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(71) Applicants: **NOVARTIS AG** [CH/CH]; Lichtstrasse 35, CH-4056 Basel (CH). **SYNTHETIC GENOMICS VACCINES, INC.** [US/US]; 11149 North Torrey Pines Road, La Jolla, California 92037 (US).

(72) Inventors: **MASON, Peter**; Novartis Vaccines, 45 Sidney Street, Cambridge, Massachusetts 02139 (US). **SUPHAPHIPHAT, Pirada**; Novartis Vaccines, 45 Sidney Street, Cambridge, Massachusetts 02139 (US). **GOMILA, Raul**; Novartis Vaccines, 45 Sidney Street, Cambridge, Massachusetts 02139 (US). **DORMITZER, Philip**; Novartis Vaccines, 45 Sidney Street, Cambridge, Massachusetts 02139 (US).

(74) Agents: **MARSHALL, Cameron John** et al.; Carpmaels & Ransford LLP, One Southampton Row, London WC1B 5HA (GB).

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(54) Title: INFLUENZA VIRUS REASSORTMENT

(57) Abstract: The invention provides reassortant influenza strains.

INFLUENZA VIRUS REASSORTMENT

This application claims the benefit of US provisional application 61/832,091 filed 6th June 2013 and European patent application no. 13179013.1 filed 26th September 2013, the complete contents of both of which are incorporated herein by reference.

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TECHNICAL FIELD

This invention is in the field of influenza virus reassortment. Furthermore, it relates to manufacturing vaccines for protecting against influenza viruses.

BACKGROUND ART

The most efficient protection against influenza infection is vaccination against circulating strains and it is important to produce influenza viruses for vaccine production as quickly as possible.

Wild-type influenza viruses often grow to low titres in eggs and cell culture. In order to obtain a better-growing virus strain for vaccine production it is currently common practice to reassort the circulating vaccine strain with a faster-growing high-yield donor strain. This can be achieved by co-infecting a culture host with the circulating influenza strain (the vaccine strain) and the high-yield donor strain and selecting for reassortant viruses which contain the hemagglutinin (HA) and neuraminidase (NA) segments from the vaccine strain and the other viral segments (*i.e.* those encoding PB1, PB2, PA, NP, M_1, M_2, NS_1 and NS_2) from the donor strain. Another approach is to reassort the influenza viruses by reverse genetics (see, for example references 1 and 2).

References 3 and 4 report that influenza viruses with a chimeric HA segment which comprises the ectodomain from a vaccine strain and the other domains from A/Puerto Rico/8/34 grew faster in eggs compared to the wild-type vaccine strain. Reference 5 teaches influenza viruses with chimeric NA proteins which contain the transmembrane and stalk domains from A/PR/8/34. References 6 and 7 teach reassortant influenza viruses which comprise chimeric HA segments that have domains from both influenza A and B viruses.

Most of the studies with chimeric HA proteins were done in eggs and reference 3 teaches that "*it is likely that the improvement seen with* [the described] *chimeric viruses is very specific to the egg substrate*". The studies which tested growth in cell culture found that the tested viruses showed poor growth in cell culture. There is therefore still a need in the art to provide high-yielding reassortant influenza viruses, especially in cell culture.

SUMMARY OF PREFERRED EMBODIMENTS

The invention provides a chimeric influenza hemagglutinin segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a signal peptide, a transmembrane domain and a

cytoplasmic domain wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are from a second influenza strain which is not A/Puerto Rico/8/34, A/WSN/33 or B/Lee/40.

Also provided is a chimeric influenza hemagglutinin segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a signal peptide, a transmembrane domain and a cytoplasmic domain, wherein the ectodomain is from a first influenza A strain which is not a H1 or H5 influenza strain, and one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are from a second influenza strain.

The invention also provides a chimeric influenza hemagglutinin segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a signal peptide, a transmembrane domain and a cytoplasmic domain, wherein the ectodomain is from a first influenza B strain, and one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are from a second influenza strain which is an influenza B strain or an influenza A strain which is not a H1 strain or a H3 strain. The chimeric hemagglutinin segment preferably comprises all of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain from the second influenza virus as the inventors have found that reassortant influenza viruses comprising such a chimeric hemagglutinin segment give particularly good HA yields in cell culture.

Also provided is a chimeric influenza hemagglutinin segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a signal peptide, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are from a second influenza strain, wherein the segment comprises one or more of: (a) guanine in the position corresponding to nucleotide 24, and/or (b) adenine in the position corresponding to nucleotide 38; and/or (c) thymine in the position corresponding to nucleotide 40; and/or (d) adenine in the position corresponding to nucleotide 44; (e) and/or thymine in the position corresponding to nucleotide 53; and/or (f) adenine in the position corresponding to nucleotide 63; and/or (g) thymine in the position corresponding to nucleotide 66; and/or (h) adenine in the position corresponding to nucleotide 69 ; and/or (i) adenine in the position corresponding to nucleotide 75; and/or (j) thymine in the position corresponding to nucleotide 78; and/or (k) adenine in the position corresponding to nucleotide 1637; and/or (l) cytosine in the position corresponding to nucleotide 1649, and/or (m) thymine in the position corresponding to nucleotide 1655, and/or (n) cytosine in the position corresponding to nucleotide 1682, and/or (o) cytosine in the position corresponding to nucleotide 1697; and/or (p) guanine in the position corresponding to nucleotide 1703, and/or (q) thymine in the position corresponding to nucleotide 1715, and/or (r) adenine in the position corresponding to nucleotide 1729, and/or (s) cytosine in the position corresponding to nucleotide

1733, and/or (t) cytosine in the position corresponding to nucleotide 1734, and/or (u) adenine in the position corresponding to nucleotide 1746, and/or (v) adenine in the position corresponding to nucleotide 1751 ; when aligned to SEQ ID NO: 15 using a pairwise alignment algorithm. Preferably, the chimeric hemagglutinin comprises all of the nucleotides of (a) to (v).

The invention also provides a chimeric hemagglutinin segment, having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a signal peptide, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are from a second influenza strain, wherein the segment encodes a protein which does not have alanine in the position corresponding to amino acid 3 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm; and/or which does not have asparagine in the position corresponding to amino acid 4 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm; and/or which does not have alanine in the position corresponding to amino acid 11 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm; and/or which does not have leucine in the position corresponding to amino acid 12 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm; and/or which does not have alanine in the position corresponding to amino acid 13 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm; and/or which does not have alanine in the position corresponding to amino acid 15 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm; and/or which does not have aspartic acid in the position corresponding to amino acid 16 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm.

In some aspects, the chimeric hemagglutinin segment may encode a protein which has one or more of valine in the position corresponding to amino acid 3 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm; and/or lysine in the position corresponding to amino acid 4 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm; and/or threonine in the position corresponding to amino acid 11 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm; and/or phenylalanine in the position corresponding to amino acid 12 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm; and/or threonine in the position corresponding to amino acid 13 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm; and/or threonine in the position corresponding to amino acid 15 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm; and/or tyrosine in the position corresponding to amino acid 16 of SEQ ID NO: 63 when aligned to SEQ ID NO: 63 using a pairwise alignment algorithm. The chimeric HA segment may comprise all of these amino acids which is preferred as reassortant influenza viruses comprising such a chimeric hemagglutinin segment give particularly good HA yields in cell culture.

