, people with egg allergies and people travelling abroad. The vaccines are not suitable solely for these groups, however, and may be used more generally in a population. For pandemic strains, administration to all age groups is preferred.

Treatment can be by a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. In a multiple dose schedule the various doses may be given by the same or different routes (e.g., a parenteral prime and mucosal boost, a mucosal prime and parenteral boost). Administration of more than one dose (typically two doses) is particularly useful in immunologically naive patients (e.g., for subjects who have never received an influenza vaccine before), or for vaccinating against a new HA subtype (e.g., in a pandemic outbreak). Multiple doses will typically be administered at least 1 week apart (e.g., about 2 weeks, about 3 weeks, about 4 weeks, about 6 weeks, about 8 weeks, about 10 weeks, about 12 weeks, about 16 weeks, etc.).

So that preferred embodiments of the present disclosure may be fully understood and put into practical effect, reference is made to the following non-limiting examples.

Examples

Example 1.

The aim of the present Example was to identify donor or seed influenza viruses whose backbone genes impart improved growth and/or yield characteristics to reassortant viruses containing such backbone genes. The influenza viruses were prepared using known methods of synthetic seed virus generation (see, e.g., PCT/US2000/009021; PCT/US2001/013656; Dormitzer *et al.*, *Sci Transl Med*, 15 May 2013 Vol 5, Issue 185, p. 185).

Methods & Results

Synthetic seed process & procedure

- MDCK cells were transfected with expression constructs for the HA and NA sequences of a range of vaccine virus strains (i.e., A/Idaho/07/2019, A/Nebraska/14/2019, A/Iowa/56/2019, A/Delaware/55/2019, A/Illinois/02/2019, A/Canberra/407/2019, A/Darwin/94/2019, A/Delaware/39/2019, A/Virginia/03/2020, A/Tasmania/503/2020 and A/Bangladesh/1002/2020) and the backbone viral segments for PA, PB1, PB2, NP, NS, and M from a range of candidate high growth donor strains (including, A/Singapore/TT1384/2016, A/South Carolina/04/2017, A/Alaska/06/2019 and A/Ohio/02/2019) that were selected based on relatively high virus yields thereof when grown in cells at wild-type virus.
- The experiments also included a cell-adapted version of A/Puerto Rico/8/1934 (PR8X) as a control donor virus for generating reassortant viruses under identical conditions, as a comparison to those based on the candidate high growth donor strains.
- Reassortant viruses were then rescued and characterized based on their HA yield at end of infection, using standard methods.
- A schematic diagram of a transfection and rescue procedure is provided in Figure 1.
- HA yields (determined by standard methods of HPLC) for each of the vaccine virus strains and their respective reassortant viruses tested are provided in Figures 2 to 12.

- A summary of the HA yield results for the A/Ohio/02/2019 donor strain is provided in Figure 13, which demonstrates the consistent improvement on HA yield of this backbone strain over a range of HA and NA sequences from vaccine virus strains.
- Percentage increases in yield versus a control vaccine virus strain for each of the candidate high growth donor strains is provided in Figure 14.

In particular aspects of the present disclosure, backbone sequences and/or HA and/or NA sequences may include any of those derived from the virus strains disclosed in the present Example.

Conclusions

The present Example identified a number of donor virus strains that are capable of supporting rescue and improved growth and yield of vaccine virus strains in MDCK cells. The A/Ohio/02/2019 and A/South Carolina/04/2017 strains demonstrated that they could consistently and significantly increase the growth and yield of reassortant viruses (both H1N1 and H3N2) over wild-type virus controls and corresponding PRX-derived reassortant viruses. The A/Singapore/TT1384/2016 and A/Alaska/06/2019 strains demonstrated that they could significantly increase the growth and yield of reassortant viruses of a H1N1 subtype over wild-type virus controls and corresponding PRX-derived reassortant viruses. Other strains showing promise from preliminary studies by the inventors as demonstrating increased growth and yield when expressed as reassortant viruses include A/Darwin/11/2021 and A/Tasmania/503/2020.

Itemized Listing of Embodiments

1. An isolated influenza virus comprising one or more of PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.
2. The isolated influenza virus of Claim 1, comprising one or more of the PB2, PB1, PA, M, NP and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a fragment, variant or derivative thereof.
3. The isolated influenza virus of Claim 1 or Claim 2, wherein the isolated influenza virus is capable of enhanced replication when grown in cells relative to a wild-type influenza virus isolate that does not comprise the one or more PA, PB1, PB2, NP, M and NS viral segments.
4. The isolated influenza virus of any one of the preceding claims, wherein the isolated influenza virus is capable of enhanced yield of a viral protein when grown in cells relative to a wild-type influenza virus isolate that does not comprise the one or more PA, PB1, PB2, NP, M and NS viral segments.
5. The isolated influenza virus of Claim 4, wherein the viral protein is a haemagglutinin (HA) protein.
6. The isolated influenza virus of any one of Claims 3 to 5, wherein the cells are MDCK cells.
7. The isolated influenza virus of any one of the preceding claims, comprising five or six of the PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from those set forth in:
 - (a) SEQ ID NOs: 9-17 or a fragment, variant or derivative thereof;
 - (b) SEQ ID NOs: 28-36 or a fragment, variant or derivative thereof;

- (c) SEQ ID NOs: 47-55 or a fragment, variant or derivative thereof;
- (d) SEQ ID NOs: 66-74, or a fragment, variant or derivative thereof;
- (e) SEQ ID NOs: 85-93, or a fragment, variant or derivative thereof; or
- (f) SEQ ID NOs: 104-112, or a fragment, variant or derivative thereof.

8. The isolated influenza virus of any one of the preceding claims, comprising five or six of the PA, PB1, PB2, NP, M and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from those set forth in:

- (a) SEQ ID NOs: 1-6 or a fragment, variant or derivative thereof;
- (b) SEQ ID NOs: 20-25 or a fragment, variant or derivative thereof;
- (c) SEQ ID NOs: 39-44 or a fragment, variant or derivative thereof;
- (d) SEQ ID NOs: 58-63 or a fragment, variant or derivative thereof
- (e) SEQ ID NOs: 77-82, or a fragment, variant or derivative thereof; or
- (f) SEQ ID NOs: 96-101, or a fragment, variant or derivative thereof.

9. The isolated influenza virus of any one of the preceding claims, further comprising a heterologous or chimeric HA viral segment and a heterologous or chimeric NA viral segment.

10. The isolated influenza virus of any one of the preceding claims, wherein the isolated influenza virus is of a N1, N2, N3, N7, or N9 subtype.

11. The isolated influenza virus of Claim 10, wherein the isolated influenza virus is of an N1 or N2 subtype.

12. The isolated influenza virus of any one of the preceding claims, wherein the isolated influenza virus is of a H1, H2, H3, H5, H7, or H9 subtype.

13. The isolated influenza virus of Claim 12, wherein the isolated influenza virus is of a H1 or H3 subtype.

14. The isolated influenza virus of any one of the preceding claims, wherein the isolated influenza virus is of a H1N1 subtype or a H3N2 subtype.

15. The isolated influenza virus of any one of the preceding claims, which is a recombinant influenza virus.
16. The isolated influenza virus of any one of the preceding claims, which is a reassortant influenza virus.
17. A method of preparing an influenza virus, said method including the step of contacting a cell with one or more genetic constructs that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.
18. The method of Claim 17, wherein the one or more genetic constructs comprise or encode one or more of PB2, PB1, PA, M, NP and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a fragment, variant or derivative thereof.
19. A method of preparing an influenza virus, said method including the step of contacting a cell with an isolated influenza virus comprising one or more of PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.
20. The method of Claim 19, wherein the isolated influenza virus is that of any one of Claims 1 to 16.
21. The method of any one of Claims 17 to 20, further including the step of isolating or harvesting the influenza virus from the cells.
22. An isolated influenza virus prepared by the method of any one of Claims 17 to 21.

23. An isolated cell infected with the isolated influenza virus of any one of Claims 1 to 16 or 22.
24. The isolated cell of Claim 23, wherein the cell is an MDCK cell.
25. A genetic construct that encodes one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.
26. A plurality of genetic constructs that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.
27. The genetic construct of Claim 25 or the plurality of genetic constructs of Claim 26, wherein the genetic construct or the plurality of genetic constructs comprise or encode one or more of PB2, PB1, PA, M, NP and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a nucleotide sequence complementary thereto, or a fragment, variant or derivative thereof.
28. A method of making an immunogenic composition, including the steps of:
- (a) providing the isolated influenza virus of any one of Claims 1 to 16 or 22 and/or a viral protein derived therefrom; and
 - (b) combining the isolated influenza virus and/or the viral protein with an adjuvant and/or treating the isolated influenza virus with an agent that inactivates the virus.
29. The method of claim 28, wherein the adjuvant comprises an immunostimulatory DNA sequence, a bacterium-derived component, an aluminium salt (alum) or a squalene oil-in-water emulsion system.
30. An immunogenic composition produced according to the method of Claim 28 or Claim 29.

31. An immunogenic composition comprising the isolated influenza virus of any one of Claims 1 to 16 or 22 and/or a viral protein derived therefrom and a pharmaceutically acceptable carrier, diluent or excipient.
32. A method of eliciting an immune response in a subject, said method including the step of administering a therapeutically effective amount of the isolated influenza virus of any one of Claims 1 to 16 or 22 and/or a viral protein derived therefrom or the immunogenic composition of Claim 30 or Claim 31 to the subject to thereby elicit the immune response in the subject.
33. A method of preventing and/or treating an influenza-associated disease, disorder or condition in a subject, said method including the step of administering a therapeutically effective amount of the isolated influenza virus of any one of Claims 1 to 16 or 22 and/or a viral protein derived therefrom or the immunogenic composition of Claim 30 or Claim 31 to the subject to thereby prevent and/or treat the influenza-associated disease, disorder or condition.
34. Use of the isolated influenza virus of any one of Claims 1 to 16 or 22 and/or a viral protein derived therefrom or the immunogenic composition of Claim 30 or Claim 31 in the manufacture of a medicament for eliciting an immune response in a subject.
35. Use of the isolated influenza virus of any one of Claims 1 to 16 or 22 and/or a viral protein derived therefrom or the immunogenic composition of Claim 30 or Claim 31 in the manufacture of a medicament for preventing and/or treating an influenza-associated disease, disorder or condition in a subject.
36. The isolated influenza virus of any one of Claims 1 to 16 or 22 and/or a viral protein derived therefrom or the immunogenic composition of Claim 30 or Claim 31 for use in therapy.
37. The isolated influenza virus of any one of Claims 1 to 16 or 22 and/or a viral protein derived therefrom or the immunogenic composition of Claim 30 or Claim 31 for use in a method of eliciting an immune response in a subject.

38. The isolated influenza virus of any one of Claims 1 to 16 or 22 and/or a viral protein derived therefrom or the immunogenic composition of Claim 30 or Claim 31 for use in a method of preventing and/or treating an influenza-associated disease, disorder or condition in a subject.

Sequence Listing

SEQ ID NO: 1	Nucleotide sequence of PB2 viral segment of A/Ohio/02/2019 agcaaaagcagggtcaattatattcagcatggaagaataaaagaacttcggaatctaattgctgcagtcctgcactcgc gagatactgacaaaaaccacagtgaccatattggccataaagaaagtagacacatcggggagacaggaaaaaac ccgtcacitaggatgaatggatgagtgcaatgaaatataatcactgctgacaaaaggataacagaaatggtcct gagagaaatgaacaaggacaaactctatggagtaaatgagtgatgctgacagatagatgagtgatcaccttt ggctgtaacatggtggaatagggaatggaccgtgacaagtagctgcattacccaaaagtgtacaaaacttattcga caaagtcgaagggttaaaacatggaaaccttggccctgtccattttagaaatcaagtcaagatagcagaagagtaga cataaaccttggtcatgcagacctcagtgccaaaggagcacaagatgtaattatggaagtgttttcccaatgaagtg ggagccagaataactaactcagaatcacagctaacaataactaaagagaaaaagaagaactccgagattgcaaa atttctccctgtagtgctgcatacatgttagagagagaactgtgctgaaaaacaagatttctccagttgctggcggaa caagcagtatatacatggaattttacatttgactcaaggaaacatgttgggaacaaatgtacactcctggtggaggtgt gaggaaatgacgatgtgaccaagcctaattattcggccagaacatagtaagaagagccgcagtgatgcagat ccactagcatcttattgagatgtgccaagcagcgaattgtggaacaaggatgtggacattcttaaacagaaac ccgactgaagaacaagctgtggatatafagcaaggctgcaatgggattgagaatcagctcatcctcagcttgggtggc tttacatttaaaagacaagcgggtcgtcagtcacaaaagaagaagggtcttacaggcaatctccaaacattgaga ataagagtacatgaggggtatgaggagttcacatgtggggaaaaagagcaacagctataactaagaaaaagcaacc agaagactgtgtcaactcatagtgagtggaagagacgaccagtcataagccgaagcaataatcgtggccatggtgtt ttcacagaagattgcataaaaagcagtttaggggtgacctgaattttgcaacagagcaaatcagcgggtgaaccc catgcatcagctttaaggcattttcagaagatgacgaaagtgtctttcaaaattggggagttgaacacatcgacagt gtgatgggaatggtgagtggttaccagatatgactccaagcacagagatgtcaatgagagggaataagagtcagca aaatgggtgtggtgaatactccagtagagaggggtgggtgttagcattgacgtgttttgagagttcgagaccaac gtgggaatgtattatctcctgaggaggtcagtgaaacacagggaactgaaggctgacaataacttattcatcgtc gatgatgtgggagattaacggtcctgagtcggtttgtgtaatacctatcaatggatcatcagggaattgggaagctgtc aaaattcaatgtgtcagaaatcctgcaatgtgtacaacaaaatggaatttgaaacgtttcaatctttagtcoccaaaggc cactagaagtcaatacagtggtgttgtagaactctattccaacaaatgagagacgtacttggaaactttgacactgcc cagataataaagcttctcccttttgagctgtctccaccgaagcaaaagcagaatgcagttctctcactgactgtgaatgt gaggggatcagggtatgagaatactgtgaagggtcaattctcctgtattcaactacaacaagaccactaaaaggctaa caattcttggaagatgacggcacttfaattgaagaccagatgaagacacatccggagtgagttccgctcttg agaggtgttcctcattatagtgaaagaagacagaagatagggaccagcaatagcatcaatgaactgagtaaaccttgc taagggtggaaggtcaatgtgtaattgggcaaggagacgtgtgtgtgtaatgaacgaaaacgggactctagt atacttactgacagccagacagcgacaaaaagaattcgatggccatcaatataactgaatagtttaaaaacgacct gttttact
SEQ ID NO: 2	Nucleotide sequence of PB1 viral segment of A/Ohio/02/2019 agcgaagcaggcaaacattgaatggatgtcaatccgactctactgttcttaaaagttccagcgcaaaatgccata agcacaacattcccttatactggagatcctcatagccatggaaacaggacagggtacactatggacacagtcac cagaacacatcaatactcagagaggggggaagtggacaacaatacagaactggggcgccccagctcaacccaa ttgatggaccactacctgaggaataatgaaccaagtggatagcacaaacagactgtgtcctggagggtatggccttcc ttgaagaatccccccaggatctttagaactcctgccttgaacaatggaagcgttcaacagacaagggtggac aaactaaccgaagtcgcccagacttatgattgacattaaacaggaaccaacccggcagcaactgcattagccaaca ccatagaagtctttatgataaacggattaacagctaataatcaggaaggctaataagatttctcaaggatgtgtagga atcaatggataaggaggaatggagataacaacacactttcaagaaaaaggagagtaagggaacaacatgacca gaaaatgtgtcacacaaagaacaatagggaagaaaaaacaagggtgaataagagaggctacctataaagagcttt gacattgaacacatgaccaaggatgcagagagaggcaattaaaaagaagggtctattgcaacacccgggagtc aaattagagggttcgtgtacttcgtgaaactttagctagaagcatttgcgaaaagcttgaacaatctggacttccggtt gggggtaatgaaaagaaggccaaactggcaaatgtgtgagaaaatgagactaatcacaagacacagagcttt ctttcacaatcactgggacaacactaagtggaaatgaaaatcaaaaccccgaaatgttttggcgatgattacatacat cacaaagaatcaacctgagtggttcagaacatcctgagcatcgaccaataatgttctcaacaaaatggcaagac

	tgggaaaaggatacatgttcgagagtaagagaatgaagctccggacacaaatacctgcagaaatgtagcaagcat tgacctgaagtaattcaatgaatcaacaaggaaagaaattgagaaaataaggcctcttctaatagatggcacagcatc attgagccctggaaatgatgagggcatgttcaacatgctaagtacagttttaggaggtctcgatactgaatcttggacaa aagaaatacaccaagacaacatactgggtgggagggctcctcctcagacgattttgcccctcatagtgaaatgcacc aaatcatgagggaatfacaagcaggagtgatgattctataggacctgcaagttagtggaatcaacatgagcaaa aagaagtcctataataaaaaacaggagacatttgaaatcactagctttttatcgatagtgatttggctaaatfttagcat ggagctaccaagtgtcggagtgctggaataaacgagtcagctgacatgagcattggagtaacagtgataaagaaca acatgataaataatgacctggaccagcaacagcccaaatggctctccaaftgttcatcaaaagattacagatacacgta tcgggtccatagaggagacacacaaatccaaacgagaagatcattcgagataaagaagctgtgggatcaaaccaca atcaaggacaggactattgtatcagatggggggccaaactatatacaatccggaaatcttcacatccctgaagtctg cttaaagtgggagctgattgagatgagaattatcggggaagactttgcaatccctgaatccctttgtcagccataaga aattgaatctgtaacaatgctgtagtaatgcagccatgggtccggccaaaagtatggaatgagccgtgtgcaact acacattcctgattcccaaaagggaaccgttccattctaaacacaagccaaagggggattcttgaggatgaacagat gtaccagaagtgtctcaactgttcgagaatttttccctagtagttcatataggagaccgattggaattcttagcatggt ggaggccatggtgtctaggcccggttgcagcagaattgacttcgagctctggaaggattaagaagggaaggttct ctgagatcatgaagatctgtccaccattgaagaactcagacggcaaaaataatgaatttagctgtcctcatgaaaa aatgcccgtgtttctact
SEQ ID NO: 3	Nucleotide sequence of PA viral segment of A/Ohio/02/2019 agcaaaagcaggactgattcagaatggaagattttgtcgacaatgcttcaaccgatgattgtgaactgcagaa aaagcaatgaaagagtatggggaggatctgaaaattgaaactaacaatttgcagcaatatgcactcacttggaggt gtgtttcatgtattcagatttcatttcatcaatgaacaaggcgcaatcaatagtagtagaacttgacgatccaaatgcact gtaaaagcacagatttgaataatcgaggggagagacagaacaatggcctggacagtagtaaacagtatctgcaac actactggagctggaaaaccgaagtgttccggatttctatgattacaagagaacagattcatgaaattggagtg acaaggagagaagtcacatataattaccttgaanaaggccaataagattaaatctgagaacacacacattcacattttt cattcactggggaggaatggccaaaaggcagactacactctcgacgaggaaagcagggctagatcaaaacc aggtctgtttacataagacaagaatggccaacagaggcctctgggattcttccgtcagtcggaagaggcggaag aaacaattgaaagaaaaattgaaatcacaggaaactatgcgcagggttgcgaccaaaagtctccaccgaacttctct gccttgagaatttttagagcctatgtggatgattcgaaccgaacggctgcattgaggggcaagcttttcaaatgtccaa agaagtgaatgcccattgaacctttctgaagacaacacgaagaccaatcaaaacttctagtgacacctctgtttat cagcgggtccaagtctcctgtagtgatgttgaattgagcattgaagaccaagtcacgaaggagaaggatccc attatatgatcgatcaagtgataaaaacattcttggatggaaagaaccttatatagtcaaacacacgaaaaggga ataaattcaattacctgctgtcatggaagcaagtactgtcagaattgcaggacattgaaaatgaggagaagattcca agaacaaaaaacatgaagaaaacgagtcactgaagtgggctctgttgaaaacatggcaccagagaaggtagac ttgaaaaactgcagagacataagcgattaaagcaatatgtagtgaagaacctgaattaaggtcactttcaagctgga tacagagtgagttcaacaaggcctgtgagctaaactgattcagctctgtagatagctcgatgagattggagaggacgta gcccacattgaacacattgcaagtatgagaaggaaattttcacagcagagggtgtccattgtagagctactgaatac ataattgaagggtatacattacactgcctgtcctaatgcatcctgtgcagcaatggacgattttcaactaattcccat gataagcaagtgcagaactaaagagggaaggcgaaaaaccaatttatattggaattcatcataaagggaagatctcatc tgagggaatgacacagacgtgtgtaactttgtgagcatggagttttctctcacagatcagagacttgagccacataat ggggagaataattgttccitgagataggagatgttactaaggagtgctataggccaaatttcaaggccgatgttctt gtatgtgaggacaaaacggaacatcaaaaggtcaaaatgaaatggggaatggagatgagacgttccctcttcagtc ctccagcagattgagagcatgattgaagccgagctcagttaaagaaaagacatgaccaaaagagtttttggagaat aaatcagaagcatggccattggggaggtcccaagagtggaagaagggttcattggaaaagctgttaggactc tattagctaagtcagtattcaatagcctgtatgcaccacaattggaagggttttcagcagagtcagaagaaactgtc ctattgttcaggtctttagggacaaactcgaacctgggacctttgatcttgggggctatatgaagcaattgaggagt gcctgattaatgacctgggtttgtctaatgctgttgggtcaactcctctctgacacatgcattaaaatgttatagca gtgctgtatttttatccgtactgtccaaaaaagtacctgtttctact
SEQ ID NO: 4	Nucleotide sequence of NP viral segment of A/Ohio/02/2019 agcaaaagcagggtgataatcactcactgagtgacatctaaatcatggcatccaaggccacaaacggctctatga acagatggaaactgafggagatcgccagaatgcaactgagattagggtcatcctcggggaagatgattgatggaatt

	gggagattctacatccaaatgtgcactgaacttaactcagtgatcatgaaggacgggtgatccaaacagcttgaca atagagaaaatggctactctctgttttgatgaagaaggaaataatacctggaaacaccccagcgcggggaaag atcccaagaaaactggggggcccatatcacaggagagtcgatgggaaatggatgagggaactcgtctttatgacaa agaagaataaaggcgaaatctggcgccaagccaacaatgggtgaggatgctacatctgggttaactcacataatgat ggcattccaatttgatgatgcaacataccagaggacaagagctctgttcggactggaaatggatccagaatgtgct ctctgatgcagggtctgactctccctagaaggctcggggctgcagggtgctgcagtcaaaaggaaatcggaacaatggt gatggaaactgacagaatggtcaaacggggatcaacgatcgaaatgttgagaggtagaattggcggaacaaac aagaagtgtctatgagagaatgtgcaacattcttaaaaggaaaatttcaaacagctgcacaaaggagcaatgttgatc aagtttagagaagtcggaacccaggaaacgctgagatcgaaatctcatatttttagcaagatctgcactgatactga gaggatcagttgctcacaatcttgcctacctgctgtgcataatggacctgcagtatccagtggttacgactttgaaa agagggtatcttctgtgggaatagacctttcaactactccaaatagccaatatacagcttaatcagacctaat gagaatccagcacacaagagtcagctgggtggtgatggcatgccaattctgctgcaattgaaatgaatgttaagct tcatcagaggaaacaaagtatctctcgggggaaactgtcaactagaggagtacaaatgcttcaaatgagaacatg gataatattggatcaagcactctgaactgagaagcgggatactggccataagaaccaggagtgagggaacact aatcaacagaggcctcgcagggccaaaccagtggtcaacctacatttctgtacaaagaaatctccattgaaaag taaccatcatggcagcattcactggaaatagcggagggaagaactcagacatgagggcagaatcataaggatga tgaaggtgcacaaaccagaagaagtgtcaattccggggaggagggttttcgagctctcagacgagaaaggcagcg aaccggtcgtgccccttttgatgatgtaacgaaggatcttatttctcgagacaatgcagaagagtagacaatt aaggaaaaataccctgttttact
SEQ ID NO: 5	Nucleotide sequence of NS viral segment of A/Ohio/02/2019 agcaaaagcagggtgacaaagacataatggatccaacactgtgtcaagttccaggtagattgcttctttggcatat ccggaaacaagttgtagacaaaaactgagtgatgccccattcctcgatcggttcgcccagatcagagggtccctaa gggggaagaggcaatfactcgtctagacatcaaaagcagccaccatgctggaaagcaaatatagaaaagattct gaaagaaatctgatgaggcacttaaaatgacctggtctcaacacctgcttcgcatataactgacatgactatt gaggaaactgtcaagaaactgttctatgctaatgcccagcagaagtggaaggacctctttgcatcagaatggacc aggcaatcatggagaaaaaatacatgttaaaagcgaatttcaatgtgattttggcgactagagaccatagtattacta agggctttaccgaagaggggagcaattgttggcgaaatcaccatgccccttttccaggacatactattgaggatg tcaaaatgcaattgggtcctcatcgaggacttgaatggaatgataacacagttcgagctctaaaaatctacaga gattcgttgagagagcagtaatgagaatggggacctccactactccaaacagaaacgggaaatggcgagaa cagctagggtcagaagtttgaaagagataagatggctaatgaaagggtgagacacagattgagaacaactgaaaata gctttgacaaataacattcatgcaagcattacaactactgttgaagtggaaacaggagataaagaactttctcaattcag cttatttaataataaaaaacacctgttttact
SEQ ID NO: 6	Nucleotide sequence of M viral segment of A/Ohio/02/2019 agcaaaagcaggtagatattgaaagatgagccttcaaccgaggtcgaaacgtatgttctctatcgttccatcaggc cccctcaaaagccgagatcgcgagagacttgaagatgtctttgctggaaaaacacagatcttgaggctctcatgga atggctaaagacaagaccaattctgtcacctctgactaaggggattttagggtttttcacgctcaccgtgccagt agcgaggactgcagcgttagacgtttgtccaaatgccctcaatgggaatggagacccaataacatggacaaag cagttaaaactgtataggaaactaagaggagataacgttccatggggccaaagaaatagctctcagttatctctg gtgcacttgcagttgcatgggctcatatacaatagaaatggggctgttaaccactgaagtggcatttggcctgtgt gtgcaacatgtgagcagattgctgattccagcacaggtctcataggcagatggtggcaacaaccaatcatttaata aaacatgagaacagaatgttttggccagcactacagctaaggctatggagcaaatggctggaatgaatgagcagg cagcgaggccatggaaatagctagtcaggccaggcagatggtgcaggcaatgagagccattgggactcatccta gttcagtagtgggttaagagatgcttctgaaaattgacagctatcagaacgaatgggggtgcagatgcaac gattcaagtgtatccgttattgttgcgcgaatatcatgggatcttgcacttgatattgtggattcttgccttttttca aatgctgtctgactcttcaaacacggccttaaaaggagcccttctacgggaaggagtacctgagctatgaggga gaatfctgaaaggaaacagcagaatgctgtggtgctgacgacagtcattttgtcagcatagagttggagtaaaaac tacctgttttact
SEQ ID NO: 7	Nucleotide sequence of HA viral segment of A/Ohio/02/2019 agcaaaagcagggtgataattctattaacatgaagactatcattgtttgagctgcattctatgtctgttttgcctcaa aaattcttgaaatgacaatagcacggcaacgctgtgcttggccaccatgcagtaccaaacgggaacgatagtgaa

	aacaatcacgaatgaccgaattgaagtactaatgctactgagctggttcagaactcctcaatagtgaaatatcgga cagtcctcatcagatccttgatggagaaaactgcacactaatagatgctctattggagaccctcagtgatggtctt caaaataagaatgggaccttttcgttgaaacggaacaaagcctacagcaactgttaccctatgatgtgccggattatg catcccttagatcactagtgtgcctcactccggcacactggagttfaacaatgaaagcttcaattgggctggagtcactca aaacgggaacaagttcttctgcataggggatctaaaagtagttctttagtagatfaaattgggtgaccacttaaac caaataccagcattaaacgtgactatgccaacaatgaacaattgacaaattgtacattgggggttcaccacccg ggtacgggacaaggacaaaatctccctgtatgcacaatcatcagggaagaatcacagtatctacaaaagaagccaac aagctgtaatcccgaatatcgatcaggccagaaataaggatccctagcagaataagcatctattggacaatag taaaaccaggagacatactttgattaacagcacagggaatctaattgctcctagggggttactcaaaatcgaagtgg gaaaagctcaataatgagatcagatgcacccattggcaagtgcagctgtaatgcatcactcaaatgggaagcattc caaatgacaaaccattccaaatgtaaacaggatcacatacggggcatgtccagatattgtaagcaagcactctg aaattggcaacagggaatgcgaatgtaccagagagacaaactagaggcatatttggcgcaatagcgggttcatag aaaatggtggagggaatgtgtaggtgtgtagcggcttcaggcatcaaaattctgagggaagaggacaagcag cagatctaaaagcactcaagcagaattgatcaaatcaatgggaagctgaatcgattgatcgggaaaaaccaacga gaaattccatcagattgaaaaagagtttcagaagtagaagggaattcaggaccctgagaaatattgtaggacac aaaaatagatctctgtcatacaacgcggagcttctgttgccttgagaaaccaacatacaattgatcactgaactca gaaatgaacaaactgtttgaaaaaacaaggaagcaactgagggaatgctgaggatattgggcaatgtgtttcaa aatataccacaatgtgacaatgcctgcatgggtcaatcagaatgggaactatgaccacaatgtatacagggatga agcattaaacaaccggtccagatcaagggaatgtgagctgaagtcagggtacaagattggatcctatggatttctt gccatatactgttttctgtgtgtctgtgtgggtcatcatgtggcctgccaaaagggaacattagggtgcaac atttgcatttgatgcattaaataaaaacacccctgtttctact
SEQ ID NO: 8	Nucleotide sequence of NA viral segment of A/Ohio/02/2019 agcgaagcaggagtaagatgaatcacaatcaaaagataataacgattggctctgtttctctaccatttcacaata tgcttctcatgcaaatggcatcctgataactactgtaacattgcatttcaagcaatattgaattcaactccccccaaac aaccaagtgtatgctgtgtgaaccaacaataatagaagaagaacataacagagatagtgtatttgaccaacaccacat agagagggaatfatgccccaaaccagcagaatacagaatgtgcaaaaccgcaatgtggcattacagatttgc ccttctctaaggacaattcaattaggcttccgctgtggggacatctgggtgacaagagaaccttatgtgcatgtga tcctgacaagtgttatcaatttgccttggacagggaacaacaataaacaacgtgcattcaataacacagcacgtga taggacccctcatcgactctattgatgtgaattgggtgttcttccatctggggaccaagcaagtgtgcatagca tgctccagctcaagtgtgacgtaggaaagcatggctgcatgtttgtataacggggatgataaaaatgcaactgct agtttcaattacaatgggaggctttagatagtgtgtttcatgtgtccaaagafattctcaggaccaggagtcagaatg cgtttgtatcaatggaactgtacagtagtaatgactgatgaaatgctacaggaaaagctgatactaaaaatattatcat tgaggaggggaaaatcgttcatactagcaaatgtcagggaagtgtcagcatgtcgaagagtgtcttctgctactcctg ataccctgtgtcagatgtgtctgcagagacaactggaaaggatccaaccggccatctgtagatataaacaataagg accatagcattgggtccagttatgtgttcaggactgttggagacacaccagaaaaaccgacagctccagcagc agccattgcttgaatcctaacaatgaaaaagggtgtcatggagtgaaggctgggcttggatgtgaaatgacgt gtggatggggagaacaatcaacgagacgtcacgcttagggatgaaaccttcaagtcgttgaaggctgtgtccaac tctaagtcacaattgcagataaattaggcaagtcatagttgacagaggtagatgtccgggtattctgtattttctgtt gaaggcaaaagctgcatcaatcgtgtctttatgtggagtgtgattagggaagaaaagggaactgaagtctgtg gacctcaaacagattgtgtgtttgtggcacctcaggtacatatggaacaggctcatggcctgatggggcgacct caatctcatgcatatataagcttgcgaattttagaaaaaaccttgtttctact
SEQ ID NO: 9	Amino acid sequence of PB2 protein of A/Ohio/02/2019 MERIKELRNLMSSQSRITREILTKTTVDHMAIIKKYTSGRQEKNPRLMKW MMAMKYPIADKRITEMVPERNEQGQTLWSKMSDAGSDRVMVSPLAV TWWNRNGPVTSTVHYPKVYKTYFDKVERLKHGTFGPVHFRNQVKIRRR VDINPGHADLSAKEAQDVIMEVVPNEVGARILTSESQLTITKEKKEELR DCKISPLMVAYMLERELVRKTRFLPVAGGTSSIIYIEVLHLTQGTCEWQM YTPGGGVRNDDVDQSLIIARNIVRRAAVSADPLASLLEMCHSTQIGGTR MVDILKQNPTEEQAVIDICKAAMGLRISSFSFGGFTFKRTSGSSVKKEEEV LTGNLQTLRIRVHEGYEFTMVGKRATAILRKATRRLVQLIVSGRDDQSI

	AEAIIVAMVFSQEDCMIAVRGDLNLFVNRRANQRLNPMHQLLRHFQKDA KVLFFQNWGVEHIDSVGMGMVGVLPDMTPSTEMSMRGIRVSKMGVDEYSS TERVVVSIDRFLRVDRDQRGNVLLSPEEVSETQGTERLTITYSSMMWEIN GPESVLVNTYQWIIRNWEAVKIQWSQNPAMLYNKMEFEPFQSLVPKATR SQYSGFVRTLFQQMRDVLGTFDTAQIHKLLPFAAAPPKQSRMQFSSLTVN VRGSGMRILVRGNSPVFNYNKTTKRLTILGKDAGTLIEDPDESTSGVESA VLRGFLIIGKEDRRYGPALSINELSNLAKGEKANVLIGQGDVVLVMKRKR DSSILTDSQTATKRIRMAIN
SEQ ID NO: 10	Amino acid sequence of PB1 protein of A/Ohio/02/2019 MDVNPSTLLFLKVPQAQNAISTTFPYTGDPYSHGTGTGYTMDTVNRTHOY SERGKWTNTTETGAPQLNPIDGPLPEDNEPSGYAQTDCVLEAMAFLEESH PGIFENSCLETMEAVQQTRVDKLTQGRQTYDWTLNRNQPAATALANTIE VFRSNGLTANESGRLIDFLKDVMESEMDKEEMEITTHFORRRVRDNMTK KMTVQRTIGKKKQVRNKRGYLIRALTNTMTKDAERGKLRRAIATPG MQIRGFVYFVETLARSICEKLEQSGLPVGGNEKKAKLANVVRKMMTNS QDTELSFTITGDNTKWNENQNPRMFLAMITYITKNQPEWFRNILSIAPIMF SNKMARLGKGYMFESKRMLRTQIPAEMLASIDLKYNFESTRKKIEKIRP LLIDGTASLSPGMMMGFMNMLSTVLGVLSILNLGQKKYTKTTYWWDGLQ SSDDFALIVNAPNHEGIIQAGVDRFYRTCKLVGINMSKKKSYINKTGTFEF TSFFYRYGFVANFSMELPSFGVSGINESADMSIGVTVIKNNMINNDLGPA TAQMALQLFIKDYRYTYRCHRGDTQIQTRRSFEIKKLWDQTSRTGLLV SDGGPNLYNIRNLHIPEVCLKWELMDENYRGRLCNPLNPFVSHKEIESVN NAVVMPAHGPAKSMEYDAVATTHSWIPKRNRSLNTSQRGILEDEQMYQ KCCNLFEKFFPSSSYRRPIGISSMVEAMVSRARIDARIDFESGRIKKEEFSEI MKICSTIEELRRQK
SEQ ID NO: 11	Amino acid sequence of PB1-F2 protein of A/Ohio/02/2019 MEQGQGTLTWTQSTEHINTQRGGSGQQIQKLRPSSTQLMDHYLRIMNQV DMHKQTVSWRLWPSLKNPTQVSLRTPALKQWKPFNRQGWTN
SEQ ID NO: 12	Amino acid sequence of PA protein of A/Ohio/02/2019 MEDFVRQCFNPMIVELAEKAMKEYGEDLKIETNKFAAICTHLEVCFMYS DFHFINEQGESIVVELDDPNALLKHFEEIIEGRDRTMAWTVVNSICNTTGA GKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKANKIKSENTIHIFSFTG EEMATKADYTLDEESRARIKTRLFTIRQEMANRGLWDSFRQSERGEETIE EKFEITGTMRRADQSLPPNFSCLENFRAYVDGFEPNGCIEGKLSQMSKE VNAQIEPFLKTTTPRIKLPSGPPCYQRSKFLMDALKLSIEDPSHEGEGIPL YDAIKCIKTFFGWKEPYIVKPHEKGINSNYLLSWKQVLSLQDIENEEKIP RTKNMKKTSQKWLALGENMAPEKVDFFENCRRDISDLKQYDSEEPRLSLS SWIQSEFNKACELTDSVWIELDEIGEDVAPIEHASMRNRYFTAEVSHCRA TEYIMKGVYINTALLNASCAAMDDFQLIPMISKCRITKEGRRKTNLYGFII KGRSHLRNDTDVVNFVSMFSLTDPRLPHKWEKYCVLEIGDMLLSAI GQISRPMFLYVRTNGTSKVKMKWGMEMRRCLLQSLQQIESMIEAESSVK EKDMTKEFFENKSEAWPIGESPKGVEEGSIGKVCRTLLAKSVFNSLYASP QLEGFSAESRKLILLIVQALRDKLEPGTFDLGGLYEAIIECLINDPWVLLNA SWFNSFLTHALK
SEQ ID NO: 13	Amino acid sequence of NP protein of A/Ohio/02/2019 MASQGTKRSYEQMETDGDRQNA TEIRASVGKMIDGIGRFYIQMCTELKL SDHEGRLIQNSLTIEKMLVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRR VDGKWMRELVLVDKEEIRRIWRQANNGEDATSGLTTHIMIWHNSLNDA T YQRTALVRTGMDPRMCSLMQGSTLPRRSGAAGAAVKGIGTMVMELIR MVKRGINDRNFWRGENGRKTRSAYERMCNILKGGFQTAAQRAMVDQV

	RESRNPGNAEIEDLIFLARSALILRGSVAHKSCLPACAYGPAVSSGYDFEK EGYSLVGIDPFKLLQNSQIYSLIRPNENPAHKSQLVWMACHSAAFEDLRL LSFIRGTKVSPRGKLSTRGVQIASNENMDNMGSSTLELRSGYWAIRTRSG GNTNQQRASAGQTSVQPTFSVQRNLPFEKSTIMAAFTGNTTEGRSDMRA EIIRMMEGAKPEEVSRGRGVFELSDEKAANPIVPSFDMSNEGSYFFGDN AEEYDN
SEQ ID NO: 14	Amino acid sequence of NS1 protein of A/Ohio/02/2019 MDSNTVSSFQVDCFLWHIRKQVVDQKLSADAPFLDRLRRDQRSRLRGRGNT LGLDIKAATHAGKQIEKILKEESDEALKMTMVSTPASRYITDMTIEELSR NWFMLMPKQKVEGPLCIRMDQAIMEKKIMLKANFNVIFGRLETIVLLRA FTEEGAIVGEISPLPSFPGHTIEDVKNAIGVLIGGLEWNDNTVRVSKNLQR FAWRSSNENGGPPLTPKQKREMARSEV
SEQ ID NO: 15	Amino acid sequence of NEP protein of A/Ohio/02/2019 MDSNTVSSFQDILLRMSKMQLGSSSEDLNGMITQFESLKIYRDSLGEAVM RMGDLHLLQNRNGKWREQLGQKFEEIRWLIEEVRHRLRTTENSFEQITF MQALQLLFEVEQEIRTFQLI
SEQ ID NO: 16	Amino acid sequence of M1 protein of A/Ohio/02/2019 MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEALMEWLKTRP ILSPLTKGILGFVFTLTVPSERGLQRRRFVQNALNGNGDPNNMDKAVKLY RKLKREITFHGAKEIALSYSAGALASCMGLIYNRMGAVTTEVAFGLVCA TCEQIADSQHRSHRQMVATTNPLIKHENRMVLASTTAKAMEQMAGSSE QAAEAMEIASQARQMVQAMRAIGTHPSSSTGLRDDLLENLQTYQKRMG VQMQRFK
SEQ ID NO: 17	Amino acid sequence of M2 protein of A/Ohio/02/2019 MSLLTEVETPIRNEWGCRCNDSSDPLIVAANIIGILHLILWILDRLFFKCVC RLFKHGLKRGPSSTEGVPESMREEYRKEQQNAVDADDSHFVSIELE
SEQ ID NO: 18	Amino acid sequence of HA protein of A/Ohio/02/2019 MKTIIALSCILCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEV TNATELVQNSSIGEICDSPHQILDGENCTLIDALLGDPQCDGFQNKKWDL FVERNKAYSNCYPYDVPDYASLRSLVASSGTLEFNNEFVWAGVTQNGT SSSIRGSKSSFFSRLNWLTHLNSKYPALNVTMPNNEQFDKLYIWGVHHP GTDKDQISLYAQSSGRITVSTKRSQQA VIPNIGSRPRIRDIPSISYWTIVK PGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCKSECITPNGSIPND KPFQNVNRITYGACPRYVKQSTLKLATGMRNVPERQTRGIFGAIAGFIEN GWEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTN EKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDL TDSEMKNLFEKTKKQLRENAEDMGNGCFKIYHKCDNACMGSIKNGTYD HNVYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCVALLGFMW ACQKGNIRCNICI
SEQ ID NO: 19	Amino acid sequence of NA protein of A/Ohio/02/2019 MNPNQKIITIGSVSLTISTICFFMQIAILITTVTLHFKQYEFNSPPNNQVMLC EPTIERNITEIVYLTNTTIEREICPKPAEYRNWSKPQCGITGFAPFSKDNSIR LSAGGDIWVTREPYVSCDPDKCYQFALGQGTINNVSNNATARDTPHR TLLMSELGVPFHLGTKQVCIAWSSSSCHDGKAWLHVCTGDDKNATASF IYNGRLVDSVVSWSKDILRTQESECVCINGTCTVVMTDGNATGKADTKI LFIEEGKIVHTSKLSGSAQHVEECSCYPRYPGVRCVCRDNWKGSNRPVD INIKDHSIGSSYVCSGLVGDTPRKTDSSSSSHCLNPNNEKGGHGVKGWAF DDGNDVWMGRTINETSRLGYETFKVVEGWSNSKSKLQINRQVIVDRGD RSGYSGIFSVEGKSCINRCFYVELIRGRKEETEVLWTSNSIVFCGTSPTY GTGSWPDGADLNLMI

SEQ ID NO: 20	Nucleotide sequence of PB2 viral segment of A/Singapore/TT1384/2016 agcaaaagcagggtcaaatatattcaatfatggagagaataaaagagctagagatctaatgtcgcagctccgcactgc cgagatactactaagaccactgtggaccatatggccataatcaaaaagtacacatcaggaaaggcaagagaagaa ccccgcactcagaatgaagtggatgagtggaatgaatacccaattacggcagacaagagaataatggacataatc ccagagagggaatgaacaaggacaaccctctggagcaaaaacaaacgagtcgtgacatcagaccgagtgatggtatca cctctggccgtaacatggtggaataggaatggcccaacaacagtacagttcaattacccaaggtatataaaacttatt tcgaaaaggctcgaagggtggaacatggtaccttcggccctgtccacttcagaatacaagttaaaataaggaggaga gttgatacaaacctggccatgcagatctcagtgccaaggaggcacaggatgtgattatggaggtgttttcccaaat gaagtgggggcaagaatactgacatcagagtcacagctggcaataacaaaaggagaagaaagagagctccagaa ttgtaaaatgtctccctgagtggtggctacatgctagaagagaaatgtgtccgttaaacaagggtttctccggtagcc ggtgggacaggcagtggttataatgaggtgtgtgacctaaccgaaggagctgctgggagcagatgtacactccagg agggaaggatggaatgagtgatgtgacaaaagctgtgattatcgtctgtaaacatagatagaagagcagcagtgct cagcagacagccattgacatctcttggaaatgtgccaacgacacagcattggagggtgtgaaagatgtgtggacatccta aacagaatccaactgaggagcaagccgtgacatatgcaaaagcagaatagggtgtgagatcagctcatctttcagt tttgggtgggttcaactttcaaaaggacaagcggatcatcagtcagaagaaagaaagaaatgctaaccgggcaacctcca aacactgaaatgaaggtacatgaagggtatgaagaattcacaatggttgggagaagagcaacagctattctcagaa aggcaaccaggagattgatccaatgaatgaagcgggagagacgagcagtcattgctgaagcaataattgtggc catggtattctcacaaggaggtgctcatgatcaaggcagttaggggcgatctgaactttgtcaatagggcaaacaga gactgaatcccatgcaccaactcttgaggccatttccaaaagatgcaaaaagtgtctttccagaactgggggaattgaaa ccatcgacaatgtgatggggatgctcggaatactgcccacatgacccaagcacggagatgtcactgagaggaa taagagtcagcaagatggggtagatgaatactccagcacggagagagtgatagttagattgaccgatttttagg gttagagatcaagagggaacgtactattgtctccgaagaagtcagtgaaaccgaaggaaactgagaagttagaca taacttattcgtcatatgatgtgggagatcaatggccctgagtcagtgctagtcacaactatcaatggataatcaga aactgggaaattgtgaaaatcaatgtgtcacaagaccaccaatgttatataataaaatggaatttgacacatttcagtc tctgtccctaaaggcaaccagaagccgtgtacagtgatctgaaggacactgtttccagcaaatgctgggagatgtgttg ggacatttgacactgtccaaataataaaactctccccccttgcgtctgctccaccagaacaggcaggatgcaattttct tcattgactgtgaatgtgagaggatcagggttaaggatactgtgaagaggcaattctccagtattcaattacaacaagg caaccaaacgacttacgggttctggaaaggatgcagggtgcattgactgaagatccagatgaaggccacatctggagtg gagtcgtgtctgtagagaggatttctattttaggcaaaagaacagaagatatggcccagcattgaagcatcaatgaa ctgagcaatcttgcaaaaggagagaagctaattgtgctaattgggcagggggacatagtggttgtaatgaacgaa aacgggactctagcatacttctgacagccagacagcgaccaaaagaattcggatggccatcaattagtgtcgaaatt gtttaaaaacgacctgtgttctact
SEQ ID NO: 21	Nucleotide sequence of PB1 viral segment of A/Singapore/TT1384/2016 agcaaaagcaggcaaacatttgaatggatgtcaatccgactctacttttctaaaaattccagcacaataatgcataa gcaccacattcccttatactggagatccctacacagccatggaaacaggatacccatggatagcagtaaac agaacacaccaatctcagaaaagggggaagtggacaacaacacagagactgtgtgcacccagctcaacccgat tgagcgaccactacctcgaagataatgaaccaatgtgggtatgcacaaacagactgtgtttctagaggctatgctttctt gaagaattcccatcaggaaatatttgacaaattacgcttggaacaatgtgaagttgttcaacaacaagggtgataa ctgactcaagggtcgcagacttatgattggacattaaacagaatacaaccggcagcaactgcattggcccaaccat agaagtttttagatcgaatgacctaacagctaacgagtcagggaaggctaatagatttctaaaggatgtgatggaatca atggacaaaggaggaatagagataacaacccacttcaagaaaaaggagagtaagagacaacataaccaagaa gatgtgtcacgcaaaagaacaatagggaagaaaaaacagagattgaataagagaggctatcttaataagagcactgac attaaatcagatgaccaaaatgctcagagagaggcgaattfaaaaagaagggtctatcgcaacacctgggagtcagatt agaggtttctgatactttgtgaaactttagctaggagcatttgcgaaaagcttgaaacagctgggctccagtagggg gcaatgaaaaagaaggccaaattggcaaatgtgtgagaagaatgatgactaattcacaagatacagagatttcttca caatcactggggacaacactaagtggaatgaaaaatcaaaatcctcgaaattgtctggcgtgattacatatatacca gaaatcaaccaggagtggttagaacaatcctgagcatggcaccataatgttctcaatataaattggcaagactagga aaaggctcatgttgcagagataaaagaatgaagggttcgaacacaataaccagcagaatgctgacgaagcattgatct gaagtatttcaatgaatcaacaagaagaaaattgagaaaataaggcctctttaaattgtgtggcacagcatcactgag tctctggatgagatgagcaterftcaacagctgaagtagcgtctctggagctcgaactcgaactagtaactcttgcaacaagaagaa