The chimeric hemagglutinin segment may comprise one or more of the 5'- NCR domain of SEQ ID NO: 110; and/or the CT domain of SEQ ID NO: 111; and/or the TM domain of SEQ ID NO: 112; and/or the 3'- NCR of SEQ ID NO: 113.

The invention also provides a chimeric hemagglutinin segment, having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a signal peptide, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are from a second influenza strain, wherein the segment comprises one or more (preferably all) of: guanine at position 24, adenine at position 38, thymine at position 40, thymine at position 53, adenine at position 63, thymine at position 66, adenine at position 69, adenine at position 75, thymine at position 78, guanine at position 1703, thymine at position 1715, adenine at position 1729, cytosine at position 1733, cytosine at position 1734, adenine at position 1746, and/or adenine at position 1751. All of these positions are relative to the corresponding position in SEQ ID NO: 15 when aligned to SEQ ID NO: 15 using a pairwise alignment algorithm.

The chimeric hemagglutinin segment may comprise one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain from the 105p30 influenza strain, which is discussed below. Preferably, the chimeric hemagglutinin segment comprises all of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain from the 105p30 influenza strain as reassortant influenza viruses comprising such a chimeric hemagglutinin segment give particularly good HA yields in cell culture.

Also provided is a chimeric HA protein which is encoded by a chimeric HA segment of the invention.

The inventors have discovered that reassortant influenza viruses which comprise a chimeric HA segment of the invention can provide HA yields which are up to 5-fold higher in the same time frame and under the same conditions compared to a reassortant influenza virus which does not comprise a chimeric HA segment.

Further provided is a chimeric influenza neuraminidase segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the transmembrane domain and the cytoplasmic domain are from a second influenza strain which is not A/Puerto Rico/8/34 or A/WSN/33.

Also provided is a chimeric influenza neuraminidase segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is a first influenza strain and the 5'- non-coding region, the 3'- non-coding region,

the transmembrane domain and the cytoplasmic domain are from a second influenza strain wherein the first and the second influenza strain are both influenza A strains or both influenza B strains.

The invention also provides a chimeric neuraminidase segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a transmembrane domain and a cytoplasmic domain, wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the transmembrane domain and the cytoplasmic domain are from a second influenza strain, wherein the segment comprises one or more (preferably all) of: adenine in the position corresponding to nucleotide 13; and/or adenine in the position corresponding to nucleotide 35; and/or adenine in the position corresponding to nucleotide 60; and/or adenine in the position corresponding to nucleotide 63; and/or adenine in the position corresponding to nucleotide 65; and/or cytosine in the position corresponding to nucleotide 67; and/or adenine in the position corresponding to nucleotide 69; and/or adenine in the position corresponding to nucleotide 75; and/or thymine in the position corresponding to nucleotide 83; and/or guanine in the position corresponding to nucleotide 89; and/or adenine in the position corresponding to nucleotide 101; and/or thymine in the position corresponding to nucleotide 107; and/or thymine in the position corresponding to nucleotide 110; and/or guanine in the position corresponding to nucleotide 120; and/or cytosine in the position corresponding to nucleotide 121; and/or thymine in the position corresponding to nucleotide 125; and/or thymine in the position corresponding to nucleotide 127. All of these positions are relative to the corresponding position in SEQ ID NO: 16 when aligned to SEQ ID NO: 16 using a pairwise alignment algorithm.

The invention also provides a chimeric neuraminidase segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a transmembrane domain and a cytoplasmic domain, wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the transmembrane domain and the cytoplasmic domain are from a second influenza strain, wherein the segment encodes a protein which does not have cysteine in the position corresponding to amino acid 14 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm, and/or which does not have leucine in the position corresponding to amino acid 15 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm; and/or which does not have valine in the position corresponding to amino acid 16 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm; and/or which does not have valine in the position corresponding to amino acid 17 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm; and/or which does not have leucine in the position corresponding to amino acid 19 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm; and/or which does not have isoleucine in the position corresponding to amino acid 23 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm; and/or which does not have isoleucine in the position corresponding to amino acid 34 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm.

In some aspects, the chimeric neuraminidase segment may encode a protein which comprises one or more of: serine in the position corresponding to amino acid 14 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm, and/or isoleucine in the position corresponding to amino acid 15 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm; and/or alanine in the position corresponding to amino acid 16 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm; and/or isoleucine in the position corresponding to amino acid 17 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm; and/or isoleucine in the position corresponding to amino acid 19 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm; and/or methionine in the position corresponding to amino acid 23 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm; and/or alanine in the position corresponding to amino acid 34 of SEQ ID NO: 64 when aligned to SEQ ID NO: 64 using a pairwise alignment algorithm. The chimeric NA segment may comprise all of these amino acids which is preferred as reassortant influenza viruses comprising such a chimeric hemagglutinin segment give particularly good HA yields in cell culture.

The chimeric neuraminidase segment may comprise one or more of the 5'- NCR domain of SEQ ID NO: 110; and/or the CT domain of SEQ ID NO: 111; and/or the TM domain of SEQ ID NO: 112; and/or the 3'- NCR of SEQ ID NO: 113.

The invention also provides a chimeric neuraminidase segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a transmembrane domain and a cytoplasmic domain, wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the transmembrane domain and the cytoplasmic domain are from a second influenza strain, wherein the segment comprises one or more (preferably all) of: adenine at position 13, adenine at position 35, adenine at position 63, adenine at position 65, cytosine at position 67, adenine at position 69, adenine at position 75, thymine at position 83, guanine at position 89, adenine at position 101, thymine at position 107, thymine at position 110, guanine at position 120, cytosine at position 121, thymine at position 125, cytosine at position 1385, thymine at position 1386, cytosine at position 1387, and/or guanine at position 1392. All of these positions are relative to the corresponding position in SEQ ID NO: 16 when aligned to SEQ ID NO: 16 using a pairwise alignment algorithm.

A chimeric neuraminidase segment may comprise one or more of the 5'- non-coding region, the 3'- non-coding region, the transmembrane domain and the cytoplasmic domain from the 105p30 influenza strain, which is discussed below. Preferably, the chimeric hemagglutinin segment comprises all of the 5'- non-coding region, the 3'- non-coding region, the transmembrane domain and the cytoplasmic domain from the 105p30 influenza strain as reassortant influenza viruses comprising such a chimeric neuraminidase segment give particularly good HA yields in cell culture.

Also provided is a chimeric NA protein which is encoded by a chimeric NA segment of the invention.

The inventors have discovered that reassortant influenza viruses which comprise a chimeric NA segment of the invention can provide HA yields which are up to 2-fold higher in the same time frame and under the same conditions compared to a reassortant influenza virus which does not comprise a chimeric NA segment.

The invention provides reassortant influenza viruses which comprise a chimeric HA and/or NA segment of the invention. Preferably, the reassortant influenza virus comprises both a chimeric HA and a chimeric NA segment of the invention as the inventors have discovered that such reassortant influenza viruses grow faster and give better HA yields than reassortant influenza viruses which comprise only a chimeric HA or a chimeric NA segment.

The invention also provides a reassortant influenza virus comprising:

a) a chimeric hemagglutinin protein having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a signal peptide, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are from a second influenza strain; and/or a chimeric neuraminidase protein having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is from a first influenza strain and one or more of the non-coding regions, the cytoplasmic domain, and the transmembrane domain are from a second influenza strain; and

(b) one or more of:

i. backbone segments from two or more different donor strains

ii. backbone segments from two or more donor strains, wherein the PB1 and the PB2 segments are from the same donor strain;

iii. backbone segments from two or three donor strains, wherein each donor strain provides more than one backbone segment;

iv. backbone segments from two or more donor strains, wherein the PB1 segment is not from the A/Texas/1/77 influenza strain;

v. backbone segments from two or more donor strains, wherein at least the PA, NP, or M segment are not from A/Puerto Rico/8/34;

vi. backbone segments from two or more donor strains, wherein the HA segment and the PB1 segment are from different influenza A strains with the same influenza virus HA subtype.

These reassortant influenza viruses are particularly useful because the inventors have discovered that influenza viruses which comprise backbone segments from two or more influenza donor strains can grow faster in a culture host compared with reassortant influenza viruses which contain all backbone segments from the same donor strain. In particular, the inventors have found that influenza viruses which comprise backbone segments from two high-yield donor strains can produce higher yield reassortants with target vaccine-relevant HA/NA genes than reassortants made with either of the two original donor strains. The first and the second influenza strains are preferably both influenza A or influenza B strains

The invention also provides a method of preparing a reassortant influenza virus comprising steps of (a) infecting a culture host with a reassortant influenza virus of the invention or a reassortant influenza virus produced by a method of the invention; (b) culturing the host from step (a) to produce the virus; and optionally (c) purifying the virus obtained in step (b).

The reassortant influenza virus may be formulated into a vaccine. The invention thus provides a method of preparing a vaccine, comprising steps of (a) preparing a reassortant influenza virus by a method according to the invention and (b) preparing a vaccine from the virus. Also, provided is a method of preparing a vaccine from a reassortant influenza virus of the invention.

Further provided is an expression system comprising one or more expression construct(s) encoding the vRNA of a reassortant influenza virus of the invention.

The chimeric HA and NA segments

The invention provides chimeric HA and NA segments.

Structurally, the influenza HA segment is composed of 5'- and 3'- non-coding regions (NCRs) which flank the HA segment's signal peptide (SP), transmembrane (TM), cytoplasmic domain (CT) and ectodomain (see Figure 4A). The HA ectodomain is the most important influenza antigen in influenza vaccines whilst the terminal domains (NCRs, SP, TM and CT) are of much less antigenic importance. The influenza NA segment also contains terminal domains which are the 5'- and 3'- NCRs, a CT domain and a TM domain, as well as an ectodomain, but NA does not contain a signal peptide (see Figure 4C). The terminal domains are of much less antigenic importance than the NA ectodomain.

A skilled person can readily determine the sequences of the terminal domains within any given HA and NA segment. Furthermore, SEQ ID NOs 105-109 and SEQ ID NOs 114-118 give the sequences of the HA terminal domains of 105p30 and PR8X, respectively. SEQ ID NOs 110-114 and SEQ ID NOs 119-122 give the sequences of the terminal domains of 105p30 and PR8X, respectively. Using this sequence information a skilled person can find the corresponding domains in other HA and NA sequences.

The chimeric HA segment of the invention comprises the ectodomain from a vaccine strain and one or more of the terminal domains from a second influenza virus. The vaccine strain can be any influenza strain and is defined as the influenza strain which provides the HA ectodomain. The second influenza strain is different to the vaccine strain. The vaccine strain and the second influenza strain are preferably both influenza A strains or both influenza B strains.

The chimeric NA segment of the invention comprises the ectodomain from a first influenza strain and one or more of the terminal domains from a second influenza virus. The 'second influenza strain' is different from the 'first influenza strain'. The first and the second influenza strain are preferably both influenza A strains or both influenza B strains.

It is preferred that the chimeric HA and NA segment comprises all of the terminal domains from the second influenza strain as the inventors have shown that reassortant influenza viruses comprising such chimeric HA and/or NA proteins can grow particularly well in cell culture.

The 'second influenza strain' can be a strain which has the influenza A virus HA subtypes H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16 or H17. It may also have the influenza A virus NA subtypes N1, N2, N3, N4, N5, N6, N7, N8 or N9. It is preferred that the second influenza virus is a H1 influenza strain as the inventors have discovered that reassortant influenza viruses which contain such chimeric HA and/or NA segments grow particularly well in cell culture. Most preferably, the second influenza strain is 105p30 or PR8-X, as discussed below.

Where the chimeric HA segment comprises one or more terminal domains from 105p30, the 5'- NCR domain may have the sequence of SEQ ID NO: 105; and/or the SP of SEQ ID NO: 106; and/or the TM domain of SEQ ID NO: 107; and/or the CT domain of SEQ ID NO: 108; and/or the 3'- NCR of SEQ ID NO: 109. Preferably, the chimeric HA segment contains all of these sequences.

Where the chimeric NA segment comprises one or more terminal domains from 105p30, the 5'- NCR domain may have the sequence of SEQ ID NO: 110; and/or the CT domain of SEQ ID NO: 71; and/or the TM domain of SEQ ID NO: 112; and/or the 3'- NCR of SEQ ID NO: 113. Preferably, the chimeric NA segment contains all of these sequences.

Where the chimeric HA segment comprises one or more terminal domains from PR8-X, the 5'- NCR domain may have the sequence of SEQ ID NO: 114; and/or the SP of SEQ ID NO: 115; and/or the TM domain of SEQ ID NO: 116; and/or the CT domain of SEQ ID NO: 117; and/or the 3'- NCR of SEQ ID NO: 118. Preferably, the chimeric HA segment contains all of these sequences.

Where the chimeric NA segment comprises one or more terminal domains from PR8-X, the 5'- NCR domain may have the sequence of SEQ ID NO: 119; and/or the CT domain of SEQ ID NO: 120; and/or the TM domain of SEQ ID NO: 121; and/or the 3'- NCR of SEQ ID NO: 122. Preferably, the chimeric NA segment contains all of these sequences.

The second influenza strain can be an influenza B strain.

The ectodomain and the one or more terminal domains may all be from an influenza A virus or an influenza B virus. It is also possible to have the ectodomain from an influenza A virus and one or more of the terminal domains from an influenza B virus and *vice versa*. It is most preferred that all the segments in the chimeric HA or the chimeric NA segments are from influenza A strains or influenza B strains.

In some embodiments, the chimeric HA segments of the invention encode a protein which does not have tyrosine in the position corresponding to amino acid 545, when aligned to SEQ ID NO: 7.