	atacaccaagacaacatactgggggatgggctccaatcatccgacgatttgcctcatagtaaatgcaccaaacca tgaaaggaatacaagcaggagtgacagattctacaggacctgcaagttagtggaatcaacatgagcaaaaagaa gtcctatataaataagacagggacattgaattcacagctttttatcgctatggatttggctaattttagcatggag ctaccagctttggagtgctggagtaaatgaatcagctgacatgagtaattggagtaaacagtgataaagaacaacatg ataaacaatgacctggacctgcaacggctcagatggctctcaattgttcatcaaaagactacagatacacataggt gcataggggagacacacaaatcagacgagaagatcattgagttgaagaagctatgggatacaacccaatcaaa ggtagggctattagatcagatggaggaccaaaactatacaacatacggaaatcttcacattcctgaagctgcttaaaat ggggagctaattgatgatgattatcggggaagactttgtaatccctgaatcccttggtagtcacaaggagattgattct gtaaacatgctgtgtaattccagccatggcccagccaaaagcatggaatatgatccgttgcactacacattcc tggattcccaagaggaaatcgttctatcctcaacacaagccaaagggggaattcttggagatgaacagatgtaccagaa gtgttgcaatttattcgagaaattttccttagcagttcatataggagaccggttggaaattttagcatgttgaggccat ggtgtctaggggcccagtgatccagagtcgacttggagctgtgacggatcaagaagaagagtgctctgagatca tgaagatctgtccaccattgaagagctcagacggcaaaaataatgaatttaactgtccttcatgaaaaaatgacctgtt tctact
SEQ ID NO: 22	Nucleotide sequence of PA viral segment of A/Singapore/TT1384/2016 agcaaaagcaggtagtgcataatggaagactttgtcgacaatgcttcaatccaatgacgtgcagcttgcgga aaaggcaatgaagaataggggaagatccaaaatcgaaactaataagtttgcgcaatttgcacacatttggaaagt ttgttcatgtattcggaattccatttcattgatgaacggggggaatcaataattgtagaattctgtgacccaatgcacta ttgaagcaccgatttgagataattgaaggaaagagaccgaatcatggcctggacagtggtgaacagtatatgtaacac aacagggatagagaagcctaaatttcttctgattgtatgattacaagagaaccggttcattgaaattggagtaaca cggagagaagtcacatatattacatagaaaaagccaacaaaataaaatctgagaagacacacattcacatctttca ttcactggagaggaaatggccccaagcggactacacccttgacgaagagagcagagcaagaatcaaaactag gcttttctactataagacaagaaatggccagtaggagtcctatgggattccttctgtagtcggaagaggcgaagaga caattgagaagaaagttagattacaggaactatgcgcaagcttgcgaccaagctccccacaaacttctccagcc ttgaaaacttcagagcctatgtagatggattcgagccgaacggctgcattgagggcaagcttccccaaatgtcaaaag aagtgaacgccaaaattgaaccattcttgaggacgacaccacgccccctcagattacctgatggaacctcttgcctac agcgggtcaaaagtctctgctgatggatgctctgaaattaagcattgaagacccgagtcacgagggggaaggaatacc actatagatgcaatcaaatgcatgaagacattcttggctggaagagcctaaaatagtcacacacatgagaaggg cgtaaatcccaattacctcatggcttgggaagcagggtctagcagagctacaggacattgaaaatgaagagaagatcc caaggcaaaagaacatgaagaaaacagccaattgaagtgggctactcgggtgaaaatagggccaccgaaaaagtgc gaactttagatgactgtaagatgttggagaccttaaacagtatgacagtgatgagccagagccagatcttagcagc tgggtccaaaatgaattcaataaggcatgtgaattgactgactcaagctggatagaactgtagtaaataggagaagat gttgcctcgattgaacatatagcaagcatgaggaggaactatttaccagcagaagtgtccactgcagggctactga atacataatgaaggagggtgtacataaatacggccttgcctaatgcatcctgtccgcatggatgacttcagctgatt ccaatgataagcaaatgtaggaccaaaagaaggaagacggaaaacaaacctgtatgggttcattataaaagggaagt ctcatttgagaaatgatactgtagtgaactttgtaagtatggagttctcactcactgatccaagactggaaccacac aaatgggaaaaaactgtgttctgaaatagagacatgctattgaggactgcgataaggtcaaggtctgaggcccatg ttcttatatgtgagaaccaatggaacctccaagatcaagatgaatggggcatggaaatgaggcgctgcttcttcaa tctcttcagcagattgagagcatgattgaagccgagcttctgtcaagagaagaacatgaccaaggaaattcttga acaaatcggaacatggccaatcggagagtcacccaggggagtgagggaaggctctattggaaaagtgtgcaga accttactggcaaaatctgtgttcaacagtctatagcgtctccacaacttgagggttttctggctgaatcaagaaat gcttctcattgttcaggcacttagggacaacctggaacctggaaccttcgatcttggggggctatatgaagcaatcga ggagtgccctgattaatgatccctgggttttgcctaatgcatcttgggtcaactccttctcacacatgactgaagtagtgc tggcaatgctactattgtctatccatattgtccaaaaagtacctgttctact
SEQ ID NO: 23	Nucleotide sequence of NP viral segment of A/Singapore/TT1384/2016 agcaaaagcaggtagataatcactcaatgagtgacatcgaaagccatggcgtctcaaggcaccacacgatcatatg aacaatggagactggtggggagcggccaggataccacagaatcagagcatctgttggaaagaatgattggaggaa tcgggagattctacatcaaatgtgcactgaactcaaaactcagtgattatgagggagactaatccagaatagcataac aatagagaggatggtgttctgtctttgatgagagaagaataaataacatagaagagcatccaagtctgtgggaaag accttaagaaaacaggaggacccatctatagaagaatagacggaaaatggacgagagaactcatcctttatgacaa

	agaagaaataaggagagttggcgccaagcaacaatggcgaagatgcaacagcaggcttactcataatcatgatttggcattccaacctgaatgatgccacatatcagaggacaagacactgttcgcactgggaatggatcccagaatgtctctctaatgcaagggtcaacacttcccagaagggtctgtgcccaggggtctgcagtaaaaggagttggaacaatagctatggaaftgatcagaatgatcaaacgtgggaatcaatgaccgaaatftctggagggggtgaaaaaggacgaaggacaaagattgcttatgaaagaatgtgcaafatcctcaaaaggaaaafttcaaacagctgccagagggaatgatgatacaagtaagagaaagtcgaaccaggaacgtgagattgaagacctattttctagcacgggtcagcactcattctgagaggatcagttgcacataaatcctgctgctgtgtgtatgggtgagtagcaagtggtccatgactttgaaagggaagggtactactggtcggtatagaccattcaaaattactccaaaacagtcagtggtcagcctgataagacaaaaatgaaaatccagctcacaagagtcgaatgtgatggatggcatgccactctgctgcatttgaagatttaagagatcaagttcataagagggaagaagtgatccaagagggaagctttccacaagagggttcagattgcttcaaatgagaatgtggaaacatggactccaataccctggaaactaagaagcagatactggccataagaaccaggagtgagggaataaccatcaacagaaggcagcagccagatcagtggtcagcctacattctcagtcagcgaatctcccttttgaagagcaaccgttatggcagcattcagcgggaacaatgaaggacggacatccgacatgcgaacagaagtataagaatgatggaaagtgcaagccagaggattgtcttcaggggcggggagctctcagctctcggacgaagaaggcaacgaaccgagctgtccttcttgcacatgagtaatgaagggtcttatttctcggagacaatgcagaggatgacaatgaaagaaaaataccctgttctactaatrtattagtagaacaagggtatttttctcaattgtcat
SEQ ID NO: 24	Nucleotide sequence of NS viral segment of A/Singapore/TT1384/2016 agcaaaagcagggtgacaaaaacataatggagtgccaacaccatgcaagctttcaggtagactgtttcttggcatatfcgcaagcgatttgcagacaatggattgggtatgcccattccttgatcggtacgccgagatcaaaagtccttaaaagggaagggaacacaccttggcctcgacatcaaaacagccactctgttgggaacaatgtggaatggatttgaaggaggaatccagcgagacacttagaatgacaattgcatctgtactacttgcgttcaatttctgacatgacctcgaaggaaatgtcacgagactgttcatgttatgcttaggcaaaagataataggccctcttgcgtgcgattggaccaggcggtcatgtgataagaacatagtacttggaagcaaaccttcagtgtaattctcaaccgattagagaccttgatactactaagggtcttctactgaggaggagcaatagttggagaatttcacactaccttctctccaggacatactatgaggatgtcaaaaatgcagttgggtctctacgcggaggacttgagtggaatggttaacacgggtcaggtctctgaaaaatacagagattcgcttggagaagctgtgatgagaatgggagaccttactacctccagagcagaatgagaatggcggggaacaattgggacagaaatttgaggaaataagggtgtaattgaagaaatacggcacagattgaagcaacagagaatagttcgaacaaataacattatgcaagccttacaactactgcttgaagtagagcaagagataagagcttctcgttcagcttattaatgataaaaaacacctgttctact
SEQ ID NO: 25	Nucleotide sequence of M viral segment of A/Singapore/TT1384/2016 agcaaaagcaggtagatatttaagatgagtccttaaccgagggtcgaaacgtacgttcttctatcatteccgtcaggcccccicaaagccgagatgcacagagactggaaagtgtcttgcaggaaagaacacagatcttgaggctctcatgggaatggctaaagacaagaccaatctgtcacctctgactaagggaattttaggattgtgttcacgtcacccgtgccagtgagcgaggactgcagcgtagacgtttatccaaaatgcctaaatgggaatggggaccggaacaacatggatagagcagttaaactatacaagaagctcaaaagagaataacgttccatggggccaaggagggtgactactaagctattcaactgtgtgcacttgcagttgcatgggcctcatatacaacaggtgggaacagtgaccacagaagctgttttgggttagtgtgtgcccactgtgaacagattgctgattcacagcatcggtctcacagacaatggctactaccaccaatccactaatcaggcatgaaaacagaatgggtgctgctagcactacggcaaggctatggaaacagggtggctggtcagtgatgaacaggcagcagaggccatggagggtgctaataagactaggcagatggttacatgcaatgagaactattgggactatcctagctccagtgctgtgtgagagatgaccttcttgaatttgcaggcctaccagaagcgaatgggagtgagatgcaagcgttcaagtgatectctcgtcattgcagcaaacatcattgggagcttgacctgatatgttgattactgactcttttttcaaatgcatttatcgtcgtttaaatacggtttgaagaggggccttctacgggaaggagtcctgagtcctagagggaagaaatatacaacaggcagcagagtgctgtgtagtgacgttcattttgcaacatagagctagagtaaaaactacctgttctact
SEQ ID NO: 26	Nucleotide sequence of HA viral segment of A/Singapore/TT1384/2016 agcaaaagcaggggaaaacaaaagcaacaaaatgaaggcaactactagtagttctgctatatacatttacaaccgc aaatgcagacacattatgtataggtatcatgcgaacaattcaacagacactgtagacacagtagaaaagaatgt aacagtaacacactctgttaaccttctggaagacaagcataacggaaaactatgcaagctaagagggttagcccat tgcatttgggtaaatgtaacattgctgctggtatcctgggaaatccagagtgtgaatcactctccacagcaagttcatg tctctacattgttgaacatctaatcagacaatggaaacgtgttaccaggagatttcaatcaattatgaggagctaaga

	gagcaattgagctcagtgatcatttgaagggttgagatatcccccaagacaacttcattggcccaatcatgactcga acaaaagggtgaacggcagcatgtctcagctggagcaaaaagcttctacaaaaacttgatatggctagttaaaaaa ggaaattcatacccaagctcaaccaatctacattaatgataaggggaaagaagtcctcgtgctggtgggcatcac catccatctactactgctgaccaaaaaagtctctatcagaatgcagatgcatatgttttggggacatcaagatacag caagaagtcaagccgggaataggaaacagaccaaaagtggaggatcaagaagggaatgaacttacttggga cactagtagagccgggagacaaaaataacattcgaagcaactggaaatctagtggtagccgagatatgcattcacaatg gaagaatgctggtatctgtattatcatttcagatacaccagtcacagattgcaatacaactgtgcagacaccggag ggtgctataaacaccagcctccatttcagaaatacaccgcatcacaattggaaaatgtccaaagtatgtaaaaagc acaaaattgagactggccacaggggttgaggaaatgtccgtctatcattcattagaggcctattcggggccattgccgg ctctattgaagggggtggacagggatgtagatggatggtacgggtatcaccatcaaaatgagcagggatcagga tatgcagccgacctgaagagcacacaaaatgccattgacaagattactaacaagaatgaattctgttattgaaaagatg aatacacagttcacagcagtggtgaaagagtcaaccacctggaaaaaagaatgagagaatcaataaaaaaagtga tgaatgttctctggacatttggacttacaatgccgaactgttgggttctattggaaaatgaaagaacttggactaccag attcaaatgtgaagaactgtatgaaaagtgaagaaccagttaaaaacaatgccaaaggaaattggaaatggctgct ttgaattttaccacaaatgcgacaacacgtgcatggaaagtgtcaaaatggacttatgactaccacaaaatactcag aggaagcaaaatgaagcagagaaaaatagatgggttaagtgggaatcaacaaggatttacagattttgctgcatc tattcaactgtcggcagttcattggtactggttagtctccctgggggcaatcagcttctggtgtagtcttaalggtctta cagtgtagaataatgtatttaacattaggatttcagaatcatgagaaaaacacacctgtttctact
SEQ ID NO: 27	Nucleotide sequence of NA viral segment of A/Singapore/TT1384/2016 agcaaaagcaggagtttaaatgaatccaaacaaaagataataaccattggttcgatctgtatgacaattggaaatgg ctaacttaattatacaaatggaaacataatctcaatattgggttagccactcaattcaaatggtaataaagccagattg aaacatgcaatcaaaagcgtcattactatgaaaacaacacttgggtaaatcagacatatgtaacatcagcaacacca actttgctgctggacagtcagtggttccgtgaaattagcgggcaattcctctctctgacctgttagtgatgggctatata acagtaaaagacaacagtgtaagaatcggttccaagggggatgtgtttgtcataagggaaacattcatatcatgctctc ccttggaaatgcagaaccttcttctgactcaaggggccttgcataatgacaaacattccaatggaaacattaaagacag gageccatatacgaacctaatgagctgtctattgggtgaagtccctctcttcaactcaagattgagtcagtcgctt ggtcagcaagtgctgtcatgatggcatcaattggctaacaattggaaattctgcccagacagtggggagtggtgctg tgttaagtaaatggcataataacagacactatcaagagttggaggaaataatattgagaacacaagagtcgaat gtgcatgtgtaaatgttctgtcttaccataatgaccgatggaccaagtgtgggcaggccctacatacaaaatcttcag aatgaaaagggaagataatcaaatcagtcgaatgaaagccctaatatcactatgaggaaatgctcctgttaacc tgattctagtgaatcacatgtgtgtgcaggggataactggcatggctcgaatcgaccgtgggtgtcttcaaccagaat ctggaaatcagatgggatacatatgcagtggtgttctggagacaatccacgccaatgataagacaggcagttgt ggtccagtatcgtcaatggagcaaatggagtaaaaggattttcattcaatacggcaatggtgttggataggggaga actaaaagcattagttcaagaaaagggtttgagatgatttgggatccgaatggatggactgggactgacaataaattct caataaagcaagataatcgtaggaaataatgagtggtcagggtatagcgggagttttgttcagcatccagaactaaca gggctggtgattgtataagaccttgccttctggttgaactaataagaggggcaccggaagagaacacaatctggactag cgggagcagcatalccttttgggtgtaaacagtgacactgtgggtgtgcttggccagacgggtgctgagttgccattt accattgacaagtaattgttcaaaaactcctgtttctact
SEQ ID NO: 28	Amino acid sequence of PB2 protein of A/Singapore/TT1384/2016 MERIKELRDLMSQSR TREILTKTTVDHMAHKKYTSGRQEKNPALRMKW MMAMKYPITADKRIMDIIPERNEQGQTLWSKTNDAGSDRVMVSP LAVT WNNRNGPTTSTVHYPKVYKTYFEKVERLKHGTFGPVHFRNKKVKKRRRV DTNPGHADLSAKEAQDVIMEVVFNPVEVGARILTSESQLAITKEKKEILQN CKIAPLMVAYMLERELVRKTRFLPVAGGTGGSVYIEVLHLTQGTWCWEQM YTPGGEVRNDDVDQSLIAARNIVRRAAVSADPLASLLEMCHSTOIGGVK MVDILKQNPTEEQA V DICKAAIGLRISSEFSFGGFTFKRTSGSSVKKEEM LTGNLQTLKLRVHEGYEEFTMVGRRATAILRKATRRLIQLIVSGRDEQSI AEAIHVAMVFSQEDCMIKAVRGDLN FVN RANQRLNPMHQLLRHFQKDA KVL FQNWGIETIDNVMGMIGILPDMTPSTEMSLRGIRVSKMGVDEYSSTE RVIVSIDRFLVRDQRGNVLLSPEEVSETQGTEKLTTITYSSSMMWEINGPE

	SVLVNTYQWIIRNWEIVKIQWSQDPTMLYNKMEFEPFQSLVPKATRSRYS GFVRTLFQQMRDVLGTFDTVQIHKLLPFAAAPPEQSRMQFSSLTVNVGRS GLRILVRGNSPVFNYNKATKRLTVLGKDGALTEDPDEGTSGVESAVLR GFLILGKEDKRYGPALSINELSNLAKGEKANVLIGQGDIVLVMKRKRDS ILTDSQTATKRIRMAIN
SEQ ID NO: 29	Amino acid sequence of PB1 protein of A/Singapore/TT1384/2016 MDVNPTLLFLKIPAQNAISTTFPYTGDPPYSHGTGTGYTMDTVNRTHQYS EKGKWTNTTETGAPQLNPIDGPLPEDNEPSGYAQTDVCLEAMAFLEESH PGIFENSCLETMEVVQQTRVDKLTQGRQTYDWTLNRNQPAATALANTIE VFRSNDLTANESGRLIDFLKDVMESMDKEEIEITTHFQRKRVRVDNITKK MVTQRTIGKKKQRLNKRGYLIRALTLNTMTKDAERGKLRRAIATPGM QIRGFVYFVETLARSICEKLEQSGLPVGGNEKKAKLANVVRKMMTNSQD TEISFTITGDNTKWNENQNPRMFLAMITYITRNQPEWFRNILSMAPIMFSN KMARLGKGYMFESKRMKVRTQIPAEMLASIDLKYNFESTKKKIEKIRPLL MDGTASLSPGMMMGMFNMMLSTVLGVSILNLGQKKYTKTTYWWDGLQS SDDFALIVNAPNHEGIIQAGVDRFYRTCKLVGINMSKKKSYINKTGTFEFT SFFYRYGFVANFSMELPSFGVSGVNESADMSIGVTVIKNNMINNDLGPAT AQMALQLFIKDYRYTYRCHRGDTQIQTRRSFELKKLWDQTQSKVGLLV DGGPNLYNIRNLHIPEVCLKWELMDDDYRGRLCNPLNPFVSHKEIDSVN NAVVMPAHGPAKSMEYDAVATTHSWIPKRNRSILNTSQRGILEDEQMYQ KCCNLFKFFPSSSYRRPVGISSMVEAMVSRARIDARVDFESGRIKKEEFS EIMKICSTIEELRRQK
SEQ ID NO: 30	Amino acid sequence of PB1-F2 protein of A/Singapore/TT1384/2016 MEQEQTDPWIQ
SEQ ID NO: 31	Amino acid sequence of PA protein of A/Singapore/TT1384/2016 MEDFVRQCFNPMIVELAEEKAMKEYGEDPKIETNKFAAICTHLEVCFMYS DFHFIDERGESHVESGDPNALLKHRFEIIEGRDRIMAWTVVNSICNTTGIE KPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKANKIKSEKTHIHIFSFTGE EMAAKADYTLDEESRARIKTRLFTIRQEMASRSLWDSFRQSERGEETIEE KFEITGTMRKLDQSLPPNFSSLENFRAYVDGFEPNGCIEGKLSQMSKEV NAKIEPFLRTTPRPLRLPDGPLCHQRSKFLMDALKLSIEDPSHEGEGIPLY DAIKCMKTFFGWKEPKIVKPHEKGVNPNYLMAWKQVLAELQDIENEKI PRTKNMKKTSQKQWALGENMAPEKVDFDDCKDVGDLDKQYDSDEPEPRS LASWVQNEFNKACELTDSSWIELDEIGEDVAPIEHASMRNYFTAQVSH CRATEYIMKGVYINTALLNASCAAMDDFQLIPMISKCRTKEGRRKTNLY GFIHGRSHLRNDTDVVNFVSMFSLTDPRLEPHKWEKYCVLEIGDMLLR TAIGQVSRPMPFLYVRTNGTSKIKMKWGMEMRRCLLQSLQQIESMIEAES SVKEKDMTKEFFENKSETWPIGESPRGVEEGSIGKVCRTLLAKSVFNSLY ASPQLEGFSAESRKLILLIVQALRDNLPGTFDLGGLYEAIEECLINDPWVL LNASWFNSFLTHALK
SEQ ID NO: 32	Amino acid sequence of NP protein of A/Singapore/TT1384/2016 MSDIEAMASQGTKRSYEQMETGGERQDTTEIRASVGRMIGGIGRFYIQM CTELKLSDYDGRLIQNSITIERMVLSAFDERRNKYLEEHPSAGKDPKKTG GPIYRRIDGKWTRELILYDKEEIRRVWRQANNGEDATAGLTHIMIWHSNL NDATYQRTRALVRTGMDPRMCSLMQGSTLPRRSGAAGA AVKGVGTIA MELIRMIKRGINDRNFWRGENGRRTVAYERM CNILKGKFQTA AQRAM MDQVRESRNP GNAEIEDLIFLARSALILRGSAHKSCLPACVYGLAVASG HDFEREGYSLVGIDPFKLLQNSQVVSLIRPNENPAHKSQLVWMACHSAA FEDLRVSSFIRGKKVIPRGKLSTRGVQIASNENVETMDSNTLELRSRYWAI RTRSGGNTNQKASAGQISVQPTFSVQRNLPFERATVMAAFSGNNEGRT

	SDMRTEVIRMMESAKPEDLSFQGRGVFELSDEKATNPVPSFDMsNEGSY FFGDNAEEYDN
SEQ ID NO: 33	Amino acid sequence of NS1 protein of A/Singapore/TT1384/2016 MESNTMSSFQVDCFLWHIRKRFADNGLGDAPFLDRLRRDQKSLKGRGN TLGLDIKTATLVGKQIVEWILKEESSETLRMTIASVPTSRYISDMTLEEMS RDWFMLMPRQKIIGPLCVRLDQAVMDKNIVLEANFSVIFNRLETILLRA FTEEGAIVGEISPLPSLPGHTYEDVKNAVGVLLIGGLEWNGNTVRVSENIQ RFAWRSCDENGRLPSLPPEQK
SEQ ID NO: 34	Amino acid sequence of NEP protein of A/Singapore/TT1384/2016 MESNTMSSFQDILMRMSKMQLGSSSEDLSGMVTRFESLKIYRDSLGEAV MRMGDLHYLQSRNEKWREQLGQKFEEIRWLIEIRHRLKATENSFEQITF MQALQLLLEVEQEIRAFSFLI
SEQ ID NO: 35	Amino acid sequence of M1 protein of A/Singapore/TT1384/2016 MSLLTEVETYVLSIIPSGPLKAEIAQRLESVFAGKNTDLEALMEWLKTRPI LSPLTKGILGFVFTLTVPSERGLQRRRFIQNALNGNGDPNNMDRAVKLYK KLKREITFHGAKEVSLSYSTGALASCMGLIYNRMGTVTTEAAFGFLVCAT CEQIADSQHRSHRQMATTNPLIRHENRMVLASTTAKAMEQVAGSSEQA AEAMEVANKTRQMVHAMRTIGTHPSSAGLRDDLLENLQAYQKRMGV QMQRFK
SEQ ID NO: 36	Amino acid sequence of M2 protein of A/Singapore/TT1384/2016 MSLLTEVETPTRSEWECRCSSDPLVIAANIIGILHLILWITDRLFFKCIYR RFKYGLKRGPSLEGVPESMREEYQQEQQSAVDVDDGHFVNIELE
SEQ ID NO: 37	Amino acid sequence of HA protein of A/Singapore/TT1384/2016 MKAILVLLYTFTTANADTLCIGYHANNSTDVDTVLEKNVTVTHSVNL LEDKHNGKLCCLRGVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVET SNSDNGTCYPGDFINYEELREQLSSVSSFERFEIFPKTTSWPNHDSNKGVT AACPHAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHH PSTTADQQSLYQNADAYVFGTSRYSKKFKPEIGTRPKVRDQEGRMNYY WTLVEPGDKITFEATGNLVVPRYAFTMERNAGSGHISDTPVHDCNTTCQ TPEGAINSTLFPQNIHPITIGKCPKYVKSTKLRLATGLRNVPSIQSRGLFGAI AGFIEGGWTGMVDGWYGYHHQNEQGSYAADLKSTQNAIDKITNKVN SVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLL ENERTLDYHDSNVKNLYEKVRNQLKNNAKEIGNGCFEFYHKCDNTCME SVKNGTYDYPKYSEEAKLSREKIDGVKLESTRIYQILAIYSTVASSLVLVV SLGAISFWMCSNGSLQCRICI
SEQ ID NO: 38	Amino acid sequence of NA protein of A/Singapore/TT1384/2016 MNPNQKIITIGSICMTIGMANLILQIGNIISIWVSHSIQIGNQSQIETCNQSVI TYENNTWVNQTYVNISNTNFAAGQSVSVKLAGNSSLCVPVSGWAIYSKD NSVRIGSKGDVFVIRETFISCSPLECRTFFLTQGALLNDKHSNGTIKDRSPY RTLMSCPIGEVPSPYNSRFESVAWSASACHDGINWLTIGISGPDSGAVAVL KYNHITDTIKSWRNILRTQESEACVNGSCFTIMTDGPSDGOASYKIFRI EKGKIISVEMKAPNYHYEECSYCPDSSEITCVCRDNWHGSRNPWVSFN QNLEYQMGYICSGVFGDNPRPNDKTGSCGPVSSNGANGVKGFSFKYGN GVWIGRTKSISSRKGFEMIWDPNGWGTGTDNKFSIKQDIVGINESGYSGS FVQHPELTGLDCIRPCFWVELIRGRPEENTIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK
SEQ ID NO: 39	Nucleotide sequence of PB2 viral segment of A/South Carolina/04/2017 agcaaaagcaggatcaattatattcagcatggaagaataaaagaactacggaatcattatgctgcagctcgcacteg cgagatactgacaaaaaccacagtggaaccatattgccataaataagaagtacacatcggggagacaggaaaagaa cccgctacttaggatgaaatggatgaggaatgaaataccaattactgctgacaaaaggataacagaaatggtcc

	ggagagaaatgaacaaggacaaactctatggagtaaaatgagtgatgctggatcagatagagtgatggtatcacctt tggctgtaacatgggtgaatagggaatggaccctgacaagtacgggtccattaccacaaagtgtacaaaacttattcg acaaagtgcgaagggttaaaacatggaccccttggccctgtccattttagaatcaagtcaagatacgcagaagagtag acataaacccctggtcatgcagaccctcagtgccaaaggaggcacaagatgtaattatggaggtgttttcccaatgaagt gggagccagaaatactaacatcagaatcacagctaacaataactaaagagaaaaagaaagaaacccgagattgcaa aattttcccttgatggtgcatacatgctagagagagaactgtgctggaaaacaagatttctccagttgctggcggga acaagcagtatatacatggaagttttactttgactcaaggaaacgtgtgggaacaaatgtactccaggtggaggga gtgaggaatgacgatgtgaccaaagcctaattattgctggccaggaaacatagtaagaagagctgcagtgctcagcag atccactcgcacttttattggagatgtccacagcagcgaattggcgggaacaaggatgggtgacattcttagacaga acccgactgaagaacaagctgtggatataatgcaaggctgcaatgggattgagaatcagctcattttcagctttgggtg gctttacatttaaaagaacaagcgggtcgtcagtgcaaaagagagaagagggtcttacaggcaatctccagacattg agaataagagtacatgaggggtatgaggaggttcacaatgggtgggaaaagagcaacagctatactaaagaaaagca accagaagattggttaactcatagtgagtggaagagacgaacagtcaatagccgaagcaataatcgtggccatgg tgttttcacaagaagattgcatgataaaagcagttagaggtgacctgaattttgtcaacagagcaaatcagcgggtgaa ccccatgcatcagcttttaaggcattttcagaaagatgcgaagactctttcaaaattgggaggtgaacacatgcag agtgtgatgggaatgggtggagtaftaccagatagctccaagcacagagatgcaatgagagggaataagagtcag caaaatgggtgtggatgaatactccagtcagagagggtgtgtgttagcattgacgtgtttttgagagttcggagacca acgtgggaatgtattatctctcagtgaggtcagtgaaacacagggaaactgaagactgaccataactttatcatca tcgatgatgtggagattaacgggtcctgagtcgtttgtgtaaatcctatcaatggatcatcaggaaattgggaagctg tcaaaattcaatgggtcagaaatcctgcaatgtgtacaacaaaatgggaattgaaccatttcaatctttagtcaccaag ccactagaagccaatacagtggtgtgacaaacttatccaacaaatgagagacgtacttggacatttgacactg cccagataataaagcttctccctttgagctgctccaccgaagcaagcagaatgcagttctctcactgactgtgaa tgtgaggggatcagggtgagaaatctgttaagggtgcaattctcctgtattcaactacaacaagaccactaaaaggct aacaattctggaaaagatgccggcactttaattgaagaccagatgaagcacatccggagtgagtcggcgtct tgagagggttccctcatfatagtaagaagacagaagatcggaccagcattgaagcaatgaactgagtaacctt gcaaaaggggaaaaggctaattgtctaattgggcaaggagacgtgtgtgttaataagaaacggaaacgggactct agtatacttactgacagccagacagcgacaaaaagaattcggatggccatcaattaactgaatgttttaaaacga cctgttttact
SEQ ID NO: 40	Nucleotide sequence of PB1 viral segment of A/South Carolina/04/2017 agcaaaagcaggcaaacatttgatgagtgatgcaatccgactctactgtttcttaaaagtccagcgcaaaatgccata agcacacaacttcccttactgagatcctccatacagccatggaaacaggggacagggtacactatggacacagtcaca cagaacacaccaatactcagagagggggaagtggacgacaaatacagaactggggctccccagctcaacccaa ttgatggaccactacctgaggaataatgaaccaagtggatagcacaaacagactgtgtcctggaggctatggccttcc ttgaagaatcccccaggtatctttgagaactcctgcttgaacaatggaagccgttcaacagacaaggggtggac aaactaacccaaggtcgcagacttatgattggacattaaacaggaaccaaccggcagcaactgcattagccaaca ccatagaagtctttaggtcgaaacggattaacagctaataatcaggaaggctaataatctcctcaaggatgtgatgg aatcaatgggataaggagggaatggagataacaacacactttcaaagaaaaaggagagtaagggaacaacatgacca agaaaatggtcacacaaagaacaatagggaagaaaaaacaaagagtgataagagaggctacctataagagctc tgacattgaacacgatgaccaaggatgcagagagaggcaatttaaaagaagggtcattgcaacacccgggatgc aaattagagggttcgtgtacttctgtgaaactttagctagaagcatttgcgaaaagcttgaacaatctggacttccggtt gggggtaatgaaaagaaggccaaactggcaaatgtgtgagaaaatgatgactaattcacaagacacagagcttt ctttcacaaactcggagacaacactaagtggatgaaaatcaaaacccccgaatgttttggcaatgattacatacat cacaaagaaccaacctgaatggttcagaaacatcctgagcatcgacccaataatgttctcaacaaaaatggcaagac tgggaaaaggatacatgttcgagagtaagagaatgaagctccggacacaaatacctgcagaatgctagcaagcat tgacctgaagtatttcaatgaatcaacaagggaagaaaattgagaaaataaggcctcttctaataagatggcacagcatc attgagccctggaatgatggtggcatgttaacatgctaagtacagttttagagctctgatactgaatttggacaa aagaaatacaccaagacaacatactgtgtggatgggtcctaactcctcagacgattttgcccctcatagtaattgcacc aatcatgagggaatacaagcagggtgtgatagtatttataggacctgcaagttagttgggaatcaacatgagcaaa aagaagtcctataataaaacaggggacatttgaattcactagcttctttatcgataggtatttggctaattttagcat ggagctgccaagtgttggagttctggaataaacagagtcagctgacatgagcattggagtaacagtgataaagaca

	acatgataaataatgacctggaccagcaacagcccaaatggctctccaatgttcatcaaaagattacagatacacgta tcgggtccatagaggagacacacaaatccaaacgagaagatcattcgagataaagaagctgtgggatcaaaccca atcaaggacaggactattggtatcagatggggaccaaactatataaatccggaaatcttcacatccctgaagctg cttaaaagtggagctgafggatgagaattatcggggagagactttgtaatccctgaatccctttgtagccataaagaa attgaatctgtaacaatgctgtagttaatgccagccatggtccggccaaaagtatggaatatgtagccgttgcgact acacactcctggattcccaaaaggaaacgcctctattctaaacacaagccaaagggaattcttgaggatgaacagat gtaccagaagtgtgcaactgttgcagaaattttccctagtagttcatataggagaccgattggaatttctagcatggt ggaggcccatggtatctaggcccgattgatccagaattgactcgagctggaggagattaagaaggagatttct ctgagatcatgaagatctgtccaccatgaagaactcagacggcaaaaataatgaatttagcttctcatgaaaa aatgacctgtttctact
SEQ ID NO: 41	Nucleotide sequence of PA viral segment of A/South Carolina/04/2017 agcaaaagcagggtactgattcaaatggaagattttgtgcgacaatgcttcaatccgatgattgtcgacttgagaaa aagcaatgaagagtatggggaggatctgaaaaattgaaaccaacaatttgcagcaatatgcactcacttggagggtg tgtttcatgtattcagatttccattcatcaatgaacaaggcgaatcaatagtagtagaacttgacatccaaatgcactg ttaaagcacagattgaaataatcgaggggagagacagaacaatggcctggacagtagtaaacagtagtctgcaaca ctactggagctggaaaaccgaagtttctaccggattttagtaftacaagaagacagattcatcgaaattggagtgga caaggagagaagtcacatatattaccttgaanaaggccaataagatcaaatctgagaacacacacattcacatttttc attcaactggggagggaatggccacaaggcagactacactctcgacgagggaagcaggcgtagggtacaaaacca ggctgtttacataagacaagaatggccaacagaggcctctggattcctttctcagtcggaaaggcgaagga aacaattgaagaaaaattgaaatcacaggaaactatgcccaggctagccgaccaaaagtctccaccgaacttctct gacctgagaatttagacctatgtggatggattcgaaacgaacggctgcattgaggggcaagctttctcaaatgtc agaagtgaatgccaaattgaaccttttctgaagacaacaccaagaccaatcaacttccctagtggaacctctgttat cagcgatccaaattcctcctgatggatgcttgaattgagcattgaagaccaagtcacgaaggagaaaggatccc attatagatgcaatcaagtgcataaaaacatttctggatggaagaaccttatatagtcacacacgaaaaggga ataaattcaaatatctgctgcatggaagcaagtactgtcagaattgcaggacattgaaatgaggagaagattccaa gaactaaaaacatgaagaaaacgagtcactgaagtgggctctgtgtgaaaacatggccaccagagaaggttagactt tgaaaactgcagagacataagcgatttgaacaatatgtagtgaagaacctgaattaagatcatttcaagctggat acagagtgaagtcaacaaggcctgtgagctaaactgattcagctctgtagagctcgatgaaattggagaggacgtag ccccgattgaacacattgcaagcatgagaagggaattatttcacagcagagggtgtccactgttagagctactgaataca taatgaaagggtgtatcaattaacactgcctgtcctaatgcatcctgtgcagcaatggacgattttcaactaattccatg ataagcaagtgcagaaactaaagagggaaggcgaaaaaccaatttatatggattcatcataaagggaagatctcatct gagggaatgacacagacgtgttaactttgtgagcatggagttttctctacagatccgagacttgaaccacataaatg ggagaataattgttcttgatagagagatgttactaaggagtgccataggccaaatttcaaggccgatgttctgt atgtgaggacaacaggacatcaaatgcaaatggaatggggaatggagatgagacgttgcctcctcagtcact ccagcagatcgagagcatgattgaagccgagtcctcagttaaagagaagacatgaccaaaagatttttgagaata aatcagaagcatggcccattggggagtcgccaaaggagtggaagaagggttcattgggaaagtctgtaggactct attggctaagtcatgtattcaatagcctgtatgcatccacaattggaaggattttcagcagagtcgaagaaactgctc cttattgtcaggctcttagggacagactcgaacctgggaccttgatcttgggggctatatgaagcaattgaggagt gcctgattaatgacccgtgtttgtctcaatgcgtctgtgttcaactcctctctgacacatgcattaaaatgattatagca gtgctactattgttatccgtactgtccaaaaagtacctgtttctact
SEQ ID NO: 42	Nucleotide sequence of NP viral segment of A/South Carolina/04/2017 agcaaaagcagggttgataatcactcactgagtgacatcaaaatcatggcgtcccaaggcaccacacgggtctatga acagatggaaactgatggagatcgccagaatgcaactgagattaggcgatcgctgggaagatgattgatggaatt gggagattctacatccaaatgtgactgaacttaaaactcagtgattatgaaggacgggttgatccaaaacagcttgaca atagagaaaatgggtactctgctttgatgaagaagggaataaatacctggagaacaccccagcgcggggaaag atcccaagaaaactgggggcccatacaggagagtcgatgggaaatggaagggaactgctctttatgacaa agaagaataaaggcgaatctggcgccaagccaacaatggtgaggatgctacatctggtctaactcacataatgattt ggcattccaattgaatgatgaacataccagaggacaagagctcttgggactggaaatggatccagaatgtgct ctctgatgcagggtcgcactctcctagaaggtcggagctgcaggtcgtgcagtcaagggaatcggaacaatggt gatggaaactgatcagaatgatcaacggggatcaacgatcgaaattttggagaggtagaattggcggaagaaac

	aagaagtgcttatgagagaatgtgcaacattctaaaggaaaatttcaaacagctgcacaaagagcaatggtggatc aagtttagaagaagtcggaacccaggaacgctgagatcgaaagatctcatatttttagcaagatctgcactgataatga gaggatcagttgctcacaatcttgctacctgctgtgcataatggacctgcagtatccagtggttacgactttgaaaa agagggaataatcttggtgggaatagacccttcaaaactacttcaaaatagccaaafatacagcttaatcagacctaat gagaatccagcacacaagagtcagctggtgtggtgcatggccattctgctgcatttgaagatttaagattgtaagct tcatacagaggacaaaaagfatctcctcgggggaaaactgtcaactagaggagtagacaattgcttcaaatgagaacatg gataatatgggatcaagcactctgaactgagaagcgggtactggccataaggaccaggagtgagggaacact aatcaacagagggcctcgcaggccaaaccagtggtgcaacctacgttttctgtacaaagaacctccatttgaaaa gtcaaccatcatggcagcattcactggaaatacggagggaagaacttcagacataggggcagaaatcataaggatg atgggaaggtgcaaaaccagaaagatttcaatccggggaggggagtttctgagctctcagacagaaaggcagcg aaccgcgacgtgcctctttgatgatgtaacgaaggatcttatttctcgagacaatgcagaagagtagcacaatt aaggaaaaatacccttggttctact
SEQ ID NO: 43	Nucleotide sequence of NS viral segment of A/South Carolina/04/2017 agcaaaagcagggtagacaagacataatggattccaacactgtgtcaagttccaggtagattgcttttggcatat ccggaaacaagttgtagaccaaaaactgagtgtatgcccatctcgcagctgcgcggagaccagaggtcccta aggggaagaggcaatactctcgtctagacatcaaaagcagccacccatgttggaagcaaatctgtagaaaagattc tgaagaagaatctgtagaggcacttaaaatgacctggtctcaaacactgcttcgcgatacataactgacatgactat tgagggaattgtcaagaaactggttcatgctaatgccacgcagaaagtgaaggccctcttgcatacagaatggacc aggcaatcatggagaaaaacatcatgttaaaagcgaatttcaatgtgttttggcggactagagaccatagtattacta agggctttcaccgaaggaggagcaatcgttggcgaaatctcaccattgcttcttttcaggacatactattgaggatg tcaaaatgcaattgggtcctcatcgaggacttgatggaatgataacacagttcagtgctctaaaaatctacaga gattcgttggagaagcagtaatgagaatggggacctccacttactccaaacagaaacgggaaatggcgagaa cagctaggtcagaagtgtgaagagataagatggctaattgaagggtgagacacagattaagaacaactgaaaata gctttgaacaaataacattcatgcaagcattacaactactgtttgaagtggacaggagataagaactttctcatttcag cttatttaatgataaaaaacacctgttctact
SEQ ID NO: 44	Nucleotide sequence of M viral segment of A/South Carolina/04/2017 agcaaaagcaggtagatattgaaagatgagccttcaaccgaggtcgaaacgtatgttctctatcgttccatcagggc cccctcaagccgagatcgcgagagactgaagatgtcttctgctggaaaaacacagatctgaggtctctatgga atggctaagacaagaccaattctgtcaccttgactaaggggatttttaggtttgttttcacgtcacccgtgccaaagt agcggaggactgcagcgttagacgtttgtccaaaatgccctcaatgggaatggagacccaaataacatggacaaaag cagttaaactgtataggaaacttaagaggggagataacgttccacggggccaaagaaatagctctcagttattctgctg gtgcaactgtgagcagattgctgattccagcacaggtctcataggcagatggtggcaacaaccaatccatttaata aaacatgagaacagaatgttttggccagcactacagctaaggctatggagcaaatggctggatcaagtgaagcagg cagcggaggccatggagattgctatgcaggccaggcagatggtgcaggcaatgagagccattgggactcattcta gttccagtagtggctaaagagatgcttctgaaaatttgcagacctatcagaacgaatgggggtcagatgcaac gattcaagtgaaccgcttgttgggtggcggaataatcattgggatcttgcacttgatattgtgattcttgatcgtctttttc aaatggctctatcagactcttcaaacacggcctttaaagaggcccttctacggaaggagtacctgagctatgaggga agaatatcgaaaggacagcagaatgctgtggtgctgacgacagtcatttgcagcatagaattggagtaaaaaa ctacctgtttctact
SEQ ID NO: 45	Nucleotide sequence of HA viral segment of A/South Carolina/04/2017 agcaaaagcaggggataattctattaacatgaagactatcattgctttgagctacattctatgctgttttgcctcaaa aaattcctggaaatgacaatgacggcaacgctgtgcttgggcaccatgcagtaccaaacggaaacgatatgtgaa aacaatcacaaatgaccgaattgaagtactaatgtactgagtttggttcagaattcctcaatagtgaaatatgcgac agtctcatcagatccttgatggagagaactgcacactaatagatgctctattgggggacccctcagttgacggcttcc aaaataagaaatgggaccttttgaacgaagcagagccctacagcaactgttacccttatgctgcccgtattatgc ctcccttaggtcactagttgctcctcctggcacactggagtttaaaaatgaagcttcaattgggactgagtcactcaa aacggaaacaagttctgttcataaggagatctagtagtatttcttagtagattaaattggttgaccacttaaacac acatatccagcactgaacgtgactatgccaacaagggaacaattgacaattgtacatttgggggttcacacccg ggtagcggacaaggaccaaatcttctgtatgctcaatcatcagggaagaatcagatctaccaaaaaggaagccaaca

[illegible]

	VLRGFLHIGKEDRRYPALSNELSNLAKGEKANVLIQGDVVLMKRKR DSSILTDSQTATKRIRMAIN
SEQ ID NO: 48	Amino acid sequence of PB1 protein of A/South Carolina/04/2017 MDVNP TLLFLKVPAQNAISTTFPYTGDPYSHGTGTGYTMDTVNRTHQY SERGKWTNTETGAPQLNPIDGPLPEDNEPSGYAQTDCVLEAMAFLEESH PGIFENSCLTMEAVQQTRVDKLTQGRQTYDWTLNRNQPAATALANTIE VFRSNGLTANESGRLIDFLKDVMESEMDKEEMEITTHFQRKRRVRDNMTK KMVTQRTIGKKKQVRNKRGYLIRALTNTMTKDAERGKLRRAIATPG MQIRGFVYFVETLARSICEKLEQSGLPVGGNEKKAKLANVVRKMMTNS QDTELSFTITGDNTKWNENQNPRMFLAMITYTTKNQPEWFRNLSIAPIMF SNKMARLGKGYMFESKRMKLRTQIPAEMLASIDLKYFNSTRKKIEKIRP LLIDGTASLSPGMMMGMFNMSTVLGVLSILNLGQKKYTKTTYWWDGLQ SSDDFALIVNAPNHEGIQAGVDRFYRTCKLVGINMSKKKSYINKTGTFFEF TSFFYRYGFVANFSMELPSFGVSGINESADMSIGVTVIKNNMINNDLGP TAQMALQLFIKDYRYTYRCHRGDTQIQTRRSFEIKKLWDQTSRTGLLV SDGGPNLYNIRNLHIPEVCLKWELMDENYRGRLCNPLNPFVSHKEIESVN NAVVMPPAHGPAKSMEYDAVATTHSWIPKRNRSILNTSQRGILEDEQMYQ KCCNLFKFFPSSSYRRPIGISSMVEAMVSRARIDARIDFESGRIKKEEFSEI MKICSTIEELRRQK
SEQ ID NO: 49	Amino acid sequence of PB1-F2 protein of A/South Carolina/04/2017 MEQGQGT LW TQSTEHTNTQRGSGRQIQKLGLPSSTQLMDHYLRIMNQ VDMHKQTVSWRLWPSLKNPTQVSLRTPALKQWKPFNRQGWNT
SEQ ID NO: 50	Amino acid sequence of PA protein of A/South Carolina/04/2017 MEDFVRQCFNPMIVELA EKAMKEYGEDLKIETNKFAAICTHLEVCFMYS DFHFINEQGESI VVELDDPNALLKHFEEI EGRDRTMAWTVVNSICNTTGA GKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKANKIKSENTHIHIFSFTG EEMATKADYTLDEESRARIKTRLFTIRQEMANRGLWDSFRQSERGEETIE EKFEITGTMRRADQSLPPNFSCLENFRAYVDGFEPNGCIEGKLSQMSKE VNAQIEPFLKTTTPRIKLPSPGPCYQRSKFLMDALKLSIEDPSHEGEGIP YDAIKCIKTFFGWKPEYIVKPHEKGINSNYLLSWKQVLSELQDIENEKIP RTKNMKKTSQKWLALGENMAPEKVD FENC RDISDLKQYDSEEP ELSLS SWIQSEFNKACELTDSVWIELDEIGEDVAPIEHASMRNRYFTA EVSHCRA TEYIMKGVYINTALLNASCAAMDDFQLIPMISK CRTKEGRRKTNLYGFII KGRSHLRNDTDVVNFVSMFSLTDPRLEPHKWEKYCVLEIGDMLLRSAI GQISRPMFLYVRTNGTSKVKMKWGMEMRRCLLQSLQQIESMIEAESSVK EKDMTKEFFENKSEAWPIGESPKGVEEGSIGKVCRTLLAKSVFNSLYASP QLEGFSAESRKL L LIVQALRDRLEPGTFDLGGLYEAIIECLINDPWVLLNA SWFNSFLTHALK
SEQ ID NO: 51	Amino acid sequence of NP protein of A/South Carolina/04/2017 MSDIKIMASQGTKRSYEQMETDGDQRNATEIRASVGK MIDGIGRFYIQM CTELKLSDYEGRLIQNSLTIEKMVLSAFDERRNKYLEEHPSAGKDPKKTG GPIYRRVDGKWMREL VLYDKEEIRRIWRQANNGEDATSGLTHIMIWHSN LNDATYQRTALVRTGMDPRMCSLMQGSTLPRRSGAAGA AVKGIGTMV MELIRMIKRGINDRNFWRGENGRKTR SAYERM CNILKGKFQTAAQRAM VDQVRESRNP GNAEIEDLIFLARSALILRGSAHKSCLPACAYGPAVSSG YDFEKEGYSLVGIDPFKLLQNSQIYSLIRPNENPAHKSQLVWMACHSAAF EDLRLLSFIRGTKVSPRGKLS TRGVQIASNENMDNMGSSTLELRSGYWAI RTRSGGNTNQQRASAGQTSVQPTFSVQRNLPFEKSTIMAAFTGNTEGRTS DMRAEIIRMMEGAKPEEV SFRGRGVFELSDEKAANPIVPSFDM SNEGSYF FGDNAEEYDN