Reassortant viruses

The invention provides a reassortant influenza virus which comprises the chimeric HA and/or NA segments of the invention. The reassortant influenza virus comprises the HA ectodomain from a vaccine strain. The vaccine strain can be any influenza strain and is defined as the influenza strain which provides the HA ectodomain, irrespective of whether the HA ectodomain is comprised in a chimeric HA segment or not. The ectodomain of the NA segment (in a chimeric or a non-chimeric NA segment) may come from the vaccine strain or it may come from a different influenza strain.

One or more of the backbone segments (*i.e.* those encoding PB1, PB2, PA, NP, M_1, M_2, NS_1 and NS_2) of the reassortant influenza virus may come from a donor strain, which is an influenza virus that provides one or more of the backbone segments but which does not provide the ectodomain of the influenza HA segment. The ectodomain of the NA segment may also be provided by a donor strain or it may be provided by the vaccine strain. The reassortant influenza strains of the invention may also comprise one or more, but not all, of the backbone segments from the vaccine strain.

The donor strain may be the same as the 'second influenza strain' which provides the one or more terminal domains of the chimeric HA or NA segments. In these reassortant influenza viruses, the PA, M and/or NS segment(s) is/are preferably from the second influenza virus. The second influenza virus may also be different to the donor strain.

The reassortant influenza virus may grow to higher or similar viral titres in cell culture and/or in eggs in the same time (for example 12 hours, 24 hours, 48 hours or 72 hours) and under the same growth conditions compared to the wild-type vaccine strain. In particular, they can grow to higher or similar viral titres in MDCK cells (such as MDCK 33016) in the same time and under the same growth conditions compared to the wild-type vaccine strain. The viral titre can be determined by standard methods known to those of skill in the art. Usefully, the reassortant viruses of the invention may achieve a viral titre which is at least 5% higher, at least 10% higher, at least 20% higher, at least 50% higher, at least 100% higher, at least 200% higher, or at least 500% higher than the viral titre of the wild-type vaccine strain in the same time frame and under the same conditions. In addition, or alternatively, the reassortant influenza viruses of the invention may achieve a viral titre which is at least 5% higher, at least 10% higher, at least 20% higher, at least 50% higher, at least 100% higher,

at least 200% higher, or at least 500% higher than the viral titre of a reassortant influenza virus which comprises the same viral segments expect that it does not have a chimeric HA or NA segment.

The reassortant influenza viruses may also grow to similar viral titres in the same time and under the same growth conditions compared to the wild-type vaccine strain. A similar titre in this context means that the reassortant influenza viruses grow to a titre which is within 3% of the viral titre achieved with the wild-type vaccine strain in the same time and under the same growth conditions (*i.e.* wild-type titre ± 3%).

The reassortant virus may also give HA yields which are at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold or at least 10-fold higher in cell culture and/or in eggs in the same time (for example 12 hours, 24 hours, 48 hours or 72 hours) and under the same growth conditions compared to the wild-type vaccine strain.

When the reassortant viruses of the invention are reassortants comprising the backbone segments from a single donor strain, the reassortant viruses will generally include segments from the donor strain and the vaccine strain in a ratio of 1:7, 2:6, 3:5, 4:4, 5:3, 6:2 or 7:1. Classical reassortants usually have a majority of segments from the donor strain, in particular a ratio of 6:2. Where only a single donor strain is used, it is preferred that all backbone segments are from PR8-X as such reassortant influenza viruses grow fast in cell culture.

The reassortant viruses of the invention can contain the backbone segments from two or more (*i.e.* three, four, five or six) donor strains. When the reassortant viruses comprise backbone segments from two donor strains, the reassortant virus will generally include segments from the first donor strain, the second donor strain and the vaccine strain in a ratio of 1:1:6, 1:2:5, 1:3:4, 1:4:3, 1:5:2, 1:6:1, 2:1:5, 2:2:4, 2:3:3, 2:4:2, 2:5:1, 3:1:2, 3:2:1, 4:1:3, 4:2:2, 4:3:1, 5:1:2, 5:2:1 or 6:1:1. The reassortant influenza viruses may also comprise viral segments from more than two, for example from three, four, five or six donor strains.

Where the reassortant influenza virus comprises backbone segments from two or three donor strains, each donor strain may provide more than one of the backbone segments of the reassortant influenza virus, but one or two of the donor strains can also provide only a single backbone segment.

Where the reassortant influenza virus comprises backbone segments from two, three, four or five donor strains, one or two of the donor strains may provide more than one of the backbone segments of the reassortant influenza virus. In general, the reassortant influenza virus cannot comprise more than six backbone segments. Accordingly, for example, if one of the donor strains provides five of the viral segments, the reassortant influenza virus can only comprise backbone segments from a total of two different donor strains.

In general a reassortant influenza virus will contain only one of each backbone segment. For example, when the influenza virus comprises the NP segment from A/California/07/09 it will not at the same time comprise the NP segment from another influenza strain.

The reassortant influenza virus may comprise the HA ectodomain from an influenza A strain. For example, the reassortant influenza virus may have the influenza A virus HA subtypes H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16 or H17. In addition, or alternatively, the reassortant influenza virus may comprise the NA ectodomain from an influenza A virus. For example, it may have the influenza A virus NA subtypes N1, N2, N3, N4, N5, N6, N7, N8 or N9. Where the vaccine strain is a seasonal influenza strain, it may have a H1 or H3 subtype. In one aspect of the invention the vaccine strain is a H1N1, a H3N2 or a H7N9 strain.

The reassortant influenza virus preferably comprises at least one backbone segment from the donor strain PR8-X. Thus, the influenza viruses of the invention may comprise one or more segments selected from: a PA segment having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of SEQ ID NO: 9, a PB1 segment having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of SEQ ID NO: 10, a PB2 segment having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of SEQ ID NO: 11, a NP segment having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of SEQ ID NO: 12, a M segment having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of SEQ ID NO: 13, and/or a NS segment having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of SEQ ID NO: 14. The reassortant influenza virus may comprise all of these backbone segments. This is particularly preferred as the inventors have shown that reassortant influenza viruses comprising a chimeric HA and/or NA segment in combination with this backbone grow particularly well in cell culture.

Alternatively, or in addition, the reassortant influenza virus may comprise one or more backbone viral segments from the 105p30 strain. Thus, where the reassortant influenza virus comprises one or more segments from the 105p30 strain, the viral segments may have sequences selected from: a PA segment having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of SEQ ID NO: 42, a PB1 segment having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of SEQ ID NO: 43, a PB2 segment having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of SEQ ID NO: 44, a NP segment having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of SEQ ID NO: 45, a M segment having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of SEQ ID NO: 46, and/or a NS segment having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of SEQ ID NO: 47. The reassortant influenza virus may comprise all of these backbone segments.

Reassortant influenza viruses with backbone segments from two or more influenza donor strains may comprise the HA segment and the PB1 segment from different influenza strains. In these reassortant influenza viruses the PB1 segment may be from donor viruses with the same influenza virus HA subtype as the vaccine strain. For example, the PB1 segment and the HA segment may both be from influenza viruses with a H1 subtype. The reassortant influenza viruses may also comprise the HA segment and the PB1 segment from different influenza strains with different influenza virus HA subtypes, wherein the PB1 segment is not from an influenza virus with a H3 HA subtype and/or wherein the HA segment is not from an influenza virus with a H1 or H5 HA subtype. For example, the PB1 segment may be from a H1 virus and/or the HA segment may be from a H3 influenza virus. Where the reassortants contain viral segments from more than one influenza donor strain, the further donor strain(s) can be any donor strain. For example, some of the viral segments may be from the A/Puerto Rico/8/34 or A/Ann Arbor/6/60 influenza strains. Reassortants containing viral segments from the A/Ann Arbor/6/60 strain may be advantageous, for example, where the reassortant virus is to be used in a live attenuated influenza vaccine.