SEQ ID NO: 52	Amino acid sequence of NS1 protein of A/South Carolina/04/2017 MDSNTVSSFQVDCFLWHIRKQVVDQKLS DAPFLDRLRRDQ RSLRGRGNT LGLDIKAATHVGKQIVEKILKEESDEALKMTMVSTPASRYITDMTIEELSR NWFMLMPTQKVEGPLCIRMDQAIMEKNIMLKANFNVIFGRLETIVLLRA FTEEGAIVGEISPLSPFPGHTIEDVKNAIGVLIGGLEWNDNTVRVSKNLQR FAWRSSNENG GPPLTPKQKRE MARTARSEV
SEQ ID NO: 53	Amino acid sequence of NEP protein of A/South Carolina/04/2017 MDSNTVSSFQDILLRMSKMQLGSSSEDLNGMITQFESLKIYRDSLGEAVM RMGDLHLLQNRNGKWREQLGQKFEEIRWLIEEVRHRLRTTENSFEQITF MQALQLLFEVEQEIRTF SFQLI
SEQ ID NO: 54	Amino acid sequence of M1 protein of A/South Carolina/04/2017 MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEALMEWLKTRP ILSPLTKGILGFVFTLTVPSEGLQRRRFVQNALNGNGDPNNMDKAVKLY RKLKREITFHGAKEIALSYSAGALASCMGLIYNRMGAVTTEVAFGLVCA TCEQIADSQHRSHRQMVATTNPLIKHENRMVLASTTAKAMEQMAGSSE QAAEAMEIASQARQMVQAMRAIGTHPSSSTGLRDDLLENLQTYQKRMG VQMQRFK
SEQ ID NO: 55	Amino acid sequence of M2 protein of A/South Carolina/04/2017 MSLLTEVETPIRNEWGCRCNDSSDPLVVAANIIGILHLILWILDRLFFKCV YRLFKHGLKRG PSTEGVPESMREEYRKEQQNAVDADDSHFVSIELE
SEQ ID NO: 56	Amino acid sequence of HA protein of A/South Carolina/04/2017 MKTIIALSYILCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEV TNATELVQNSSIGEICDSPHQILDGENCTLIDALLGDPQCDGFQNKKWDL FVERSRAYSNCYPYDVPDYASLRSLVASSGTLEFKNESFNWGTGTQNGT SSACIRRSSSSFFSRLNWLTHLNYTYPALNVTMPNKEQFDKLYIWGVVHHP GTDKDQIFLYAQSSGRITVSTKRSQQA VIPNIGSRPRIRDIPSRSIYWTVIK PGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGCKKSECITPNGSIPND KPFQNVNRITYGACPRYVKQSTLKLATGMRNVPEKQTRGIFGAIAAGFIEN GWEGMVDGWYGFGRHQNSEGRGQAADLKSTQA AIDQINGKLNRLIGKTN EKFHQIEKEFSEVEGRVQDLEKYVEDTKIDLWSYNAELLVALENQHTIDL TDSEMKNLFEKTKQLRENAEDMGNGCFKIYHKCDNACIGSIRNETYDH NVYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCVALLGFIMWA CQKGNIRCNICI
SEQ ID NO: 57	Amino acid sequence of NA protein of A/South Carolina/04/2017 MNPNQKIITIGSVSLTISTICFLMQIAILITTVTLHFKQYEFNSPPNNQVMLC EPTIERNITEIVYLTNTTIEKEICPKPAEYRNWSKPQCGITGFAPFSKDNSIR LSAGGDIWVTREPYVSCDPDKCYQFALGQGTTLNNVHSNNTVRDRTPYR TLLMNELGVPFHLGTKQVCIAWSSSSCHDGKAWLHV CITGDDKNATASII YNGRLVDSVVSWSKDILRTQES ECV CINGTCTVVM TDGNATGKADTKIL FIEEGKIVHTSKLSGSAQHVEECSCYPRYPGVRCVCRDNWKGSNRPVDI NIKDHSIVSSYVCSGLVGDTPRKNDSSSSSHCLNPNNEEGGHGVKGWAF DDGNDVWMGRTINETSRLGYETFKVVEGWSNPKSKLQINRQVIVDRGD RSGYSIGFSVEGKSCINRCFYVELIRGRKEETEVLWTSNSIVVFCGTS GTY GTGSWPDGADLNLMIH
SEQ ID NO: 58	Nucleotide sequence of PB2 viral segment of A/Alaska/06/2019 agcaaaagcagg tcaattatattcagcatggaaagaataaaagaacttcggaatctaattgcgcagctctgcactcgc gagatactgacaaaaaccacagtggaaccatattgccataaftaagaagtacacatcgaggagacaggaaaagaac ccgtcacttaggatgaaatggatgatggcaatgaaataccaatcactgctgacaaaaaggataacagaaatggttct gagagaaatgaacaaggacaaaacttatggagtaaaatgagtgatgctggatcagatagagtgatgatcaccttt ggctgtaacatgggtggaatagggaatggacccgtgacaagtacgggtccattacccaaaaagtgacaaaacttatttcga

	caaagtcgaaaggtaaacaatggaaccttggccctgtccattttagaaatcaagtaacgacagagagtaga cataaaccttggtcatgcagacctcagtgccaaaggccacaagatgtaattatggaagttgtttcccaatgaagt ggagccagaataactaacatcagaatcacagctaacaataactaaagagaaaaagaagaactccgagattgcaaa atttcccttgatggtcgatatactgtagagagagaacttggcgaaaaacaagatttccagttgctggcgga caagcagtatatactgaagtgttactatgactcaaggaaatgttgggaacaaatgtactactctgggaggtgt gaggaatgacgatgttgaccaagcctaattattgcccaggaacatagtaagaagagccgagtgtagcagat ccactagcatcttttggagatgtgcccagcacgcaaatgttgggaacaagatgtagacattctaaacagaaac ccgactgaagaacaagctgtgatatatgcaaggctgcaatgggattgaggatcagctccttcagcttgggtg ttacattttaaagaacaagcgggtcgtcagtcataaaagaagaagggttcttacaggcaatctccaaacattgaga ataagagtacatgaggggtatgaggttcacaatgttggggaagaagagcaacagctataactaagaaaagcaacc agaagactgtgtcaactcatagtgagtggaagagacgaacagtcataagccgaagcaataatctgtggccatgtgt ttcaacaagaagattgcatgataaaagcagttagaggtgacctgaatttgcacagagcaaatcagcgggtgaaccc catgcatcagcttfaaggcatgttcagaagaatgctgaaagtgtctttcaaaattggggagtgaacacatgcagat gtgatgggaatgttggagtgttaccagatatgactccaagcacagagatgcaatgagaggaataagagtcagca aatgtgggtgtgataactccagtagagaggggtgtgttagcattgacgtgtttgagagttcgagaccaac gtgggaatgtattattatctctgagggaagtcagtgaaacacagggaactgaagactgacaataacttattctgtc gatgatgtgggagattaacggtcctgagtcggtttgtcaatcctatcaatggatcaggaattgggaagctgtc aaaattcaatgtctcagaatctgcaatgtgtacaacaaaatggaattgaaaccgttcaatctttagtcccgaagc cactagaagtcaatagctgggttgcagaactctattccaacaaatgagagacgtacttgggaacttgcactgcc cagataataaagcttctccctttgagctgtctccaccgaagcaaaagcagaatgcagttctctcactgactgtgaatg gaggggagcagggatgagaatactgtaaggggcaattctctgtattcaactacaacaagaccactaaaaggctaa caattcttgaaaagatgccgacatttaattgaagaccagatgaagcacatccgagtgagtgccgcctctg agaggggtcctcattataggtaaagaagacagaagatacggaccagcattgaagcatcaatgaactgagtaacctgtc taaggggaaaaggctaatgtgctaattgggcaaggagacgtgtgtgttaagtaaacgaaaacgggactctagt atacttactgacagccagacagcgacaaaagaattcggatggccatcaattactgaatgttataaacgacct tgtttctact
SEQ ID NO: 59	Nucleotide sequence of PB1 viral segment of A/Alaska/06/2019 agcraaagcaggcaaacatttgaatggatgtcaatccgactctactgttctttaaagtccagcgcaaaatgccataa gcacaacattcccttatactggagatcctccatagcagccatggaacaggagcaggggtacactatggacacagtaac agaacacataactcagagagggggaagtggacaacaatacagaacactggggcgccccagctgaacccaat tgaatgaccactactcagagataatgaaccaagtggatagcacaaacagactgtgtcctggagctatgtgcttct tgaagaatccaccaggtatctttagaactcctgcttgaacaatggaagccgttcaacagacaagggtggaca aactaacccaaggtcgcagacttatgttggacattaaacagggaaccaaccgagcaactgcaatgccaacac catagaagtctttagatcaaacggattaacagctaatgaatcagggaaggctaatagatttctcaaggatgtgatgaa tcaatggataaggaggaaatggagataacaacacacttcaagaaaaaggagagtaagggaacaacatgaccaag aaaatggtcacacaagaacaatagggaagaaaaacaagggtgaataagagaggctacctaataagagctttag acattgaacacgatgaccaaggatgcagagagaggcaactaaaaagaagagctattgcaacaccgggagtgca aattagagggttcgtgtacttctgaaactttagctagaagcatttgcgaaaagcttgaaacatctgacttccggtg ggggtaatgaaaagaaggccaacttggcaaatgttggagaaaaatgatgactaattcacaagacacagagcttct ttcaaatcactggggacaacactaagtgaatgaaaatcaaaaccccgaatgttttggcgatgattacatacatca caaagaatcaacctgagtggttcagaacatctgagcatcgaccataatgttctcaacaaaatggcgaagactg ggaaaaaggatacatgttcgagagtaagagaatgaagatccggacacaaatccctgcagaatgctagcaagcattg acctgaagtatttcaatgaatcaacaagggaagaaaattgagaaaataaggcctcttctaataatggtgcacagcatatt gagccctggaatgatgtggcatgttcaacatgctaagtagagtttaggagtcctcgatactgaatcttggacaaaag aaatacaccaagacacatactgttggatgggtcctcaatcctcagacgatttgcctcctatagtgatgaccgaat catgaggggaatacaagcaggagtgatagattctataggacctgcaagttagtggaatcaacatgagcaaaaaga agtctctataataaaaacagggaacatttgaattcactagctttttatcgataggttggctaatgtttagcatggag ctgccaagtctggagtgctggaataaacgagtcagctgacatgagcattggagtaacagtgataaagaacaacat gataaataatgaccttggaccagcaacagcccaatggctctcaattgttcatcaagattacagatacagtatcg gtgccatagaggagacacacaatccaaacgagaagatcattcgagataaagaagctgtgggatcaaacccaatc

	aaggacagactattggtatcagatggggggccaaacttatacaaatatccggaaatttcacatccctgaagtctgtta aagtgggagctgatggatgagaattatcgggggaagactttgcaatccccctgaatccctttgtcagccataaagaaatt gaatctgtaaacaatgctgtagttaatgccagccatgggtccggccaaaagtatggaatatgatccgttgcaactaca catttcctggattccaaaaggaaacgtgtctattctaaacacaagccaaaagggggatcttgaggatgaacagatgtac cagaagtgtctgcaactgttctgagaaattttccctagtagtcatataggagaccgattggaaatttctagcatgtgtgga ggccatgtgtctagggcccgtatgatccagaattgtactcgaagtctggaaggattaagaaggaaagaggtctctg agatcatgaagaatctgttccaccattgaagaactcagacggcaaaaataatgaatttagctgtctctcatgaaaaaat gccttttttctact
SEQ ID NO: 60	Nucleotide sequence of PA viral segment of A/Alaska/06/2019 agcaaaagcagggtactgattcagaatggaagattttgtgcgacaatgcttcaatccgatgattgtcgaaacttgcagaa aaagcaatgaaagagatgtggggaggtatctgaaaattgaaactaacaatttgcagcaatatgcactcatttggagggtg tgtttcatgtattcagatttcatttcataatgaacaggcgaaatcaatagtagtagaactgtacgactcaaatgtcactg ttaaagcacagatttgaataatcggatggagacagaaacagtcctgtgcagtagtaaacagatactgcaca ctactgtgagctggaaaaaccgaagtgttctaccggattttagattacaaagaaacagattatcgaatttggagtg caaggaggaagttccacatatattaccttggaaaaggccaaataagattaaatctgagaaacacacacattcacattttt attcactggggagggaattggccacaaggcagactacacitctgcagcaggaaagcagggtcaggatcaaaacca ggctgtttaccataagacaagaattggcccaacagaggcctctgggattccttctcagtcgcgaaagaggcgaaga aacaattgaagaaaaatttgaatcacaggaaactatgcgaggcttgcgaccaaagtctccaccgaacttctctg ccttgagaattttagagcatatgtgtgattggaaccgaaccgctgcattgagggcaagcttctcaaatgtccaaa gaagtgaatgcccaattgaaccttttctgaagacaacaccaagaccaatcaacttcttagtggacctctgtttatc agcgtgccaaagtctctctgatggatgctttgaaattgagcattgaagaccaagtcacgaaggagaaaggatccca ttatatgatgcgatacagtcataaaaacattcttggatggaagaaaccttatatagtcacacacagaaaagggaa taaatcaaatcctgctgtcatggaagcaagtagctcagaattgcaggacattgaaatgaggagaagattccaa gaacaaaaaacatgaagaaaacgagtcactgaagtggctcttgggtgaaaacatggcaccagagaaggtagact ttgaaaaactgcagagacataacgctattgaaacataatgatagtgaagaacctgaattaaagctcatttcaagctgact acagagtgtattcaacaaggcctgtgagctaaactgattcagctctgataagatcgcgatgagattggagaggacatg ccccattgaacacattgcaagtatgagaaggaaattttcagacagagggttccattgtagagctactgaatacat aatgaaagggggtatacattaacactgcctctcctaatgcacactgtgcagcaatggacgattttcaactaattccatg ataagcaagtgcagaactaaagagggaaggcgaaaaaccaatttatatggattcatataaagggaagatctcatct gaggaaatgacacagacgtgtgtaaaactttgtgacatggagtttctctcagatccgagacttgagccacataaatg ggagaataattgtgtccttgagataggagatattgtactaaggagtgtctataggccaaattcaaggccgatgttctgt atgtgaggacaaaacggaaatcaaaaggtcaaaatgaaatggggaattgagatgagacgttgcctctcagtcact ccagcagattgagagcatgattgaagccgagtcctcagttaaagaaaaagacatgaccaagagtttttggagata aatcagaagcatggccattgggggagtcccccaaaggagtggaagaagggttcattggaaaagtctgtaggactc attagctaaagtcagtattcaatagcctgtatgcataccacaattggaaggattttcagcagagtcagaagaaactgtc cttatgttcaggctcttaggggacaacctgaacctgggaccttggcttctgggggctatatgaagcaattgaggagt gctgatattgatccctgggtttgtcctaattgtctgtgttcaactcttctcgtacacatgcattaaaatagttatagca gtgtgtctatttgtatccgtactgttccaaaaaaagctacattgttctact
SEQ ID NO: 61	Nucleotide sequence of NP viral segment of A/Alaska/06/2019 agcaaaagcagggttgataatcactcactgagtgacatctaaatcatggcgtcccaaggcaccaaaacggtcttatga acagatggaactgatggagatgccagaatgcaactgagattagggtcatccgtcgggaagatgattgatggaatt gggagattctacatccaatgtgactgaacttaaaactcagtgatcgaaggacggttgatccaaaacagcttgaca atagagaaaatggtactctgtctttgatgaagaaggaaataaatacctggaagaacacccagcgcgggggaaag atcccaagaaaactgtggggggccaaatatacaggagagtcgatgggaaatggatgggggaactcgtctcttatgaca agaagaataaaggcgaaatctggcggcgaaggccaaatggtgaggatgctacatctgtgttcaactcacataatgatt ggcattccaattgaaatgatgcaacataccagaggacaagagctctgtctcggactggaatggatccagaatgtgct ctctgatgcagggtctgactctccctagaagggtccggggctgcagggtgtcgcagtcgcaagggaatcggaaacaatggt gatgcaactatcagaatgttcaacgggggctatcaacgatcgaaaattttgagagtgagaatgggcgggaaaca agaagtgtcttagtagagaattgtcaaaccttttaaggaaaaattcaaacagctgcacaaagatgcgaatgttgatca aatttagagaaaagtcggaaccaggaacgtctgaatcgaagatctcatatttttagcaagatgtcactgaataca

	aggatcagttgctcacaaatcttgcctacctgacctgcatatggacctgcagtatccagtggttacgactttgaaaaa gagggatattccttgggtgggaatagaccttcaactactccaaaatagccaaatatacagcttaatcagacctaatg agaatccagcacacaagagtcagctggttgatggcatgccattctgctgcatttgaaagttaagattgtaagctt catcagagggacaaaagtatctcctcggggaaactgtcgaactagaggagtagacaattgcttcaalagagaactg gataatattggatcaagcactctgaactgagaagcgggatactgggccataagaaccaggagtgagggaacact aatcaacagagggcctcgcaggccaaaccagtgtgcaacctacattttctgtacaagaatctccattgaaaag tcaacctcatggcagcattcactggaaatacggagggaagaactcagacatgagggcagaatcataaggtga tggaagggtgcaaacagagaagtgtcattcgggggaggggagtttgcagctctcagacgagaaggcagcg aaccgatcgtgcctctttgatatgagtaacgaaggatcttatttctcgagacaatgcagaaggtacgacaatt aaggaaaaataccttgtttctact
SEQ ID NO: 62	Nucleotide sequence of NS viral segment of A/Alaska/06/2019 agcaaaagcagggtgacaaagacataatggattccaactgtgtcaagttccaggtagattgctttcttggcatat ccggaacaagtgttagaccaaaaactaagtgtatccccattctcgtatcggttcgagatcagaggtccctaa ggggagaggcaatactctcggtctagacatacaaaagcagcccccattgctggaagcaaattatagaaaagattct gaaagaagaatctgataggcacttaaaatgacctgggtctcaaacctgcttcgcatataactgacatgactatt gaggaaactgtcaagaactgggtcatgtctaatgcccagcagaagaaggaggacctctttgcatcagaatggacc aggccttccacggaagaggagcaaatgttggcgaaatctcaccattgcttctttccaggacatactattgaggatg tcaaaatgcaattgggtcctcatcgaggagctgaatggaatgataacacagttcagttctctaaaaatctacaga gattcgttggagaagcagtaattgagaatgggggacctccactactccaaaacagaaacgggaatggcgagaa cagctaggtcagaagtgtgagagataagatggctaattgaagaggtgagacacagattgagaacaactgaaaata gctttgaacaataacattcatgcaagcattacaactactgttgaagtggacaggagataagaactttctcatttcag cttatttaataataaaaaacacctgtttctact
SEQ ID NO: 63	Nucleotide sequence of M viral segment of A/Alaska/06/2019 agcaaaagcaggtagatattgaaagatgagccttctaaccgagggtcgaacgtatgttctctatcgtttccatcaggc cccctcaagccgagatcgcgcagagacttgaaagtgtcttctggtggaaaaacacagatcttgaggtctcatgga atggctaagacaagaccaattctgtcactctgactaaggggattttaggggtttttttacgctcaccgtgccagtg agcaggagactgcagcgttagacgtttgtccaaaatgcctcaatgggaatggagacccaataacatggacaaaag cagttaaactgtataggaaacttaagaggagataacgttcctggtggccaaagaaatagctctcagttattctgtct gtgcacttgcagttgcatgggctcatatacaatagaatgggggctgttaaccactgaagtggcatttggcctgtgt gtgcaacatgtgagcagattgtgattccagcacaggtctcataggcagatgggtgcaacaaccaatcattaata aaacatgagaacagaatgttttggccagcactacagctaaaggctatggagcaaatggctggatcaagttagcagg cagcggaggccatggagatagctagtcaggccaggcagatggtgcaggcaatggagagccattgggactcactcta gttccagtactgttctaagagatgattcttgaataattgcagacctatcagaaacgaatgggggtgcagatgcaac gattcaagtgtacgttattgttgcgcaaatatcattgggacttgcacttgatattgttgattcttgatcttttttca aatgctgtctgactctcaaacacggccttaaaaggcccttctacggaaggagtacctgagcttatgaggga gaatctgaaagggaacagcagaatgtgtgtgctgacgacagtcattttgtcagcatagagttggagttaaaaac taactgtttctact
SEQ ID NO: 64	Nucleotide sequence of HA viral segment of A/Alaska/06/2019 agcaaaagcaggggataattctattaacctgaagactatcattgctttgagctgcattctatgtcaggttttgcctaaa aaattccttgaaatgacaatagcacggcaacgtgtgcttgggcaccatgcagtaccaaacggaacgatagtga aacaatcacgaatgaccgaattgaagttaactaatgctactgagctgttcagaaactcctcaataggtgaaatagcga cagtcctcatcagatcctgtatggagaaaactgcacactaatagatgctctattgggagacctcagttgtatgcttt caaaataagaaatgggaccttttctgtgaacgggaacaaagcctacagcaactgttaccttatgtatgtccggattatg catccttagatcactagttgctcatccggcacactggagtttaacaatgaaagcttcaattgggctggagtcactca aaacggaacaagtcttctgtcataaggggatctaaaagtgttcttttagtagattaaattggtgaccacttaaacct caataaccagcattaaacgtgactatgccaacaatgaacaatttgacaattgtacatttgggggtgttcaccacccg gttacggacaaggacaaaatctccgttatgcacaatcatcagggaagaatcacagatctaccaaaagaagccaac aagctgtaatcccgaatcgtatgtagaccagaataaggatccctagcagaataagcatctatttgacaatag taaaaccaggagacatacttttgattaacgacaggggaatctaatgtctcctaggggttacttcaaaatcacgaagtgg

[illegible]

SEQ ID NO: 67	Amino acid sequence of PB1 protein of A/Alaska/06/2019 MDVNPTLLFLKVPQAQNAISTTFPYTGDPPYSHGTGTGYTMDTVNRTHQY SERGKWTNTTETGAPQLNPIDGPLPEDNEPSGYAQTDVCLEAMAFLEESH PGIFENSCLTMEAVQQTRVDKLTQGRQTYDWTLNRNQPAATALANTIE VFRSNGLTANESGRLIDFLKDVMESEMDKEEMEITTHFQRKRRVRDNMTK KMVTQRTIGKKKQVRNKRGYLIRALTNTMTKDAERGKLRRAIATPG MQIRGFVYFVETLARSICEKLEQSGLPVGGNEKKAKLANVVRKMMTNS QDTELSFTITGDNTKWNENQNPRMFLAMITYITKNQPEWFRNLSIAPIMF SNKMARLGKGYMFESKRMKIRTQIPAEMLASIDLKYFNESTRKKIEKIRP LLIDGTASLSPGMMMGFMNMLSTVLGVSLNLGQKKYTKTTYWWDGLO SSDDFALIVNAPNHEGIAQGVDRFYRTCKLVGINMSKKKSYINKTGTFEF TSFFYRYGFVANFSMELPSFGVSGINESADMSIGVTVIKNNMINNDLGA TAQMALQLFIKDYRYTYRCHRGDTQIQTRRSFEIKKLWDQTSRTGLLV SDGGPNLYNIRNLHIPEVCLKWELMDENYRGRLCNPLNPFVSHKEIESVN NAVVMPAHGPAKSMEYDAVATTHSWIPKRNRSILNTSQRGILEDEQMYQ KCCNLFKFFPSSSYRRPIGISSMVEAMVSRARIDARIDFESGRIKKEEFSEI MKICSTIEELRRQK
SEQ ID NO: 68	Amino acid sequence of PB1-F2 protein of A/Alaska/06/2019 MEQGQGTLTWTQSTEHINTQRGGSGQQIQKLGRPSSTQLMDHYLRIMNQV DMHKQTVSWRLWPSLKNPTQVSLRTPALKQWKPFNRQGWNT
SEQ ID NO: 69	Amino acid sequence of PA protein of A/Alaska/06/2019 MEDFVRQCFNPMIVELAEKAMKEYGEDLKIETNKFAAICTHLEVCFMYS DFHFINEQGESIVVELDDPNALLKHRFEIIEGRDRTMAWTVVNSICNTTGA GKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKANKIKSENTHIHIFSFTG EEMATKADYTLDEESRARIKTRLFTIRQEMANRGLWDSFRQSERGEETIE EKFEITGTMRRLADQSLPPNFSCLENFRAYVDGFEPNGCIEGKLSQMSKE VNAQIEPFLKTTTPRIKLPSPGPCYQRSKFLMDALKLSIEDPSHEGEGIPL YDAIKCIKTFFGWKEPYIVKPHEKGINSNYLLSWKQVLSLQDIENEEKIP RTKNMKKTSQLKWALGENMAPEKVDNFENCRDISDLKQYDSEEPRLSLS SWIQSEFNKACELTDSVWIELDEIGEDVAPIEHASMRNRYFTAENVSHCR TEYIMKGVYINTALLNASCAAMDDFQLIPMISKCRCKEGRRTNLYGFII KGRSHLRNDTDVVNFVSMFSLTDPRLPHKWEKYCVLEIGDMLLRSAI GQISRPMFLYVRTNGTSKVKMKWGMEMRRCLLQSLQQIESMIEAESSVK EKDMTKEFFENKSEAWPIGESPKGVEEGSIGKVCRTLLAKSVFNSLYASP QLEGFSAESRKLILLIVQALRDKLEPGTFDLGGLYEAIIECLINDPWVLLNA SWFNSFLTHALK
SEQ ID NO: 70	Amino acid sequence of NP protein of A/Alaska/06/2019 MASQGTKRSYEQMETDGDQRNATEIRASVGKIDGIGRFYIQMCTELKL SDHEGRLIQNSLTIEKMVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRR VDGKWMRELVLVDKEEIRRIWRQANNGEDATSGLTTHIMIWHSNLNDAT YQRTALVRTGMDPRMCSLMQGSTLPRRSGAAGAAVKGIGTMVMELIR MVKRGINDRNFWRGENGRKTRSAYERMCNILKGFQTAQGRAMVDQV RESRNPNGNAEIEDLIFLARSALILRGVVAHKSLCPACAYGPAVSSGYDFEK EGYSLVGIDPFKLLQNSQIYSLIRPNENPAHKSQVLVWMACHSAAFEDLRL LSFIRGTKVSPRGKLSTRGVQIASNENMDNMGSSTLELRSGYWAIRTRSG GNTNQQRASAGQTSVQPTFSVQRNLPFEKSTIMAAFTGNTGRTSDMRA EIIRMMEGAKPEEVSRGRGVFELSDEKAANPIVPSFDMSEGSYFFGDN AEEYDN
SEQ ID NO: 71	Amino acid sequence of NS1 protein of A/Alaska/06/2019