The reassortant influenza virus may also comprise backbone segments from two or more influenza donor strains, wherein the PB1 segment is from the A/California/07/09 influenza strain. This segment may have at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity or 100% identity with the sequence of SEQ ID NO: 24. The reassortant influenza virus may have the H1 HA subtype. It will be understood that a reassortant influenza virus according to this aspect of the invention will not comprise the HA and/or NA segments from A/California/07/09.

The reassortant influenza strain may comprise the HA ectodomain and/or the NA ectodomain from an A/California/4/09 strain. Thus, for instance, the HA gene segment may encode a H1 hemagglutinin whose ectodomain is more closely related to SEQ ID NO: 70 than to SEQ ID NO: 50 (*i.e.* has a higher degree sequence identity when compared to SEQ ID NO: 70 than to SEQ ID NO: 50 using the same algorithm and parameters). SEQ ID NOs: 70 and 50 are 80% identical. Similarly, the NA gene may encode a N1 neuraminidase which is more closely related to SEQ ID NO: 99 than to SEQ ID NO: 51. SEQ ID NOs: 99 and 51 are 82% identical.

The reassortant influenza virus may also comprise at least one backbone viral segment from the A/California/07/09 influenza strain. When the at least one backbone viral segment is the PA segment it may have a sequence having at least 95%, at least 96%, at least 97% or at least 99% identity with the sequence of SEQ ID NO: 23. When the at least one backbone viral segment is the PB1 segment, it may have a sequence having at least 95%, at least 96%, at least 97% or at least 99% identity with the sequence of SEQ ID NO: 24. When the at least one backbone viral segment is the PB2 segment, it may have a sequence having at least 95%, at least 96%, at least 97% or at least 99% identity with the sequence of SEQ ID NO: 25. When the at least one backbone viral segment is the NP segment it may have a sequence having at least 95%, at least 96%, at least 97% or at least 99% identity with the

sequence of SEQ ID NO: 26. When the at least one backbone viral segment is the M segment it may have a sequence having at least 95%, at least 96%, at least 97% or at least 99% identity with the sequence of SEQ ID NO: 27. When the at least one backbone viral segment is the NS segment it may have a sequence having at least 95%, at least 96%, at least 97% or at least 99% identity with the sequence of SEQ ID NO: 28.

Where a reassortant influenza virus comprises the PB1 segment from A/Texas/1/77, it preferably does not comprise the PA, NP or M segment from A/Puerto Rico/8/34. Where a reassortant influenza A virus comprises the PA, NP or M segment from A/Puerto Rico/8/34, it preferably does not comprise the PB1 segment from A/Texas/1/77. In some embodiments, the invention does not encompass reassortant influenza viruses which have the PB1 segment from A/Texas/1/77 and the PA, NP and M segments from A/Puerto Rico/8/34. The PB1 protein from A/Texas/1/77 may have the sequence of SEQ ID NO: 29 and the PA, NP or M proteins from A/Puerto Rico/8/34 may have the sequence of SEQ ID NOs 30, 31 or 32, respectively.

Particularly preferred are reassortant influenza viruses which comprise a chimeric HA and/or NA segment according to the invention (preferably both), the NP, PB1 and PB2 segments from 105p30 and the M, NS and PA segments from PR8-X. Also particularly preferred are reassortant influenza viruses which comprise a chimeric HA and/or NA segment according to the invention (preferably both), the PB1 segment from A/California/4/09 and the other backbone segments from PR8-X. Such reassortant influenza viruses are preferred because the inventors have found that they grow very well in cell culture and provide very good HA yields.

The backbone viral segments may encode viral proteins which are optimized for culture in the specific culture host. For example, where the reassortant influenza viruses are cultured in mammalian cells, it is advantageous to adapt at least one of the viral segments for optimal growth in the culture host. For instance, where the expression host is a canine cell, such as a MDCK cell line, the viral segments may encode proteins which have a sequence that optimises viral growth in the cell. Thus, the reassortant influenza viruses of the invention may comprise a PB2 segment which encodes a PB2 protein that has lysine in the position corresponding to amino acid 389 of SEQ ID NO: 3 when aligned to SEQ ID NO: 3 using a pairwise alignment algorithm, and/or asparagine in the position corresponding to amino acid 559 of SEQ ID NO: 3 when aligned to SEQ ID NO: 3 using a pairwise alignment algorithm. Also provided are reassortant influenza viruses in accordance with the invention in which the PA segment encodes a PA protein that has lysine in the position corresponding to amino acid 327 of SEQ ID NO: 1 when aligned to SEQ ID NO: 1 using a pairwise alignment algorithm, and/or aspartic acid in the position corresponding to amino acid 444 of SEQ ID NO: 1 when aligned to SEQ ID NO: 1, using a pairwise alignment algorithm, and/or aspartic acid in the position corresponding to amino acid 675 of SEQ ID NO: 1 when aligned to SEQ ID NO: 1, using a pairwise alignment algorithm. The reassortant influenza strains of the invention may also have a NP segment which encodes a NP protein with threonine in the position corresponding to

amino acid 27 of SEQ ID NO: 4 when aligned to SEQ ID NO: 4 using a pairwise alignment algorithm, and/or asparagine in the position corresponding to amino acid 375 of SEQ ID NO: 4 when aligned to SEQ ID NO: 4, using a pairwise alignment algorithm. Variant influenza strains may also comprise two or more of these mutations. It is preferred that the variant influenza virus contains a variant PB2 protein with both of the amino acids changes identified above, and/or a PA protein which contains all three of the amino acid changes identified above, and/or a NP protein which contains both of the amino acid changes identified above. The influenza virus may be a H1 strain.

Alternatively, or in addition, the reassortant influenza viruses may comprise a PB1 segment which encodes a PB1 protein that has isoleucine in the position corresponding to amino acid 200 of SEQ ID NO: 2 when aligned to SEQ ID NO: 2 using a pairwise alignment algorithm, and/or asparagine in the position corresponding to amino acid 338 of SEQ ID NO: 2 when aligned to SEQ ID NO: 2 using a pairwise alignment algorithm, and/or isoleucine in the position corresponding to amino acid 529 of SEQ ID NO: 2 when aligned to SEQ ID NO: 2 using a pairwise alignment algorithm, and/or isoleucine in the position corresponding to amino acid 591 of SEQ ID NO: 2 when aligned to SEQ ID NO: 2 using a pairwise alignment algorithm, and/or histidine in the position corresponding to amino acid 687 of SEQ ID NO: 2 when aligned to SEQ ID NO: 2 using a pairwise alignment algorithm, and/or lysine in the position corresponding to amino acid 754 of SEQ ID NO: 2 when aligned to SEQ ID NO: 2 using a pairwise alignment algorithm.