	MDSNTVSSFQVDCFLWHIRKQVVDQKLS DTPFLDRLRRDQ RSLRGRGNT LGLDIKAAATHAGKQIEKILKEESDEALKMTMVSTPASRYITDMTIEELSR NWFMLMPKQKVEGPLCIRMDQAIMEKKIMLKANFN VIFGRLETIVLLRA FTEEGAIVGEISPLSPFPGHTIEDVKNAIGVLIGGLEWNDNTVRVSKNLQR FAWRSSNENG GPPLTPKQKRE MARTARSEV
SEQ ID NO: 72	Amino acid sequence of NEP protein of A/Alaska/06/2019 MDSNTVSSFQDILLRMSKMQLGSSSEDLNGMITQFESLKIYRDSLGEAVM RMGDLHLLQNRNGKWREQLGQKFEEIRWLIEEVRHRLRTTENSFEQITF MQALQLLFEVEQEIRTF SFQLI
SEQ ID NO: 73	Amino acid sequence of M1 protein of A/Alaska/06/2019 MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEALMEWLKTRP ILSPLTKGILGFVFTLTVPSERGLQRRRFVQNALNGNGDPNNMDKAVKLY RKLKREITFHGAKEIALSYSAGALASCMGLIYNRMGAVTTEVAFGLVCA TCEQIADSQHRSHRQMVATTNPLIKHENRMVLA STTAKAMEQMAGSSE QAAEAMEIASQARQMVQAMRAIGTHPSSSTGLRDDLLENLQTYQKRMG VQMQRFK
SEQ ID NO: 74	Amino acid sequence of M2 protein of A/Alaska/06/2019 MSLLTEVETPIRNEWGCRCNDSSDPLIVAANIIGILHLILWILDRLFFKCV C RLFKHGLKRG PSTEGVPESMREEYRKEQQNAVDADDSHFVSIELE
SEQ ID NO: 75	Amino acid sequence of HA protein of A/Alaska/06/2019 MKTIIALSCILCQVFAQIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEV TNATELVQNSSIGEICDSPHQILDGENCTLIDALLGDPQCDGFQNKKDWL FVERNKAYSNCYPYDVPDYASLRSLVASSGTLEFNNE SFNWAGVTQNGT SSS CIRGSKSSFFSRLNWLTHLNSKYPALNVTMPNNEQFDKLYIWGVHHP GTDKDQISLYAQSSGRITVSTKRSQQA VIPNIGSRPRIRDIPSRIYWTIVK PGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGCKKSECITPNGSIPND KPFQNVN RITYGACPRYVKQSTLKLATGMRNVPERQTRGIFGAIAGFIEN GWGLVDGWYGRFHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTN EKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDL TDSEMNKLF EKTKKQLRENAEDMGNCGFKIYHKCDNACMG SIRNGTYD HNVYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCVVLLGFIMW ACQKGNIRCNICI
SEQ ID NO: 76	Amino acid sequence of NA protein of A/Alaska/06/2019 MNPNQKIITIGSVSLTISTICFFMQIAILITTVTLHFKQYEFNSPPNNQVMLC EPTIIERNITEIVYLTNTTIEREICPKPAEYRNWSKPQCGITGFAPFSKDNSIR LSAGGDIWVTREPYVSCDPDKCYQFALGQGTINN VHSNNTARDRTPHR TLLMSELGV PFHLGTKQVCIAWSSSSSCHDGKAWLHVCITGDDKNATASF IYNGRLVDSVVSWSKDILRTQESECV CINGTCTV VMTDGNATGKADTKI LFIEEGKIVHTSKLSGSAQHVEECSCYPRYPGVRCVCRDNWKG SNRP IVD INIKDHSIVSSYVCSGLVGDTPRKTDSSSSSHCLNPNNEKGGHGVKGWAF DEGNDVWMGR TINETSRLGYETFKVVEGWSNSKSKLQINRQVIVDRGDR SGYSGIFSVEGKSCINRCFYVELIRGRKEETEVLWTSNSIVVFCGTS GTYG TGSWPDGADLNL MHI
SEQ ID NO: 77	Nucleotide sequence of PB2 viral segment of A/Darwin/11/2021 agcgaagcaggtcaattatattcagcatggaagaataaaagaactacggaatctaattgcgcagtc ccgcactcg cgagatactgacaaaaccacagtg gaccatatggccataaftaagaagtacacatcggggagacaggaaaagaa cccgtcacttagaatgaaatg gatgagcaatgaaatccaattactgctgacaaaaagataacagaaatggttc ggagagaaatgaacaaggacaaactctatggagtaaaatg agtgatgctggatcagacagagtgatggtatcacctt tgctgtaacatggtggaataggaaatggaccgtgacagatacggtcattacccaaaagtgtacaaaactatttcg acaaagtcgaaagggttaaacatggaacctttggccctgtccattttagaaatcaagtc aaaaatacgaagaagagtag

	acataaatcctggtcatgcagacctcagtgccaagaggcacaagatgtaattatggaagtgttttccaatgaagt gggagccagaataactaacatcagaatcacagctaacaataactaaagagaaaaagaagaactccgagattgcaa aatttctcccttgatggtcgcatacatgctagagagagaacttgtgcggaaaacaagatttctccagttgctggcgga acaagcagtaatacatggaagtgttacttgaactcaaggaaacgtgttgggaacaaatgtacactccaggtggagga gtgaggaatgacgatgttgacaaagcctaattatgtgcggccagggaacatagtaagaagagccgaggtgacagcag atccactagcatcttattggagatgtgccacagcacgcaaattggcggaacaaggatgtgagacattcttagacaga acccgactgaagaacaggctgtggatataatgcaaagctgcaatgggattgagaatcagctcatctttagccttgggtg gtctcacattaaaagaacaggcgggtcgtcagtaaaagagaggaggttctacaggcaatctccagacattg agaataagagtagaggggtacaggagttcacatgtgtgggaaaagagcaacagctatactagaagaagca accagaagactgtgtcaactcatagtgagtggaagagacgaacagtcattagccgaagcaataatcgtggccatgg tgttttcacaagaagattgcgtgataaaagcagttagaggtgacctgaatttgtcaacagagcaaatcagcggttgaa ccccatgcatcagcttttaaggcattttcagaagatgcgaaagtactcttcaaaattgggaggtgaacacatcgac agtgtgatgggaatgtgtgagatattaccagatatgactccaagcacagagatgtcaatgagaggaataagagtcag caaatgtgggtggtgataaactccagtagagaggggtgtgttagcattgatcgggttttagagagttcagacca acgtgggaatgtattatctctcagggaggtcagtgaaacacagggaactgaaagactgaccataacttattcatca tcgatgatgtggagattaacggccctgagtcgggtttgtcaatactatcaatggatcatcaggaaattgggaagctg tcaaaattcaatgtgtcagaacctgcgaatgtgtacaacaaatggaattgaaacatttcaatcttagtcccaag gccactagaagccaatacagtggtttgtcagaactctattccaacaaatgagagacgtacttgggacatttgacact gcccagataataaagcttctcccttttcagctgctccaccgaagcaaaagcagaatgcagttcttctactgactgtga atgtgaggggtacagggtatgagaatactgttaaggggcaatttctctgtattcaactacaacaagaccactaaaagg ctaacaattcttgaaaagatgcggcacttaattgaagaccagatgaagcacatccggagtgaggtccgctgt cttgagaggggttctctattataggttaaggagacagaagatacggaccagcattaagcatcaatgaactgagtaacc ttgcaaaaggggaaaaggctaatgtgtaattgggcaaggagacgtgtgtgtgtaatgaaacggaaacgggactc tagtatacttactgacagccagacagcgacaaaagaattcggatggccatcaatatactgaaatgtttaaaccg acctgtgttctact
SEQ ID NO: 78	Nucleotide sequence of PB1 viral segment of A/Darwin/11/2021 agcgaagcaggcaaacatttgaatggatgtaateccgactctattgttctaaaagttccagcgcaaaatgccataa gcacaacattcccttatactggagatcctccatacagccatggaacaggggacagggtacactatggacacagtcac agaacacaccaatactcagagaggggggaagtggacgacaaatacagaactgggctccccagctcaacccaat tgatggaccactacctcagagataatgaaccaagtggatgacacaacagactgtgtcctggaggtctatggcccttct tgaagaatccccaccaggtatctttgaaaactcctgcctgaaacaatggaagccgttcaacagacaagggttagaca aactgacccaaggctcgcagacttatgactggacattaaacaggaaccaaccggcagcaactgcattagccaacac catagaagtcttagatcgaacggattaacagctaataatcaggaaggctaataagatttctcaaggatgtgatgga atcaatggataaggagggaatagagataacaacacacttcaaaagaaaaggagagtaagggaacaacatgacca gaaaatgtgcacacaagaacaatagggaagaaaaacaagagtgataagagaggctacctaataagagctct gacattgaacacgatgaccaaggatgcagagagaggcaattaaaaagaagggtctattgcaacaccggggtgc aaatttagaggatctgtgtatttgcgtgaaactttagctagaagcatttgcgaaaaactgaacaatctgacttccggtg ggggtaatgaaaagaggccaactcgcgaatgtgtgagaaaaatgatgactaattcacaagacacagagcttct ttcacaatcactggagacaacactaagtggaatgaaaatcaaaaccccgaaatgttttggcaatgattacatatca caaagaaccaacctgaatgttcagaacatcctgagcatcgaccaataatgttctcaacaaaatggcaagactg ggaaaaggatacatgttcgagagtaagagaatgaagctccggacacaaatacctgcagaatgtctagcaagcattg acctgaagtatttcaatgaatcaacaagggaagaaattgagaaataaggcccttcttaatagatggaacagcatcatt gagccctggaaatgatgaggtcatgttcaacatgctaagtacagttttaggagtcctgatactgaaatcttgacaaaag aaatacaccaagacaacgtactgtgtggatgggtccaatcctcagacgatttgcctcatagtgtaagcaccat catgagggaataacaagcaggagtgatagattctataggacctgcaagtttagtggaatcaacatgagcaaaaaga agtcctatataataaaacaggacatttgaaactagcttctttatcgatatgatttggctaattttagcatggag ctgccaagtgttgaggtctggagtaaacgagtcagctgacatgagcattggagtaacagtgaataagaacaacat gataataatgaccttggaccagcaacagcccaatggctctcaattgttcaaaagattacagatacagtatcg gtgccatagaggagacacacaatccaacaagaagatcattgaaataaagaagctgtgggatcaaacccaatca aagacaggattattgtatcagatggggacaaactatataatccgaatcttcaatccctgaagtctgcttga

	agtgaggagctgagacgacaattatcgagggaagacttgaatccccgaatcccttgcagccataaagaaatg aatctgtaaacatgctgtagtaatccagcccattggtcggccaaaagtatggaatatgacggttgcgactacac actcctggattccaaaagggaaccgctctattctaaacacaagccaaaggggaattcttgaggatgaacaaatgtac cagaaaatgctgcaacttctcgagaaattttccctagtagttcatatagagagaccgattggaaattctagcatgttgga ggccatggatctagggcccgattgatgccagaattgacttcgagcttggaaggattaagaagggaagagttctctg agatcatgaagatctgtccaccattgaagaactcagaccggcaaaaataatgaatttagctgtcctcatgaaaaaat gccttgtttctact
SEQ ID NO: 79	Nucleotide sequence of PA viral segment of A/Darwin/11/2021 agcgaagcagggtactgattcaaaatggaagatttgtgcggcaatgctcaaccgatgattgcaactgcagaa aaagcaatgaagagatggagaggatctgaaaatgaaaccaaaaattgacgaatatgcactcacttggagggt gtgtttcatgtattcagattccatttcatcaatgaacaaggcgaaatcaatagtagtagaacttgacgatccaaatgcact gttaaaacacagattgaaataatcagagggagagacagaacaatggcctggacagtagtaaacgatctgcaac actactggagctggaaaaccgaagtgtttacgggatctgtatgattacaagaaaacagatcactgaaattggagtga caaggagagaagtcacatatattaccttgaaaaggccaataaaataaatacagagacacacattcactgtttttc attcactggggagggaatggccacaaggggcagactacactctcgacgaggaaagcaggggctaggtataaaacca ggctatttaccataagacaagaatggccaacagaggcctctgggattcttctgcaatccgaaaggaggcgaagaa acaattgaagaaaaatttgaatcacagggaactatgcgaggcttgcggaccaagttctccaccgaacttctctgc cttgagaaatttagagcctatgtgagtgattcgaaccgaacggctgcatgaggggcaagctttctcaaatgtccaag aagtgaatgccaaattgaacttttctgaagacaacccaagaccaatcaacttctagtggactcctgttatca gcgatccaaattctcctgatggatgcttgaattgagcatgaagaccgaagtcaggaaggagaaggatccatt atatgatgcaatcaagtgcataaaaacatttcttgatgaaagaacctatatagtcaaacacacgaaaaggggat aaattcaaatcctgctgtcatggaagcaagtactgtcagaattgcaggacattgaaaatgaggagaagattccaag aactaaaaacatgaagaaaacgagtcactgaagtgggctcttggtgaaaacatggcaccagagaaggtagacttt gaaaactgcagagacataagcgattgaagcaatgatcagtggaagaacctgaattgaaggacacttcaagctgggt acaaagtgaattcaacaaggcctgtgagctaactgattcagctctgtagagctcgatgaattggagaggacgtag cccaattgaacacattgcaagcatgagaagggaattacttcacagcagagggtgtccattgcagagctactgaatac ataatgaagggggtatacataactgcccgtcctaatgcacactgtgcggcaatggacgattttcaactaatcccat gataagcaagtgcagaactaaaggagggaaggcgaaaaaccaatttatatggattcatcataaagggaagatctc tgagggaatgacacagacgtggttaactttgtgagcatggagtttcttcacagatccgagactgaaccacataaat gggagaaaattgtgtccttgatagtaggatatgttactaaggagtgccataggccagatttcaaggccgagtgttctt tctgtgaggacaacacggaacatcaaaagtcaaaatgaaatggggaatggagatgagacgttgcctccttcagtca ctccagcagatcgagagcatgattgaagccgagtcctcagttaaagagaagacatgaccaagagttttttgagaa taaatcagaagcatggccattggggagtgccccaaaggagtggaagaagggtccattgggaaggtctgtaggact ctattggctaagtcatgtattcaatagcctgtatgcatcaccacaattggaaggattttcagcagagtcagaaaaactgct ccttattgtcaggctcttagggacaaactcgaaacctgggaccttgcatttgggggctatatgaagcaattgaggag tgccgtgattaatgatccctgggttttgcataatgcatcttggttcaactccttctgacacatgcatlaaatagttatagc agtgctactatttgcacccgtactgtccaaaaaagtaccttgtttctact
SEQ ID NO: 80	Nucleotide sequence of NP viral segment of A/Darwin/11/2021 agcaaaagcagggttgataatcactcactgagtacatcaaaatcatggcgcccaaggcaccacacggctttatga acagatggaaactgagggatcgccagaatgcaactgagattagggcatcgctgggaagatgattgatggaatt gggagattctacatccaaatgtgcactgaacttaactcagtatcatgaaggacgggtgatccaaaatagcttgaca atagagaaaatgggtactctgtctttgatgaagaagggaataataacctgggaagaacacccagcgcgggggaag atcccaagaaaactggggggcccatatacagaagagtcgatggaaaatggatgagggaactcgtctttatgacaa agaggaataaaggcgaatctggcgccaagccaacaatggtaggagtgctacatctggtctaacccacatgagattt ggcattccaatttgaatgatgcgacataccagaggacaagagcttcttgcggactggaaatggatccagaatgtgct ctctgatgcagggtgctgactcctctagaaggfccggagctgcagggtctgcagtcagggaatcggaacaatgggt gatggaaactgatcaggatgatcaaacgggggatcaacgatcgaaattttggagagggtgagaatggcggaac aagaagtgttatgataaatgtcaacattctaaaggaaaatttcaaacagctgcacaaagagcaatgggtgatca agttagagaagtcggaaccagggaacgctgagatcgaagatctcatttttagcaagatctgcactgatattgag aggatcagttgctcacaatcttgctacctgctgtgcatatggacctgcagtatccagtggttacgacttgaacaaa

	gagggatattcttgggtgggaatagaccctttcaaaactactcaaaatagccaaatafatacagcttaatacagacctaat agaatccagcacacagagtcagctgtgtgtagtgcatgccattctgctgcatttgaagatttaagattttaagtttc atcagaggggacaaaagtatctctcgtgggggaaactgtcaactagaggagtacaaattgcttcaaatgagaacatgg ataatattggatcaagcactcttgaactgagaagcgggtactcggccataaggaccaggagcggaggaaacacta atcaacagagggtctccgaggccaaaccagtgtgcaacctactgttctgtacaaagaacatccatttgaag tcacccatcatggcagcattcactggaatacggagggaagaactcagacatgaggggcagaatcataaggatga tggagggtgcaaaaccagaagaagtgtctcctcgggggaggggagtttctgagctctcagacgagaaggcagcg aacccgatcgtgccctctttgtatagtagtaacgaaggatcttattctcggagacaatgcagaagagtacgacaatt aagaaaaaatacccttgttctact
SEQ ID NO: 81	Nucleotide sequence of NS viral segment of A/Darwin/11/2021 agcaaaagcagggtgacaaagacataatgattccaactgtgtcaagttccaggtagattgcttcttggcattat ccggaaacaagttgtggaccacaaaactcagatgatgccccattctcgtatcgcttccagatcagaggtccctaa gggggaaggagcaatactcgtgtagacataaactcagccacctattgtggaaagccagcttagaaaagattctg aaaggagaatctgtagtggccacttaaatgacctggtctcaacacctgtcttcgcgatacataactgacatgactatt gagggaattgtcaagaactgtgtcattgctaatgcccaagcagaaggtgggaaggaccttctgcatcagaatggacca ggcaatcatggagaaaaacatcatgtttaaagcgaatttcaatgtgattttggccggcttagagaccatagattacta agggtcttcaccgafgaggggagcaattgttggcgaatctaccattgcttcttccaggacatactattgaggatgt caaaaatgcaattgggtctctatcggaggacttgaaatggaatgataacacagttcaggtctctaaaatctacagag attcgttggagaagcagtcattgagaatgggggacctccacttactcctcaaaacagaaacgggaatggtcgagAAC agctaggtcagaagtgtgaagagataagatggctgattgaagggtgagacacagattagaacaaactgaaaatag ctttgaacaaataacattcatgcaagcattacaactactgttgaagtgggaacaggagataagaactttctcatcttcagct tatttaafgataaaaaacaccttgttctact
SEQ ID NO: 82	Nucleotide sequence of M viral segment of A/Darwin/11/2021 agcaaaagcaggtagatattgaagatgagccttctaccgaggtcgaacgtatgttctctctatcgttccatcaggc cccccaagccgagatcgccgagagacttgaagatgtcttctgtgggaaaaacacagatcttggagctctcatgga attgttaaagacaagaccacttctgaccttgcataagggtatttttaggttttttttttttttttttttttttttttt agcgaggactgcagcgttagacgttctcctcaaatgggaatggagaccacaaataacatfgacaaag cagttaaactgtataggaaactaagaggagataacgttccacggggccaaagaatagctctcagttattctgctg gtgcacttgcagggtgcatgggctcatatacaataggatgggggtgtgaacctgaagtggcatttggcctgtgt gtgcaacatgtgagcagattgtgattccagcacagggtctcataggcagatgggtggcaacaaccaatccattata aaacatgagaacagaatgttttggccagcactacagctaaggctatggagcaaatggctgtgctcaagtggagcaag cagcagaggccatggagattgttagtcaggccaggcagatgggtgaggaatggagccattgggactcatccta gttcagcaactgtgtcagaatgattcttgaataattgacagacatcagaaacgaatgggggtgcagatgcaac gattcaagtaacccgtgtgtgtccgcgaatatcattgggactgtgactgtatattgattcttctgctctttttt aaatgcgtctatgcactctcaaacacggccctaaagaggcccttctacgggaaggtgtacctgagctctatgaggga gaataccgaaggaacagcagaatgctgtggatgctgacgacagctattttgtcagcatagaattggagtaaaaaac taccttgttctact
SEQ ID NO: 83	Nucleotide sequence of HA viral segment of A/Darwin/11/2021 agcaaaagcaggggataattctattaacctgaagactatcattgcttggagcaacattctatgtctgttttgcctaaa aaataacctggaaatgacaatagcacggcaacgtgtgcttgggtaccatgcagtagcaaacgggaacgatgtgaa aacaatcacaaatgaccgaattgaagttactaatgctacigagtgtggtcagaattcatcaataggtgaaatattcgac agtctcatcagatccttgatggagggaactgcacactaatagatgctctattgggggaccctcagttgtgacggcttcc aaaataagggaatgggaccttttgtgaacgaagcagagccaacagcaactgttacccttatgatgtgcccggattatg cctcctttaggtcactagttgctctatccggcacactggagttaaaaatgaagcttcaattggactggagtcacaaca aaacgggaacagttctgctgctgataagggtatctagtagtatttttttagtagattaattgtgtgaccagcttaaca acatataccagcacagaacgtgactatgccaacaagggaacaatttgacaattgtacatttgggggggttcaccacc cggataccggacaagaaccaaactctcctgttgtctcaatcatcagggaagaatcacagtatccacaaaagaagccaa caagctgtaatcccaatatcgatctagaccagaataagggtatcccttagcagaataagcatctattggacaata gtaaaacccgggagacatacttttgattaaacgacacagggaatctaattgtctcctagggtttacttcaaaatacgaatg ggaaaaagctcaatgaatgaatgacatgacacccattggcaaatgtaactgaatcctacatcccaaatgaagcatt

	ccaatgacaaaccgttccaaatgtaaacaggatcacatacggggcctgtcccagatatgttaagcaaaagcaccctg aaattggcaacagggaatgcgaatgtaccagagaacaaaccagaggcataattggcgcaatagcgggttcacag aaaatggatggagggaatgggtgatgggttacgggttcaggcatcaaaattctgagggaagaggacaagcag cagatctcaaaagcactcaagcagcaatcgatcaaatcaatgggaagctgaatcgattgacggaaaaaccaacga gaaattccatcagattgaaaaagaattctcagaagtagaagggaaggttcaagatctgagaaatatgttgaggacac taaaatagatctctggcatacaacgggagcttctgttgccttggaacacacatacgaattgacctaactgactca gaaatgaacaaactgttgaacaaacaaagaagcaactgagggaatgtcaggatattgggaatgtgtttcaa aatatatcacaaatgtgacaatgctgcataaggatcaataagaatgaaacttatgaccacaatgtgtacaggatga agcactaaacaaccgggtccagatcaagggaagtgagctgaagtcagggtacaaagattggatcctatggatttctt tggcatgtcatgttttcttctgttattgttctgttgggttcacatgttgggcctgccaaaagggaacattagatgcaac atttgcatttgagtgcaataaaaaacacctgttttctact
SEQ ID NO: 84	Nucleotide sequence of NA viral segment of A/Darwin/11/2021 agcaaaagcaggagtaagatgaatccaaatcaaaagataataacgattgctctgtttctctcacatttccacaata tgccttctcatgcaaatgcatcctgataactactgttaacattgcatttcaagcaatataaattcaactcccccaata accaagtgtatgctgtgtaaccaacaataatagaagaaacataacagagatagtgatttggaccaacaccaccata gagaaggaaatatgccccaaaccagcagaatacagaattggcgaacacgcaatgtggcattacaggatttgcac ctctctcaaggacaaattgattaggcttctcgctgggtgggacactctgggtgacaagagaacctatgtgtcatgctgat ctgtacaagtgttatcaatttgccttggacaggggaacacactaaacaatgtgcattcaataaacacagtaagtata gaaccccttatcgactctattgatgaatgagttgggttcttccatctggggaccaagcaagtgtgcatagcatg gtccagctcaagtgtgcacgatggcaagcatggctgcatgtttgtataacgggggatgataaaatgcaactgctag ctctattacaatggaggctgttagatagtggtttcatgttccaacgataattctcagaaccaggagtcagaatgcg ttgtatcaatggaactgtacagtagtaagtactgtgaaatgctacaggaaaagctgatactaaatactattcattg aggaggggaaaatcgctcactatagcaaatgtcagggaagtgtcagcatgtcgaagagtgctctgtctatctcgat atcctgtgtcagatgtgtctgcagagacaactggaaggatccaaccggcccatcatagataaaacataaaggat catagcattgtttccaggtatgtgttctgtgactgtttggagacacaccagaaaaagcgacagctccagcagtagc cattgtttgaacctaaatgaaaaagtgatcatggagtgaaaggctggccttggatgtggaatgacgtgtgg atgggggagaacaatcaacgagacgtcacgcttagggatgaaccttcaaaagtcgtgaaggctgtgcaacctta agtccaaatgcatgataataggaagtcagtagtgacagaggcgataggtccggtattctgttatttctgttgaa ggcaaaagctgcatcaatcggtgttttatgtggagtgattagggggaagaaaagaggaaactgaagctctgttgact tcaaacagttattgtgtgtttgtggcaccacaggtacatatggaacaggtcatgtgacctgatggggcgaaacctcagtc tcatgcatatataagcttctgcaattttagaaaaaactctgtttctact
SEQ ID NO: 85	Amino acid sequence of PB2 protein of A/Darwin/11/2021 MERIKELRNLSQSRTREILTKTTVDHMAIHKKYTSGRQEKNPSLRMKW MMAMKYPITADKKITEMVPERNEQGQTLWSKMSDAGSDRVMSPLAV TWWNRNGPVTDTVHYPKVYKTYFDKVERLKHGTFGPVHFRNQVKIRRR VDINPGHADLSAKEAQDVIMEVVPNEVGARILTSESQLTITKEKKEELR DCKISPLMVAYMLERELVRKTRFLPVAGGTSSIIYIEVLHLTQGTWCWEQM YTPGGGVRNDDVDQSLIIARNIVRRAAVSADPLASLLEMCHSTQIGGTR MVDILRQNPTEEQAVDICKAAMGLRISSSFSFGGFTFKRTSGSSVKREEEV LTGNLQTLRIRVHEGYEFTMVGKRATAILRKATRRLVQLIVSGRDEQSI AEAIIVAMVFSQEDCVIKAVRGDLNFDVNRANQRLNPMHQLLRHFQKDA KVLVFNQWGVHIDSVMGMLVGLPDMTPSTEMSMRGIRVSKMGVDEYSS TERVVVSIDRFLRVRDQGRGNVLLSPEEVSETQGTERTLTITYSSMMWEIN GPESVLVNTYQWIIRNWEAVKIQWSQNPAMLYNKMEFEPFQSLVPKATR SQYSGFVRTLFFQMRDVLGTFDTAQIIKLLPFAAAPPKQSRMQFSSLTVN VRGSGMRILVRGNSPVFNYNKTTKRLTILGKDAGTLIEDPDESTSGVESA VLRGFLHIGKEDRRYGPALSINELSNLAKGEKANVLIGQGDVVLVMKRKR DSSILTDSQTATKRJRMALN
SEQ ID NO: 86	Amino acid sequence of PB1 protein of A/Darwin/11/2021