The choice of donor strain for use in the methods of the invention can depend on the vaccine strain which is to be reassorted. As reassortants between evolutionary distant strains might not replicate well in cell culture, it is possible that the donor strain and the vaccine strain have the same HA and/or NA subtype. In other embodiments, however, the vaccine strain and the donor strain can have different HA and/or NA subtypes, and this arrangement can facilitate selection for reassortant viruses that contain the HA and/or NA segment from the vaccine strain. Therefore, although the 105p30 and PR8-X strains contain the H1 influenza subtype these donor strains can be used for vaccine strains which do not contain the H1 influenza subtype.

Thus, an influenza virus may comprises one, two, three, four, five, six or seven viral segments from the 105p30 or PR8-X strains and a HA segment which is not of the H1 subtype. The reassortant donor strains may further comprise an NA segment which is not of the N1 subtype.

Strains which can be used as vaccine strains include strains which are resistant to antiviral therapy (*e.g.* resistant to oseltamivir [8] and/or zanamivir), including resistant pandemic strains [9].

The reassortant influenza virus may be an influenza B virus. For example, the reassortant influenza virus may comprises the HA ectodomain from a first influenza B virus and the NP and/or PB2 segment from a second influenza B virus which is a B/Victoria/2/87-like strain. The B/Victoria/2/87-like strain may be B/Brisbane/60/08.

The reassortant influenza B virus may comprise the HA ectodomain from a first influenza B virus and the NP segment from a second influenza B virus which is not B/Lee/40 or B/Ann Arbor/1/66 or B/Panama/45/90. For example, the reassortant influenza B virus may have a NP segment which does not have the sequence of SEQ ID NOs: 80, 100, 103 or 104. The reassortant influenza B virus may also have a NP segment which does not encode the protein of SEQ ID NOs: 19, 23, 44 or 45. The reassortant influenza B virus may comprise both the NP and PB2 segments from the second influenza B virus. The second influenza B virus is preferably a B/Victoria/2/87-like strain. The B/Victoria/2/87-like strain may be B/Brisbane/60/08.

The reassortant influenza B virus may comprise the HA ectodomain from a B/Yamagata/16/88-like strain and at least one backbone segment from a B/Victoria/2/87-like strain. The reassortant influenza B virus may comprise two, three, four, five or six backbone segments from the B/Victoria/2/87-like strain. In a preferred embodiment, the reassortant influenza B virus comprises all the backbone segments from the B/Victoria/2/87-like strain. The B/Victoria/2/87-like strain may be B/Brisbane/60/08.

The reassortant influenza B virus may comprise viral segments from a B/Victoria/2/87-like strain and a B/Yamagata/16/88-like strain, wherein the ratio of segments from the B/Victoria/2/87-like strain and the B/Yamagata/16/88-like strain is 1:7, 2:6, 4:4, 5:3, 6:2 or 7:1. A ratio of 7:1, 6:2, 4:4, 3:4 or 1:7, in particular a ratio of 4:4, is preferred because such reassortant influenza B viruses grow particularly well in a culture host. The B/Victoria/2/87-like strain may be B/Brisbane/60/08. The B/Yamagata/16/88-like strain may be B/Panama/45/90. In these embodiments, the reassortant influenza B virus usually does not comprise all backbone segments from the same influenza B donor strain.

The reassortant influenza B virus may comprise:

a) the PA segment of SEQ ID NO: 71, the PB1 segment of SEQ ID NO: 72, the PB2 segment of SEQ ID NO: 73, the NP segment of SEQ ID NO: 74, the NS segment of SEQ ID NO: 76 and the M segment of SEQ ID NO: 75; or

b) the PA segment of SEQ ID NO: 71, the PB1 segment of SEQ ID NO: 78, the PB2 segment of SEQ ID NO: 73, the NP segment of SEQ ID NO: 74, the NS segment of SEQ ID NO: 82 and the M segment of SEQ ID NO: 81; or

c) the PA segment of SEQ ID NO: 71, the PB1 segment of SEQ ID NO: 78, the PB2 segment of SEQ ID NO: 79, the NP segment of SEQ ID NO: 74, the NS segment of SEQ ID NO: 76 and the M segment of SEQ ID NO: 75; or

d) the PA segment of SEQ ID NO: 30, the PB1 segment of SEQ ID NO: 72, the PB2 segment of SEQ ID NO: 73, the NP segment of SEQ ID NO: 74, the NS segment of SEQ ID NO: 76 and the M segment of SEQ ID NO: 75, or

e) the PA segment of SEQ ID NO: 71, the PB1 segment of SEQ ID NO: 72, the PB2 segment of SEQ ID NO: 73, the NP segment of SEQ ID NO: 74, the NS segment of SEQ ID NO: 82 and the M segment of SEQ ID NO: 81.

Influenza B viruses currently do not display different HA subtypes, but influenza B virus strains do fall into two distinct lineages. These lineages emerged in the late 1980s and have HAs which can be antigenically and/or genetically distinguished from each other [10]. Current influenza B virus strains are either B/Victoria/2/87-like or B/Yamagata/16/88-like. These strains are usually distinguished antigenically, but differences in amino acid sequences have also been described for distinguishing the two lineages *e.g.* B/Yamagata/16/88-like strains often (but not always) have HA proteins with deletions at amino acid residue 164, numbered relative to the 'Lee40' HA sequence [11]. In some embodiments, the reassortant influenza B viruses of the invention may comprise viral segments from a B/Victoria/2/87-like strain. They may comprise viral segments from a B/Yamagata/16/88-like strain. Alternatively, they may comprise viral segments from a B/Victoria/2/87-like strain and a B/Yamagata/16/88-like strain.

Where the reassortant influenza B virus comprises viral segments from two or more influenza B virus strains, these viral segments may be from influenza strains which have related neuraminidases. For instance, the influenza strains which provide the viral segments may both have a B/Victoria/2/87-like neuraminidase [12] or may both have a B/Yamagata/16/88-like neuraminidase. For example, two B/Victoria/2/87-like neuraminidases may both have one or more of the following sequence characteristics: (1) not a serine at residue 27, but preferably a leucine; (2) not a glutamate at residue 44, but preferably a lysine; (3) not a threonine at residue 46, but preferably an isoleucine; (4) not a proline at residue 51, but preferably a serine; (5) not an arginine at residue 65, but preferably a histidine; (6) not a glycine at residue 70, but preferably a glutamate; (7) not a leucine at residue 73, but preferably a phenylalanine; and/or (8) not a proline at residue 88, but preferably a glutamine. Similarly, in some embodiments the neuraminidase may have a deletion at residue 43, or it may have a threonine; a deletion at residue 43, arising from a trinucleotide deletion in the NA gene, which has been reported as a characteristic of B/Victoria/2/87-like strains, although recent strains have regained Thr-43 [12]. Conversely, of course, the opposite characteristics may be shared by two B/Yamagata/16/88-like neuraminidases *e.g.* S27, E44, T46, P51, R65, G70, L73, and/or P88. These amino acids are numbered relative to the 'Lee40' neuraminidase sequence [13]. The reassortant influenza B virus may comprise a NA segment with the characteristics described above. Alternatively, or in addition, the reassortant influenza B virus may comprise a viral segment (other than NA) from an influenza strain with a NA segment with the characteristics described above.

The backbone viral segments of an influenza B virus which is a B/Victoria/2/87-like strain can have a higher level of identity to the corresponding viral segment from B/Victoria/2/87 than it does to the corresponding viral segment of B/Yamagata/16/88 and *vice versa*. For example, the NP segment of

B/Panama/45/90 (which is a B/Yamagata/16/88-like strain) has 99% identity to the NP segment of B/Yamagata/16/88 and only 96% identity to the NP segment of B/Victoria/2/87.

Where the reassortant influenza B virus of the invention comprises a backbone viral segment from a B/Victoria/2/87-like strain, the viral segments may encode proteins with the following sequences. The PA protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 83. The PB1 protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 84. The PB2 protein may have at least 97%, at least 98%, at least 99% or 100% identity with the sequence of SEQ ID NO: 85. The NP protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 86. The M_1 protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 87. The M_2 protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 88. The NS_1 protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 89. The NS_2 protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 90. In some embodiments, the reassortant influenza B virus may also comprise all of these backbone segments.

Where the reassortant influenza B viruses of the invention comprise a backbone viral segment from a B/Yamagata/16/88-like strain, the viral segment may encode proteins with the following sequences. The PA protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 91. The PB1 protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 92. The PB2 protein may have at least 97%, at least 98%, at least 99% or 100% identity with the sequence of SEQ ID NO: 93. The NP protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 94. The M_1 protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 95. The M_2 protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 96. The NS_1 protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 97. The NS_2 protein may have at least 97% identity, at least 98%, at least 99% identity or 100% identity to the sequence of SEQ ID NO: 98.

The invention can be practised with donor strains having a viral segment that has at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95% or at least about 99%, or 100% identity to a sequence of SEQ ID NOs 71-76 or 77-82. Due to the degeneracy of the genetic code, it is possible to have the same polypeptide encoded by several nucleic acids with different sequences. For example, the nucleic acid sequences of SEQ ID NOs: 33 and 34 have only 73% identity even though they encode the same viral protein. Thus, the invention may be practised with viral segments that encode the same polypeptides as the sequences of SEQ ID NOs 71-76 or 77-82.

The reassortant influenza virus may comprise segments from a vaccine strain which is an inter-pandemic (seasonal) influenza vaccine strain. It may also comprise segments from a vaccine strain which is a pandemic strain or a potentially pandemic strain. The characteristics of an influenza strain that give it the potential to cause a pandemic outbreak are: (a) it contains a new hemagglutinin compared to the hemagglutinins 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 hemagglutinin; (b) it is capable of being transmitted horizontally in the human population; and (c) it is pathogenic to humans. A vaccine strain with H5 hemagglutinin type is preferred where the reassortant virus is used in vaccines for immunizing against pandemic influenza, such as a H5N1 strain. Other possible strains include H5N3, H9N2, H2N2, H7N1, H7N7 and H7N9, and any other emerging potentially pandemic strains. The invention is particularly suitable for producing reassortant viruses for use in vaccine for protecting against potential pandemic virus strains that can or have spread from a non-human animal population to humans, for example a swine-origin H1N1 influenza strain.

Expression constructs

The invention provides an expression construct which encodes the chimeric HA or NA segments of the invention. Further provided are expression constructs which encode the viral segments of a reassortant influenza virus of the invention.

The invention also provides an expression construct encoding the HA and/or NA terminal domains of the chimeric HA and/or NA segments of the invention. These expression constructs are useful because the HA and NA ectodomains which need to be included in influenza vaccines change every season. The expression construct of this aspect of the invention may further encode one or more of the backbone segments. By including the terminal domains in the expression construct, it is necessary only to clone the ectodomain of the HA and/or NA segments of the circulating strain in order to provide the chimeric HA and/or NA molecule. The expression construct may comprise a restriction site between the SP and the TM domain which is useful as it facilitates cloning of the ectodomain. It is understood that the ectodomain needs to be cloned in frame with the terminal domains but this is well within the capabilities of a skilled person.

Expression constructs may be uni-directional or bi-directional expression constructs. Where more than one expression construct is used to express the viral segments of a reassortant influenza virus, it is possible to use uni-directional and/or bi-directional expression.

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. Thus, the method of the invention may utilise at least one bi-directional expression construct wherein a 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 vRNA. The bi-directional expression construct may be a bi-directional expression vector.

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. Preferably, one of the promoters is a pol I promoter and at least one of the other promoters is a pol II promoter. This is useful as the pol I promoter can be used to express uncapped vRNAs while the pol II promoter can be used to transcribe mRNAs which can subsequently be translated into proteins, thus allowing simultaneous expression of RNA and protein from the same construct. Where more than one expression construct is used within an expression system, the promoters may be a mixture of endogenous and non-endogenous promoters.

The pol I and pol II promoters used in the expression constructs may be endogenous to an organism from the same taxonomic order from which the host cell is derived. Alternatively, the promoters can be from an organism in a different taxonomic order than the host cell. The term "order" refers to conventional taxonomic ranking, and examples of orders are primates, rodentia, carnivora, marsupialia, cetacean, *etc.* Humans and chimpanzees are in the same taxonomic order (primates), but humans and dogs are in different orders (primates *vs.* carnivora). For example, the human pol I promoter can be used to express viral segments in canine cells (*e.g.* MDCK cells) [14].

The expression construct will typically 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.

An expression construct may be a vector, such as a plasmid or other episomal construct. Such vectors will typically comprise at least one bacterial and/or eukaryotic origin of replication. Furthermore, the vector may comprise a selectable marker which allows for selection in prokaryotic and/or eukaryotic cells. Examples of such selectable markers are genes conferring resistance to antibiotics, such as ampicillin or kanamycin. The vector may further comprise one or more multiple cloning sites to facilitate cloning of a DNA sequence.

As an alternative, an expression construct may 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 invention. Reference 15 describes a linear expression construct which describes individual linear expression constructs for each viral segment. It is also possible to include more than one, for example two, three four, five or six viral segments on the same linear expression construct. Such a system has been described, for example, in reference 16.

Expression constructs can be generated using methods known in the art. Such methods were described, for example, in reference 17. Where the expression construct is a linear expression construct, it is possible to linearise it before introduction into the host cell utilising a single restriction enzyme site. Alternatively, it is possible to excise the expression construct from a vector using at least two restriction enzyme sites. Furthermore, it is also possible to obtain a linear expression construct by amplifying it using a nucleic acid amplification technique (*e.g.* by PCR).

The expression constructs may be non-bacterial expression constructs. This means that the construct can drive expression in a eukaryotic cell of viral RNA segments encoded therein, but it does not include components which would be required for propagation of the construct in bacteria. Thus the construct will not include a bacterial origin of replication (ori), and usually will not include a bacterial selection marker (e.g. an antibiotic resistance marker). Such expression constructs are described in reference 18 which is incorporated by reference.