	MDVNP TLLFLKVPAQNAISTTFPYTGDPYSHGTGTGYTMDTVNRTHQY SERGKWTNTTETGAPQLNPIDGPLPEDNEPSGYAQTDCVLEAMAFLEESH PGIFENSCLTMEAVQQTRVDKLTQGRQTYDWTLNRNQPAATALANTIE VFRSNGLTANESGRLLIDFLKDVME SMDKEEIEITTHFQRKRRVRDNMTK KMVTQRTIGKKKQRVNKRGYLIRALTNTMTKDAERGKLRRAIATPG MQIRGFVYFVETLARSICEKLEQSGLPVGGNEKKAKLANVVRKMMTNS QDTELSFTITGDNTKWNENQNPRMFLAMITYITKNQPEWFRNILSIAPIMF SNKMARLGKGYMFESKRMKLRTQIPAEMLASIDLKYNESTRKKIEKIRP LLIDGTASLSPGMMMGFMNMLSTVLGV SILNLGQKKYTKTTYWWDGLO SSDDFALIVNAPNHEGIQAGVDRFYRTCKLVGINMSKKKSYINKTGTFEF TSFFYRYGFVANFSMELPSFGVSGVNESADMSIGVTVIKNNMINNDLGPA TAQMALQLFIKDYRYTYRCHRGDTQIQTRRSFEIKLWDQTSKGTGLLV SDGGPNLYNIRNLHIPEVCLKWELMDDNYRGRLCNPLNPFVSHKEIESVN NAVVM PAHGPAKSM EYDAVATTHSWIPKRNRSILNTSQRGILEDEQMYQ KCCNLF EKFFPSSSYRRPIGISSMVEAMVSRARIDARIDFESGRIKKEEFSEI MKICSTIEELRRQK
SEQ ID NO: 87	Amino acid sequence of PB1-F2 protein of A/Darwin/11/2021 MEQGQGT LW TQSTEHTNTQRGGSGRQIQKLGLPSSTQLMDHYLRIMNQ VDMHKQTVSWRLWPFLKNPTQVSLKTPALKQWKPFNRQG
SEQ ID NO: 88	Amino acid sequence of PA protein of A/Darwin/11/2021 MEDFVRQCFNPMIVELAEKAMKEYGEDLKIETNKFAAICTHLEVCFMYS DFHFINEQGESIVVELDDPNALLKHFEEHGRDRTMAWTVVNSICNTTGA GKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKANKIKSENTHIHVFSFT GEEMATRADYTLDEESRARIKTRLFTIRQEMANRGLWDSFRQSERGEETI EEKFEITGTMRR LADQSLPPNFSCLENFRAYVDGFEPNGCIEGKLSQMSK EVNAQIEPFLKTTPRPIKLPSGPPCYQRSKFLLMDALKLSIEDPSHEGEGIP LYDAIKCIKTFFGWKEPYIVKPHEKGINSNYLLSWKQVLSLQDIENEEKI PRTKNMKKTSQKWLALGENMAPEKVD FENC RDISDLKQYDSEEP ELRTL SSWVQSEFNKACELTDSVWIELDEIGEDVAPIEHASMRNRNYFTA EVSHC RATEYIMKGVYINTALLNASCAAMDDFQLIPMISK CRTKEGRRKTNLYG FIIKGRSHLRNDTDVVNFVSMFSLTDPRLPHKWEKYCVLEIGDMLLRS AIGQISRP MFLYVRTNGTSKVKMKWGMEMRRCLLQSLQQIESMIEAESS VKEKDMTKEFFENKSEAWPIGESPKGVEEGSIGKVCRTLLAKSVFNSLYA SPQLEGFSAESRKL LLLIVQALRDKLEPGTFDLGGLYEAIEECLINDPWVLL NASWFNSFLTHALK
SEQ ID NO: 89	Amino acid sequence of NP protein of A/Darwin/11/2021 MASQGTKRSYEQMETDGDQRNATEIRASVGKMIDGIGRFYIQMCTELKL SDHEGRLIQNSLTIEK MVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRR VDGKWMREL VLYDKEEIRRIWRQANNGEDATSGLTHMMIWHSNLND TYQRTRALVRTGMDPRMC SLMQGSTLPRRSGAAGAAVKGIGTMVMELI RMIKRGINDRNFWRGENG RKT RSAYDRMCN ILKGKFQTAAQRAMVDQ VRESRNP GNAEIEDLIFLARSALIRGSVAHKSCLPACAYGPAVSSGYDFE KEGYSLVGIDPFKLLQNSQIYSLIRPNENPAHKSQLVWMACHSAAFEDLR LLSFIRGTVKVS PRGKLSTRGVQIASNENMDNMGSSTLELRSGYWAIRTRS GGNTNQQRASAGQTSVQPTFSVQRNIPFEKSTIMAAFTGNTEGRTSDMR AEIIRMMEGAKPEEV SFRGRGVFELSDEK AANPIVPSFDMSNEGSYFFGD NAEEYDN
SEQ ID NO: 90	Amino acid sequence of NS1 protein of A/Darwin/11/2021 MDSNTVSSFQVDCFLWHIRKQVVDQKLS DAPFLDRLRRDQ RSLRGRGNT LGLDIKSATHVGKQIVEKILKGESDEALKMTMVSTPASRYITDMTIEELSR

	NWFMLMPKQKVEGPLCIRMDQAIMKNIMLKANFNVIFGRLETIVLLRA FTDEGAIVGEISPLPSFPGHTIEDVKNAIGVLIGGLEWNDNTVRVSKNLQR FAWRSSHENGGPPLTPKQKREMARSEV
SEQ ID NO: 91	Amino acid sequence of NEP protein of A/Darwin/11/2021 MDSNTVSSFQDILLRMSKMQLGSSSEDLNGMITQFESLKIYRDSLGEAVM RMGDLHLLQNRNGKWREQLGQKFEEIRWLIEVRHRLRTTENSFEQITF MQALQLLFEVEQEIRTFQFLI
SEQ ID NO: 92	Amino acid sequence of M1 protein of A/Darwin/11/2021 MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEALMEWLKTRP ILSPLTKGILGFVFTLTVPSERGLQRRRFVQNALNGNGDPNNMDKAVKLY RKLKREITFHGAKEIALSYAGALASCMGLIYNRMGAVTTEVAFGLVCA TCEQIADSQHRSHRQMVATTNPLIKHENRMVLASTTAKAMEQMAGSSE QAAEAMEIASQARQMVQAMRAIGTHPSSSTGLRDDLLENLQTYQKRMG VQMQRFK
SEQ ID NO: 93	Amino acid sequence of M2 protein of A/Darwin/11/2021 MSLLTEVETPIRNEWGCRCNDSSNPLVVAANIIGILHLILWILDRLFFKCV YRLFKHGLKRGPESTEGVPESMRREYRKEQNAVDADDSHFVSIELE
SEQ ID NO: 94	Amino acid sequence of HA protein of A/Darwin/11/2021 MKTIIALSNI LCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEV TNATELVQNSSIGEICDSPHQILDGNCNTLIDALLGDPQCDGFQNKEDWL FVERSRANSNCYPYDVPDYASLRSLVASSGTLEFKNESFNWTGVKQNGT SSACIRGSSSSFFSRLNWLTSLNNIYPAQNVTMPNKEQFDKLYIWGVHHP DTDKNQISLFAQSSGRITVSTKRSQQA VIPNIGSRPRIRDIPSISIWYVTKP GDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCKSECITPNGSIPNDK PFQNVNRITYGACPRYVKQSTLKLATGMRNVPEKQTRGIFGAAGFIENG WEGMVDGWYGFRRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNE KFHQIEKEFSEVEGRVQDLEKYVEDTKIDLWSYNAELLVALENQHTIDL DSEMKNLFKTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNETYDHN VYRDEALNNRFQIKGVELKSGYKDWILWISFAMSCFLLCIALLGFMWAC QKGNIRCNICI
SEQ ID NO: 95	Amino acid sequence of NA protein of A/Darwin/11/2021 MNPNQKIITIGSVSLTISTICFFMQIAILITTVTLHFKQYEFNSPPNNQVMLC EPTIERNITEIVYLTNTTIEKEICPKPAEYRNWSKPQCGITGFAPFSKDNSIR LSAGGDIWVTREPYVSCDLDKCYQFALGQGTTLNNVHSNNTVSDRTPYR TLLMNELGVPFHLGKQVCIAWSSSSCHDGKAWLHVCITGDDKNATASF IYNGRLVDSVVSWSNDILRTQESECVCINGTCTVMTDGNATGKADTKI LFIEEGKIVHTSKLSGSAQHVEECSCYPYPGVRCVCRDNWKGSNRPIDI NIKDHISVSRYSGLVGDTPRKSDSSSSSHCLNPNNEKGDHGVKGWAF DDGNDVWMGRTINETSRLGYETFKVVEGWSNPKSKLQINRQVIVDRGD RSGYSGIFSVEGKSCINRCFYVELIRGRKEETEVLWTSNSIVVFCGTSGTY GTGSWPDGANLSLMHI
SEQ ID NO: 96	Nucleotide sequence of PB2 viral segment of A/Tasmania/503/2020 agcgaagcaggtcaattatattcagcatggaaagaataaaagaactacggaatctaattgctgcagctcgcactcg cgagatactgacaaaaaccacagtgacccatattggccataaataagaagtacacatcggggagacaggaagaa cccgactactagaatgaatggatgatggcaatgaatatccaattactctgacaaaaggataacagaaaatgggtcc ggagagaaatgaacaaggacaaactctatggagtaaaatgagtgatgctggatcagacagagtgatggatcacctt tggtgtaacatgggtgaatagggaatggaccgtgacagatacgggtccattacccaaaagtgtacaaaacttactcg acaaagtcgaaaggttaaacatggaaccttggccctgtccattttagaaatcaagtcaaaatcgaagaagagtag acataaatcctggtcatgcagacctcagtgccaaagaggcacaagatgtaattatgaagttgttttcccaatgaagt gggagccagaataactaacatcagaatcacagctaacaataactaaagagaaaaagaagaactccgagattgcaa

	aatttctccctgatggtcgcatatgctagagagagaacttgtgcggaaaacaagatttctccagttgctggcgga acaagcagtatatacattgaagtgttacttgaactcaaggacgtgttgggaacaaatgtacactccaggtggaggga gtgagggaatgacgatgttgaccaagcctaattattgcggccagggaacatagtaagaagagccgcaggtctagcgg atccactcgcacttltattggagatgtgccacagcagcaaatggcggaacaaggatgggtgacattcttagacaga acccgactgaagaacaagctgttgatatacaagctgcaatgggattgagaatcagctcatcttcagcttgggtg gcttcacatttaaaagaacaagcgggtcgtcagtcaaaagagagggaagaggttctacaggcaatctccagacattg agaataagagtacatgaggggtatgagggttcacaatgtgtgggaaaagagcaacagctatactaaagaaagca accagaagactggtcaactcatagtgatggaagagacgaacagtcataagctgaagcaataatcgtggccatgg tgttttcacaagaagattgcgtgataaaagcagttagagggtgacctgaattttgtcaacagagcaaatcagcgggtgaa ccccatgcatcagctttaaaggcatttccagaagaatgcgaaagtactcttcaaaatggggaggtgaacacatcgac agtggtgatgggaatggttgagttaccagatattgactccaagcacagagatgtcaatgagagggaataagagtcag caaaatgggtgtggtatgataactccagtcagagagggtgtgtgttagcattgatcggtttttgagagttcgagacca acgtgggaatgtattatctctcagagggtcagtgaaacacagggaactgaagactgaccataacttattcatca tcgatgatgtgggagattaacggccctgagtcggtttgtcaatacctatcaatggatcatcaggaaattgggaagctg tcaaaattcaatggtctcagaacctgcattgtgtacacaaaatggaattgaaccatttcaatcttttagtcccgaag ggccactagaagccaatacagtggtgttcagaaactctattccaacaaatgagagacgttaattgggacatttgcact ggccagataaataagcttctcccttttcagctgtctccaccgaagcaagcagaatgcagttctctcactgactgtga atgtgaggggtatcagggtgagaatactgttaaggggaatttctctgtattcaactacaacaagaccactaaaggg ctaacaattcttggaaaagatgccggcactttaaattgaagaccagatgaagcacatccggagtgaggtccgcgt cttgagagggttctcattataggtgaaggaagacagaagatacggaccagcattaagcatcaatgaactgagtaacc ttgcaaaaggggaaaaggctaattgtctaattgggcaaggagacgtgtgtgtgtatgaatgaacggaaacgggactc tagtatacttactgacagccagacagcgaccaaagaattcggtggccatcaattaactgaaatgtttaaaccg acctgtttctact
SEQ ID NO: 97	Nucleotide sequence of PB1 viral segment of A/Tasmania/503/2020 agcgaagcaggcaaacatttgaatggatgtcaatccgactctattgttcttaaaagtccagcgcaaaatgccataa gcacaacattcccttatactggagatcctccatagccatggaaacaggacagggtacactatggacacagtcac agaacacaccaatactcagagaggggggaagtggacgacaaatacagaactggggctcccagctcaacccaat tgatggaccactacctgaggataatgaaccaagtggatagcacaacagactgtgtcctggaggctatggccttct tgaagaatcccaccagggtatcttgaaaactcctgcttgaacaaatggaagccgttcaacagacaagggttagaca aactaactcaaggtcgcagactatgttgacattaaacagggaaccacggcagcaactgcattggccaacac catagaagctttagatcgaacgggattaacagcctaataatcagggaaggctaataagatttctcaaggatgtgatga atcaatggataaggaggaatggagataaacacacacttcaaaagaaaagaagagtaagggaacaacatgacca gaaaatggtcacacaagaacaatagggaagaaaaacaagagtgataaagagaggctacctaataagagctct gacattgaacacgatgacaaaggatgcagagagaggcaaataaaaagaagggtattgcaacaccgggatgc aaatttagaggatctgttattcgttgaactttagctagaagcatttgcgaaaaactgaacaatctggacttccgggtg ggggtaatgaaaagaaggccaactggcgaatgtgtgagaaaaatgafgactaattcacaagacacagagcttct ttcacaatcactggagacaacactaagtggaatgaaaatcaaaaccccgaaatgttttggcaatgattacatataca caaagaaccaacctgaatggttcagaacatcctgagcatcgaccaataatgttctcaaaacaaatggcaagactg ggaaaaggatatactgtcagagtaagagaatgaagctccggacacaaatacctgcagaatgttagcaagcattg acctgaagtatttcaatgaatcaacaaggagaagaattgagaaaataaggcctcttctaatagatggaacagcatcatt gagccctggaatgatgatgggcatgttcaacatgctaagtacagttttaggagtcctgatactgaatcttggacaaaag aaatacaccaagacaacgtactgtgggtgggtcctaactcctcagacgatttggccctcagatgaatgcacaaaat catgagggaatacaagcaggtggtgtagatgtatagacctgcaagtttagtgggaatcaacatgagcaaaaaga agtcctatataaataaaacaggggacattgaattcactagcttctttatcgatatggatttggcctaattttagcatggag ctgccaagtttggagtgcttggaataaacgagtcagctgacatgagcattggagtaacagtgataaagaacaacat gataaataatgacctggaccagcaacagcccgaatggctctccaattgttcatcaagattacagatacacgtatcg gtgccatagaggagacacacaaatcaacaagaagatcattcgagataaagaagctgtgggtatcaaacccaatc aaagacaggattattatagatgaggggaccaaacttatacaatcgcgaatcttcacatccctgaagtctgttg aagtgggagctgatgatgataatfatcggggaagacttgaatccctgaatcccttgcagccataaagaatgt aatctgtaaacatctgtagtaatccagccatggtcggccaaaagtatgaatgatgacgttgccgactacac

	actcctggattccaaaagggaaccgctctattctaaacacaagccaaaaggggaattcttgaggatgaacaaatgtac cagaagtgctgcaactgttcgagaaattttccctagtagttcatataggagaccgattggaattctagcatgggtga ggccatggatctagggccggatgagccagaattgacttcgagctcggaggattaagaagggaagagttctctg agatcatgaagatctgtccaccattgaagaactcagacggcaaaaataatgaatttagcttgccttcctatgaaaaat gcctgttttact
SEQ ID NO: 98	Nucleotide sequence of PA viral segment of A/Tasmania/503/2020 agcaaaagcagggtactgattcaaaatggaagatttctgacgaatgctcaaccgatgattgtgaactgcagaa aaagcaatgaaagagtatggagaggatctgaaaattgaaaccaaaatttgcagcaatgacactcacttggaggt gtgttcatgtatcagattccatttcatcaatgaacaaggcgaatcaatagtagtagaactgacgacccaatgcact gttaaacacagattgaaataatcaggggagagacagaaacaaatggcctggacagtagtaaacgatctgcac actactggagctggaaaaccgaagttttaccggatctgtatgtattacaaagaaaacagattcctgaaattggagtga caaggagagaaagtcacatatattaccttgaagggccaataagtaaatcagagaacacacattcacattttt attcactggggaggaatggccacaagggcagactacactctcagcagggaagcagggttaggattaaaacca ggctatttaccataagacaagaatggccaacagaggcctctgggattccttctcagtcggaagaggcgaaga aacaattgaagaaaattgaaatcacaggaaactatgcagcaggcttgcgaccaaagtctccaccgaacttctctg ccttgagaattttagagcctatgtgagtgattcgaaccgaacggctgcattgaggcgaagctttcctcaatgtccaaa gaagtgaatgccaaaattgaacctttctgaagacaacacaaagaccaatcaacttctagtggacctcctgttact agcgtaccaaaattcctcctgatggatgcttgaattgagcattgaagaccaagtcacgaagggaagggatccca ttatatgatgaatcaagtgcataaaaacattcttggatggaaagaaccttatatagtaaacacacagaaaaggga taaatcaaataccctgctgtcatgggaagcaagtactgtcagaattgcaggacattgaaaatgaggagaagattccaa gaactaaaaacatgaagaaacgagtcactgaagtgggctcttgggtaaaacatggcaccagagaaggtagactt tgaaaactgcagagacataagcgatttgaagcaatgacagtgaagaacctgaattgaaggtcactttcaagctggat acaaagtgaagttcaacaaggcctgtgagctaacgtattcaatctgtagagctcgatgaattggagaggacgtag ccccaattgaacacattgcagcatgagaaggaaattatttcacagcagagggtgccattgtagagctactgaataca taattgaagggtatataattaactgcctgtcaatgcactctgtgcgcaatggacgattttcaactaatcccatg ataagcaagtcagaaactaaagagggaaggcgaaaaaccaatttatatggattcatcataaagggaagatctcatct gaggaaatgacacagacgtgtaaaacttctgagcatggagtttcttcacagatccgagactgaaccacataaatg ggagaataattgtctctgagataggagatatttactaaggagtgccataggccaaatttcaaggccgatgtcttctgt atgtgaggacaacggacatcaaaagtcaaaatgaaatggggaatggagatgagacgttgcctcctcagtcact ccagcagatcgagagcatgttgaagccgagtcctcagttaaagagaagacatgaccaagaggtttttgagaata aatcagaagcatggccattggggagtcgcccaaggagtggaagaagggttcattgggaaggtctgttagactct attggctaagtcagtattcaatagcctgtatgcaccacaattggaaggatttcagcagaatcaagaaaactgtctc cttattgttcaggctcttagggacaaactgaacctgggaccttgccttgggggctatatgaagcaattgaggagt gcctgattaatgacctgggtttgtctaatgcattgttgaactcctcctgacacatgcattaaaatagttatagca gtgctactattgttatccgtactgtcaaaaaagtacctgttttact
SEQ ID NO: 99	Nucleotide sequence of NP viral segment of A/Tasmania/503/2020 agcaaaagcagggttgataatcactcactgagtgacatcaaaatcatggcgtcccaaggcaccaaaagggtcttga acagatggaaactgatggagatcgccagaatgcaactgagattagggcatcctcgggaagatgattgatggaatt gggagattctacatccaaatgtgcactgaacttaactcagtgatcatgaaggacgggttgatccaaaatagcttgaca atagagaaaatggtactctctgtttgatgaagaagggaataataacctgggaagaacaccccagcgggggaag atcccaagaaaactggggggcccatatacagaagagtcgatgaaaatgatgagggaactgctctttatgacaa agaagaataaaggcgaaatctggcgccaagccaacatggtgaggatgctacatctggtctaaccacataatgatt ggcattccaatttgaatgatgcacataccagaggacaagagctcttcttggactggaatggaatccagaatgtct ctctgatgcagggatcactcctcctagaaagtcgggagctgcagggtctgcagtcagaagggaatcggaaatggt gatggaaactgatcagaatgatcaaacggggatcaacgatcgaaattttggagaggtgagaatggcggaac aagaagtgcttatgagagaattgcaacattctaaaggaaaatttcaaacagctgcacaaagagcaatgggtgac aagtcagagaaagtcggaaccaggaaacgctgagatcgaagatctcataattttagcaagatctgcactgatattga gaggatcagttgctcaaaatcttgcctacctgcctgtgcatatggacctgcagtatcagtggttacgactttgaaa agagggaattccttgggtgggaatagaccttcaactacttcaaatagccaaatatacagcttaatacagacctaat gagaatccagcacacaagagtcagctgggtggtgatggcatgccattctgctgcaattgaagattgaagattgaagct

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SEQ ID NO: 100	Nucleotide sequence of NS viral segment of A/Tasmania/503/2020 agcaaaagcagggtgacaaagacataatggaftccaacactgtgtcaagttccaggtagattgcttcttggcatat ccggaaacaagttgtggaccaaaaactgagtgtgccccattcctcgtatcggttcgagatcagagggtccctaa gggggaagaggcaatactcgggtctagacatcaaatcagccaccatgttggaaagcaaatctagtaaaagattctg aaaggagaatctgatgaggcaactaaaaatgacctgggtctcaacacctgtctcgcatacataactgacatgactatt gaggaattgtcaagaaactggtcatgtaatgccaaagcagaaggtggaggacctcttgcacagaatggacca ggcaatcatggagaaaaacatcatgttaaaagcgaatttcaatgtattttggccggctagagaccatagattgcta agggctttcaccgaagaggaggcaattgttggcgaatctcaccattgcctctttccaggacatactattgaggtatg tcaaaatgcaattgggtcctcatcgaggagcttgaatggaaatgataacacagttcaggtctctaaaaatctacaga gattcgttggagaagcagtcagagaatgggggacctccacttactcacaacagaacggggaatggcgagaa cagctagggtcagaagttgaagagataagatggctgattgaaggtgagacacagattaagaaacaactgaaaaata gctttgaaacaataacattcagcagcattacaactactgttgaagtggacaggagataagaactttctcatttcag cttatttaatgataaaaaacacctgttttact
SEQ ID NO: 101	Nucleotide sequence of M viral segment of A/Tasmania/503/2020 agcaaaagcaggtagatattgaaagatgagccttctaccagggtcgaacgtatgttctctatcgttccatcaggc cccctcaagccgagatgcgcagagactgaagatgtctttgctgggaaaaacacagatcttgaggctctcatgga atggttaagacaagaccaattctgtcactttgactaaggggattttagggtttgttttcacgctcaccgtgcccagtg agcagaggactgcagcgtagacgtttgtcctcaaatgcccctcaatgggaatggagaccacaataacatggacaaag cagttaaactgtataggaaacttaagaggagataacgttcacggggccaaagaaatagctctcagttattctgtctg gtgcaactgtgagcagattgtgattccagcacagggtctcataggcagatgttggcaacaaccaatccattaata aaacatgagaacagaatgttttggccagcactacagctaaggctatggagcaaatggctggatcaagtgagcaag cagcagaggccatggagattgctagtcaggccaggcagatgtgcaggcaatgagagccattgggacatcctca gttcagcactgtctaaagagatgactcttgaataattgcagacctatcagaacgaatgggggtgcagatgcaac gattcaagtgaccctgtgtgttgcgcgaataatcattggatctgtcacttgatattgtgattctgtatcttttttc aaatgcgtctatcactcttcaaacacggcccttaaaaggagccattctacggaaggtgtacctgagctatgagggaa gaataccgaaaggaaacagcagaatgctgtggatgtgacgacagtcattgtcagcatagaattggagtaaaaaac tacctgttttact
SEQ ID NO: 102	Nucleotide sequence of HA viral segment of A/Tasmania/503/2020 agcaaaagcaggggataattctattaacctgaagactatcattgctttgagctacattctatgtcttcttctcaaaa aatccctggaaatgacaatagcacggcaacgctgtgcttgggcaccatgcagtaccacaacgggaacgatagtga aacaatcacaatgaccgaattgaagtactaatgctactgagttggttcagaattctcctaaggtgaaatatcgac agtctcatcagatccttgatggagggaactgcacactaatagatgctctatttggggaccctcagtgtagcggcttctc aaaataagggaatgggaccttttgtgaacgaagcagagccaacagcaactgttacccttatgatgtgcccggattatg cctcccttaggtcactagttgctcctcctcgacactggagtttaaaatgaagcttcaattggactggagtcacaaca aacgggaacaagttctgctgcataaggggatctagtagtatttttagtagaataattgttgaccacttaacta cacatatccagcactgaacgtgactatgccaacaagggaacaatttgacaattgtacatttgggggttaccacccc gagtacggacaaggaccaaatctccctgtttgctcaaccatcagggaagaatcacagtatctacaaaagaagccaa caagctgtaatccaaatatcgatctagaccagaataagggaatatccctagcagaataagcatctatttggacaata gtaaaaccgggagacatacttttgaataacagcacagggaatctaattgctcctagggggttacttcaaaatacgaagtg ggaaaagctcaataatgagatcagatgcacccattggcaaatgcaagtctgaatgcactcctcaaatggaagcatt cccaatgacaaccgttcaaaatgtaaacagatcacatacggggcctgtccagatatgtcaagcaaacaccc tgaatttggcaacagggaatgcaaatgtaccagagaacaaaccagggcataatttggcgcaataggcgggttcat

	agaaaatggatgggaggggaatgggtgatgggtgtacgggttcaggcatcaaaattctgaggggaagaggacaagc agcagatctcaaaagcactcaagcagcaatcgatcaaatcaatgggaagctgaatcgattgacggaaaaaccaac gagaaaattccatcagattgaaaaagaattctcagaagtagaagggaaggttcaagacctgagaaatatgttgaggga cactaaaatagatctctggctafacaacgctgagcttctgttggccctggagaaccaacafacaattgacctaaactgact cagaaatgaacaaactgtttgaaaaaacaagaagcaactgagggaaaatgctgaggatatgggaaatggtgtttc aaaatataccacaatgtgacaatgcctgcataggatcaataagaatgaaacttatgaccacaatgtgtacagggat gaagcattaaacaaccgggtccagatcaaggaggttgagctgaagtcagggtacaaagattgacatctatgatttc ctttgcatgtcatgtttttgtttgtattgtttgttgggttcacatgttggcctgccaagggcaacattagatgca acatttgcatttgagtgcatatataaaacacctgttttctact
SEQ ID NO: 103	Nucleotide sequence of NA viral segment of A/Tasmania/503/2020 agcaaaagcaggagtaagatgaatcaaatcaaaagataataacgattggctctgtttcttcacaaattccacaata tgctttctcatgcaaatggcatcctgataactactgttaacattgcatttcaagcaaatgaattcaactccccccaaata accaagtgtgctgtgtgaaccaacaataatagaagaacatgacagagatagtgatttggaccaacaccaccata gagaaggaaatatgccccaaaccagcagaatacagaattgttcaaaaccgcaatgtggcattacaggatttgcac ctttctcaaggacaattcgattaggcttccgctgtgggtgacatctgggtgacaagagaacctatgtgtcatcgat cttgacaagtggtatcaatttgccttggacaggggaacaacactaaacaacgtgcattcaataaacacagtacgtgata gaacctctatcgactctattgataatgagttgggtgttcttccatctggggaccaagcaagtgtgcatagcatg gtccagctcaagttgacagatggaaaagcatggctgcatgtttgtataacgggggatgataaaaatgcaactgctag cttcattacaatggaggctgtgtagatagtggtttcatggtccaacgatatttcagaaccaggagtcagaaatgcg tttgatcaatggaactgtacagtagtaatgactgaggaatgctacagggaaaagctgatactaaaatactattcattg aggaggggaaaatcgctacatactagcaaatgtcagggaagtgtcagcatgtcgaagagtgctctgtctatctcgat atcctgtgtcagatgtgtctgacagacaactggaaagatccaaccggcccatcatagataataacataaaggat catagcattgtttccaggatgtgtgttctgactgttggagacacaccagaaaaagcgacagctccagcagtagc cattgtttgaaccctaacaatgaaaaagggtatcatgagtgaaaggctgggctttgatgtggaatgacgtgttg atgggggagaacaatcaacgagacgtcacgcttaggtatgaaccttcaaaagctgtgaaggctgtgccaacctata agtccaaattgcagataaataggcaagtcatagttgacagaggtgatagggtccgggtattctgtattttctgttgaa ggcaaaagctgcataatcggtgcttttatgtggagttgattaggggaagaaaagggaactgaagctgtgtggac ctcaaacagttattgtgtttgtggcacctcagggtacatatggaacaggctcatggcctgatggggcgaacctcagt ctcatgcatataaagcttctgcaatttttagaaaaactcctgttttctact
SEQ ID NO: 104	Amino acid sequence of PB2 protein of A/Tasmania/503/2020 MERIKELRNLSQSRTREILTKTTVDHMAHKKYTSGRQEKNP SLRMKW MMAMKYPIADKRITEMVPERNEQGQTLWSKMSDAGSDRVMVSPLAV TWWNRNGPVTDTVHYPKVYKTYFDKVERLKHGTFGPVHFRNQVKIRRR VDINPGHADLSAKEAQDVIMEVVPNEVGARILTSESQLTITKEKKEELR DCKISPLMVAYMLERELVRKTRFLPVAGGTSSIIYIEVLHLTQGTWCWEQM YTPGGGVRNDDVDQSLIIARNIVRRAAVSADPLASLLEMCHSTQIGGTR MVDILRQNPTEEQAVIDICKAAMGLRISSSFSFGGFTFKRTSGSSVKREEEV LTGNLQTLRIRVHEGYEEFTMVGKRATAILRKATRRLVQLIVSGRDEQSI AEAIIVAMVFSQEDCVIKAVRGDLNLFVNRRANQRLNPMHQLLRHFQKDA KVL FQNWGVEHIDSVMGMVGVL PDMTPSTEMSMRGIRVSKMGVDEYSS TERVVVSIDRFLRVRDQQRGNVLLSPEEVSETQGTERLTITYSSSMWWEIN GPESVLVNTYQWIIRNWEAVKIQWSQNPAMLYNKMEFEPFQSLVPKATR SQYSGFVRTL FQQMRDVIGTFDTAQHKLLPFAAAPKQSRMQFSSLTVN VRGSGMRILVRGNSPVFNYNKTTKRLTILGKDAGTLIEDPDESTSGVESA VLRGFLIIGKEDRRYGPALSINELSNLAKGEKANVLIGQGDVVLVMKRKR DSSILTDSQTATKRIRMAIN
SEQ ID NO: 105	Amino acid sequence of PB1 protein of A/Tasmania/503/2020 MDVNPTLLFLKVP AQNAISTTFPYTGDPYSHGTGTGYTMDTVNRTHQY SERGKWTNTTETGAPQLNPIDGPLPEDNEPSGYAQTD CVLEAMAFLEESH PGIFENSCLTMEAVQQTRVDKLTQGRQTYDWTLNRNQPAATALANTIE

	VFRSNGLTANESGRLIDFLKDVME\$MDKEEMEITTHFORKRRVRDNMTK KMVTQRTIGKKKQVRNKRGYLIRALTNTMTKDAERGKLRRAIATPG MQIRGFVYFVETLARSICEKLEQ\$GLPVGGNEKKAKLANVVRKMMTNS QDTELSFTITGDNTKWNENQNP\$RMFLAMITYITKNQPEWFRNILSIAPIMF SNKMARLGKGYMFESKRMKLRTQIPAEMLASIDLKYFNSTRKKIEKIRP LLIDGTASLSPGMMMGMFNM\$STVLGVSILNLGQKKYTKTTYWWDGLQ SSDDFALIVNAPNHEGIQAGVDRFYRTCKLVGINMSKKKSYINKTGTFEF TSFFYRYGFVANFSMELPSFGV\$GINESADMSIGVTVIKNNMINNDLGPA TAQMALQLFIKDYRYTYRCHRGDTQIQTRRSFEIKKLWDQTQSKTGLLV SDGGPNLYNIRNLHIPEVCLKWELMDDNYRGRLCNPLNPFVSHKEIESVN NAVVM\$PAHGPAKSMEYDAVATTHSWIPKRNR\$ILNTSQRGILEDEQMYQ KCCNLFEKFFPSSSYRRPIGI\$SMVEAMVSRARIDARIDFESGRIKKEEFSEI MKICSTIEELRRQK
SEQ ID NO: 106	Amino acid sequence of PB1-F2 protein of A/Tasmania/503/2020 MEQGQGT\$LTWQT\$TEHTNTQ\$RGSGRQIQKLG\$LP\$STQLMDHYLRIMNQ VDMHKQTV\$SWRLWPSLKNPTQVSLKTPALKQWKPFNRQG
SEQ ID NO: 107	Amino acid sequence of PA protein of A/Tasmania/503/2020 MEDFVRQCFNPMIVELAEKAMKEYGEDLKIETNKFAAIC\$HLEVC\$FMYS DFHFINEQGESIVVELDDPNALLK\$HFEIIEGRDRTMAWTVVNSICNTTGA GKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKANKIK\$ENTHIHIFSFTG EEMATRADYTLDEESRARIKTRLFTIRQEMANRGLWDSFRQ\$SERGEETIE EKFEITGTMRR\$ADQSLPPNF\$CLENFRAYVDGFEPNGCIEGKLSQMSKE VNAQIEPFLKTT\$PRPIKLPSGPPCYQ\$RSKFL\$MDALKLSIEDPSHEGEGIP YDAIKCIK\$TFFGWKEPYIVKPHEKGINSNYLLSWKQVLSELQDIENEKIP RTKNM\$KTSQLK\$WALGENMAPEKVD\$FENC\$RDISDLKQYDSEEP\$ELRSL\$ SWIQSEFNKACELTDSIWIELDEIGEDVAPIEHASMR\$NYFTA\$EVSHCRA TEYIMKGVYINTALLNASCAAMDDFQLIPMISKCR\$TKEGRRKTNLYGFII KGRSHLRNDTDVVNPFVSM\$EFSLTDPRLEPHKWEKYCVLEIGDMLL\$SAI GQISRPMFLYVRTNGTSKVKMKWGMEMRRCLLQSLQ\$QIESMIEAESSVK EKDMTKEFFENKSEAWPIGESPKGVEEGSIGKVCRTLLAK\$SVFNSLYASP QLEGFSAESRKL\$LLIVQALRDKLEPGTFDLGGLYEAIEECLINDPWVLLNA SWFNSFLTHALK
SEQ ID NO: 108	Amino acid sequence of NP protein of A/Tasmania/503/2020 MASQGT\$KRSYEQMETD\$GDRQ\$NATEIRASVGK\$MIDGIGRFYIQMCTELKL SDHEGR\$LIQNSLTIEK\$MVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRR VDGKWMRELVL\$YDKEEIRRIWRQ\$ANNGEDAT\$SGLTHIMIWH\$SNLNDAT YQRT\$RALVRTGMDPRMCSLMQ\$GSTLPRR\$SGAAGA\$AVKGIGTMV\$MELIR MIKRGINDRNFWRGENGRKTR\$SAYERMCN\$ILKGKFQTA\$AQRAMVDQVR ESRNPGNAEIEDLIFLARSALILRG\$VAHKSCLPACAYGPAVSSGYDFEKE GYSLVGIDPFKLLQNSQIYSLIRPNENPAHKSQ\$LVW\$MACH\$AA\$FEDLRL\$ SFIRG\$TKV\$SPRGKLSTRGVQIASNENMDNMG\$SSTLELR\$SGYWAIRTRSGG NTNQQRASAGQTSVQPTFSVQRNIPFEKSTIMAAFTGNTEGRTSDMRAEH RMMEGAKPEEV\$SFRGRGVFELSDEK\$AANPIVPSFDM\$SNEG\$SYFFGDNAE EYDN
SEQ ID NO: 109	Amino acid sequence of NSI protein of A/Tasmania/503/2020 MDSNTVSS\$FQVDCFLWHIRKQVVDQKLS\$DAPFLDRLRRDQ\$RSLRGRGNT LGLDIK\$ATHVGKQIVEKILKGE\$DEALKMTMVSTPASRYITDMTIEELSR NWFMLMPKQKVEG\$PLCIRMDQAIM\$KNIML\$KANFNVIFGRLETIVLLRA FTEEGAIVGEISPL\$SPFGHTIEDVKNAIGV\$LIGGLEW\$NDNTVRV\$SKNLQR FAWRSSHENG\$GPPLTPKQKRE\$MARTARSEV

SEQ ID NO: 110	Amino acid sequence of NEP protein of A/Tasmania/503/2020 MDSNTVSSFQDILLRMSKMQLGSSSEDLNGMITQFESLKIYRDSLGEAVM RMGDLHLLQNRNGKWREQLGQKFEEIRWLIIEVRHRLRTTENSFEQITF MQALQLLFEVEQEIRTFSFQLI
SEQ ID NO: 111	Amino acid sequence of M1 protein of A/Tasmania/503/2020 MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEALMEWLKTRP ILSPLTKGILGFVFTLTVPSEGLQRRRFVQNALNGNGDPNNMDKAVKLY RKLKREITFHGAKEIALSYSAGALASCMGLIYNRMGAVTTEVAFGLVCA TCEQIADSQHRSHRQMVATTNPLIKHENRMVLASTTAKAMEQMAGSSE QAAEAMEIASQARQMVQAMRAIGTHPSSSTGLRDDLLENLQTYQKRMG VQMQRFK
SEQ ID NO: 112	Amino acid sequence of M2 protein of A/Tasmania/503/2020 MSLLTEVETPIRNEWGCRCNDSSDPLVVAANIIGILHLILWILDRLFFKCV YRLFKHGLKRGHSTEGVPESMREEYRKEQQNAVDADDSHFVSIELE
SEQ ID NO: 113	Amino acid sequence of HA protein of A/Tasmania/503/2020 MKTIIALSYILCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEV TNATELVQNSSIGEICDSPHQILDGGNCTLIDALLGDPQCDGFONKEWDL FVERSRANSNCYPYDVPDYASRLSLVASSGTLEFKNESFNWTGVKQNGT SSACIRGSSSSFFSRLNWLTHLNYTYPALNVTMPNKEQFDKLYIWGVHHP STDKDQISLFAQPSGRITVSTKRSQAVIPNIGSRPRIRDIPSISIIYWTIVKP GDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCKSECITPNGSIPNDK PFQNVNRITYGACPRYVKQSTLKLATGMRNVPEKQTRGIFGAIAGFIENG WEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNE KFHQIEKEFSEVEGRVQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLT DSEMKNLFEKTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNETYDHN VYRDEALNNRFQIKGVELKSGYKDWILWISFAMSCFLLCIALLGFMWAC QKGNIRCNICI
SEQ ID NO: 114	Amino acid sequence of NA protein of A/Tasmania/503/2020 MNPNQKIITIGSVSLTISTICFFMQAILITTVTLHFKQYEFNSPPNNQVMLC EPTIERNMTEIVYLTNTTIEKEICPKPAEYRNWSKPQCGITGFAPFSKDNSI RLSAGGDIWVTREPYVSCDLDKCYQFALGQGTTLNNVHSNNTVRDRTP YRTLLMNELGVPFHLGTKQVCIAWSSSSCHDGKAWLHVCITGDDKNAT ASFIYNGRLVDSVVSWSNDILRTQESECVCINGTCTVVMTDGNATGKAD TKILFIEEGKIVHTSKLSGSAQHVEECSCYPRYPGVRCVCRDNWKGSNRPI IDINIKDHSIVSRYVCSGLVGDTPRKSDSSSSSHCLNPNNEKGDHGVKGW AFDDGNDVWMGRTINETSRLGYETFKVVEGWSNPKSKLQINRQVIVDRG DRSGYSGIFSVEGKSCINRCFYVELIRGRKEETEVLWTSNSIVVFCGTSST YGTGSWPDGANLSLMHI
SEQ ID NO: 115	Nucleotide sequence of 5' non-coding region AGC[A/G]AAAGCAGG
SEQ ID NO: 116	Nucleotide sequence of 3' non-coding region CCTTGTTTCTACT

CLAIMS:

1. An isolated influenza virus comprising one or more of PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.
2. The isolated influenza virus of Claim 1, comprising one or more of the PB2, PB1, PA, M, NP and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a fragment, variant or derivative thereof.
3. The isolated influenza virus of Claim 1, wherein the isolated influenza virus is capable of enhanced replication when grown in cells relative to a wild-type influenza virus isolate that does not comprise the one or more PA, PB1, PB2, NP, M and NS viral segments.
4. The isolated influenza virus of Claim 1, wherein the isolated influenza virus is capable of enhanced yield of a viral protein when grown in cells relative to a wild-type influenza virus isolate that does not comprise the one or more PA, PB1, PB2, NP, M and NS viral segments.
5. The isolated influenza virus of Claim 4, wherein the viral protein is a haemagglutinin (HA) protein.
6. The isolated influenza virus of Claim 3, wherein the cells are MDCK cells.
7. The isolated influenza virus of Claim 1, comprising five or six of the PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from those set forth in:
 - (a) SEQ ID NOs: 9-17 or a fragment, variant or derivative thereof;
 - (b) SEQ ID NOs: 28-36 or a fragment, variant or derivative thereof;
 - (c) SEQ ID NOs: 47-55 or a fragment, variant or derivative thereof;
 - (d) SEQ ID NOs: 66-74, or a fragment, variant or derivative thereof;

- (e) SEQ ID NOs: 85-93, or a fragment, variant or derivative thereof; or
 - (f) SEQ ID NOs: 104-112, or a fragment, variant or derivative thereof.
8. The isolated influenza virus of Claim 1, comprising five or six of the PA, PB1, PB2, NP, M and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from those set forth in:
- (a) SEQ ID NOs: 1-6 or a fragment, variant or derivative thereof;
 - (b) SEQ ID NOs: 20-25 or a fragment, variant or derivative thereof;
 - (c) SEQ ID NOs: 39-44 or a fragment, variant or derivative thereof;
 - (d) SEQ ID NOs: 58-63 or a fragment, variant or derivative thereof;
 - (e) SEQ ID NOs: 77-82, or a fragment, variant or derivative thereof; or
 - (f) SEQ ID NOs: 96-101, or a fragment, variant or derivative thereof.
9. The isolated influenza virus of Claim 1, further comprising a heterologous or chimeric HA viral segment and a heterologous or chimeric NA viral segment.
10. The isolated influenza virus of Claim 1, wherein the isolated influenza virus is of a N1, N2, N3, N7, or N9 subtype.
11. The isolated influenza virus of Claim 10, wherein the isolated influenza virus is of an N1 or N2 subtype.
12. The isolated influenza virus of Claim 1, wherein the isolated influenza virus is of a H1, H2, H3, H5, H7, or H9 subtype.
13. The isolated influenza virus of Claim 12, wherein the isolated influenza virus is of a H1 or H3 subtype.
14. The isolated influenza virus of Claim 1, wherein the isolated influenza virus is of a H1N1 subtype or a H3N2 subtype.
15. The isolated influenza virus of Claim 1, which is a recombinant influenza virus.
16. The isolated influenza virus of Claim 1, which is a reassortant influenza virus.

17. A method of preparing an influenza virus, said method including the step of contacting a cell with one or more genetic constructs that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.
18. The method of Claim 17, wherein the one or more genetic constructs comprise or encode one or more of PB2, PB1, PA, M, NP and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a fragment, variant or derivative thereof.
19. The method of Claim 17, further including the step of isolating or harvesting the influenza virus from the cells.
20. A method of preparing an influenza virus, said method including the step of contacting a cell with an isolated influenza virus comprising one or more of PA, PB1, PB2, NP, M and NS viral segments that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.
21. The method of Claim 20, wherein the isolated influenza virus is that of Claim 1.
22. The method of Claim 20, further including the step of isolating or harvesting the influenza virus from the cells.
23. An isolated influenza virus prepared by the method of Claim 17.
24. An isolated influenza virus prepared by the method of Claim 20.
25. An isolated cell infected with the isolated influenza virus of Claim 1.

26. The isolated cell of Claim 25, wherein the cell is an MDCK cell.
27. A genetic construct that encodes one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.
28. A plurality of genetic constructs that encode one or more viral proteins that independently comprise, consist of or consist essentially of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, 28-36, 47-55, 66-74, 85-93 and 104-112, or a fragment, variant or derivative thereof.
29. The genetic construct of Claim 27 or the plurality of genetic constructs of Claim 26, wherein the genetic construct or the plurality of genetic constructs comprise or encode one or more of PB2, PB1, PA, M, NP and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a nucleotide sequence complementary thereto, or a fragment, variant or derivative thereof.
30. The plurality of genetic constructs of Claim 28, wherein the genetic construct or the plurality of genetic constructs comprise or encode one or more of PB2, PB1, PA, M, NP and NS viral segments that independently comprise, consist of or consist essentially of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6, 20-25, 39-44, 58-63, 77-82 and 96-101, or a nucleotide sequence complementary thereto, or a fragment, variant or derivative thereof.
31. A method of making an immunogenic composition, including the steps of:
- (a) providing the isolated influenza virus of Claim 1 and/or a viral protein derived therefrom; and
 - (b) combining the isolated influenza virus and/or the viral protein with an adjuvant and/or treating the isolated influenza virus with an agent that inactivates the virus.

32. The method of Claim 31, wherein the adjuvant comprises an immunostimulatory DNA sequence, a bacterium-derived component, an aluminium salt (alum) or a squalene oil-in-water emulsion system.
33. An immunogenic composition produced according to the method of Claim 31.
34. An immunogenic composition comprising the isolated influenza virus of Claim 1 and/or a viral protein derived therefrom and a pharmaceutically acceptable carrier, diluent or excipient.
35. A method of eliciting an immune response in a subject, said method including the step of administering a therapeutically effective amount of the isolated influenza virus of Claim 1, a viral protein derived therefrom or an immunogenic composition comprising the isolated influenza virus to the subject to thereby elicit the immune response in the subject.
36. A method of preventing and/or treating an influenza-associated disease, disorder or condition in a subject, said method including the step of administering a therapeutically effective amount of the isolated influenza virus of Claim 1, and/or a viral protein derived therefrom or an immunogenic composition comprising the isolated influenza virus to the subject to thereby prevent and/or treat the influenza-associated disease, disorder or condition.
37. Use of the isolated influenza virus of Claim 1, a viral protein derived therefrom or an immunogenic composition comprising the isolated influenza virus in the manufacture of a medicament for eliciting an immune response in a subject.
38. Use of the isolated influenza virus of Claim 1, a viral protein derived therefrom or an immunogenic composition comprising the isolated influenza virus in the manufacture of a medicament for preventing and/or treating an influenza-associated disease, disorder or condition in a subject.
39. The isolated influenza virus of Claim 1, a viral protein derived therefrom or an immunogenic composition comprising the isolated influenza virus for use in therapy.

40. The isolated influenza virus of Claim 1, a viral protein derived therefrom or an immunogenic composition comprising the isolated influenza virus for use in a method of eliciting an immune response in a subject.

41. The isolated influenza virus of Claim 1, a viral protein derived therefrom or an immunogenic composition comprising the isolated influenza virus for use in a method of preventing and/or treating an influenza-associated disease, disorder or condition in a subject.