The expression constructs may be prepared by chemical synthesis. The expression constructs may either be prepared entirely by chemical synthesis or in part. Suitable methods for preparing expression constructs by chemical synthesis are described, for example, in reference 18.

The expression constructs of the invention can be introduced into host cells using any technique known to those of skill in the art. For example, expression constructs of the invention can be introduced into host cells by employing electroporation, DEAE-dextran, calcium phosphate precipitation, liposomes, microinjection, or microparticle-bombardment.

The expression construct(s) can be introduced into the same cell type which is subsequently used for the propagation of the influenza viruses. Alternatively, the cells into which the expression constructs are introduced and the cells used for propagation of the influenza viruses may be different.

In some embodiments, cells may be added following the introduction of the expression construct(s) into the cell, as described in reference 19. This is particularly preferred because it increases the rescue efficiency of the viruses further and can thus help to reduce the time required for viral rescue. The cells which are added may be of the same or a different cell type as the cell into which the expression construct(a) is/are introduced, but it is preferred to use cells of the same cell type as this facilitates regulatory approval and avoids conflicting culture conditions.

The invention also provides an expression system which comprises one or more of the expression constructs of the invention. The expression system may comprise one or more expression constructs which encode all the viral segments of a reassortant influenza virus of the invention.

The expression system may comprise at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven or at least twelve expression constructs.

Reverse genetics

The invention is particularly suitable for producing the reassortant influenza viruses of the invention through reverse genetics techniques where the viruses are produced in culture hosts using an expression system which comprises one or more of the expression constructs of the invention. In these techniques, it is understood that the virus is produced from the expression construct(s) in the expression system.

Reverse genetics for influenza A and B viruses can be practised with 12 plasmids to express the four proteins required to initiate replication and transcription (PB1, PB2, PA and NP) and all eight viral genome segments. To reduce the number of constructs, however, a plurality of RNA polymerase I transcription cassettes (for viral RNA synthesis) can be included on a single plasmid (*e.g.* sequences encoding 1, 2, 3, 4, 5, 6, 7 or all 8 influenza vRNA segments), and a plurality of protein-coding regions with RNA polymerase II promoters on another plasmid (*e.g.* sequences encoding 1, 2, 3, 4, 5, 6, 7 or 8 influenza mRNA transcripts) [20]. It is also possible to include one or more influenza vRNA segments under control of a pol I promoter and one or more influenza protein coding regions under control of another promoter, in particular a pol II promoter, on the same plasmid. This is preferably done by using bi-directional plasmids.

Preferred aspects of the reference 20 method involve: (a) PB1, PB2 and PA mRNA-encoding regions on a single expression construct; and (b) all 8 vRNA encoding segments on a single expression construct. Including the neuraminidase (NA) and hemagglutinin (HA) segments on one expression construct and the six other viral segments on another expression construct is particularly preferred as newly emerging influenza virus strains usually have mutations in the NA and/or HA segments. Therefore, the advantage of having the HA and/or NA segments on a separate expression construct is that only the vector comprising the HA and NA sequence needs to be replaced. Thus, in one aspect of the invention the NA and/or HA segments of the vaccine strain may be included on one expression construct and the vRNA encoding segments from the donor strain(s) of the invention, excluding the HA and/or NA segment(s), are included on a different expression construct. The invention thus provides an expression construct comprising one, two, three, four, five or six vRNA encoding backbone viral segments of a donor strain of the invention. The expression construct may not comprise HA and/or NA viral segments that produce a functional HA and/or NA protein.

Known reverse genetics systems involve expressing DNA molecules which encode desired viral RNA (vRNA) molecules from pol I promoters, bacterial RNA polymerase promoters, bacteriophage polymerase promoters, *etc.* As influenza viruses require the presence of viral polymerase to initiate the life cycle, systems may also provide these proteins *e.g.* the system further comprises DNA

molecules that encode viral polymerase proteins such that expression of both types of DNA leads to assembly of a complete infectious virus. It is also possible to supply the viral polymerase as a protein.

Where reverse genetics is used for the expression of influenza vRNA, it will be evident to the person skilled in the art that precise spacing of the sequence elements with reference to each other is important for the polymerase to initiate replication. It is therefore important that the DNA molecule encoding the viral RNA is positioned correctly between the pol I promoter and the termination sequence, but this positioning is well within the capabilities of those who work with reverse genetics systems.

In order to produce a recombinant virus, a cell must express all segments of the viral genome which are necessary to assemble a virion. DNA cloned into the expression constructs of the present invention preferably provides all of the viral RNA and proteins, but it is also possible to use a helper virus to provide some of the RNA and proteins, although systems which do not use a helper virus are preferred. As the influenza virus is a segmented virus, the viral genome will usually be expressed using more than one expression construct in the methods of the invention. It is also envisioned, however, to combine one or more segments or even all segments of the viral genome on a single expression construct.

In some embodiments an expression construct will also be included which leads to expression of an accessory protein in the host cell. For instance, it can be advantageous to express a non-viral serine protease (*e.g.* trypsin) as part of a reverse genetics system.

Cells

The culture host for use in the invention can be any eukaryotic cell that can produce the virus of interest. The invention will typically use a cell line although, for example, primary cells may be used as an alternative. The cell will typically be mammalian or avian. 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, lung cells, *etc.* Examples of suitable hamster cells are the cell lines having the names BHK21 or HKCC. Suitable monkey cells are e.g. African green monkey cells, such as kidney cells as in the Vero cell line [21-23]. Suitable dog cells are *e.g.* kidney cells, as in the CLDK and MDCK cell lines.

Further suitable cells include, but are not limited to: CHO; 293T; BHK; MRC 5; PER.C6 [24]; FRhL2; WI-38; etc. Suitable cells are widely available e.g. from the American Type Cell Culture (ATCC) collection [25], from the Coriell Cell Repositories [26], or from the European Collection of Cell Cultures (ECACC). For example, the ATCC supplies various different Vero cells under catalogue numbers CCL 81, CCL 81.2, CRL 1586 and CRL-1587, and it supplies MDCK cells under catalogue number CCL 34. PER.C6 is available from the ECACC under deposit number 96022940.

Preferred cells for use in the invention are MDCK cells [27-29], derived from Madin Darby canine kidney. The original MDCK cells are available from the ATCC as CCL 34. It is preferred that derivatives of MDCK cells are used. Such derivatives were described, for instance, in reference 27 which discloses MDCK cells that were adapted for growth in suspension culture ('MDCK 33016' or '33016-PF', deposited as DSM ACC 2219). Furthermore, reference 30 discloses MDCK-derived cells that grow in suspension in serum free culture ('B-702', deposited as FERM BP-7449). In some embodiments, the MDCK cell line used may be tumorigenic. It is also envisioned to use non-tumorigenic MDCK cells. For example, reference 31 discloses non tumorigenic MDCK cells, including 'MDCK-S' (ATCC PTA-6500), 'MDCK-SF101' (ATCC PTA-6501), 'MDCK-SF102' (ATCC PTA-6502) and 'MDCK-SF103' (ATCC PTA-6503). Reference 32 discloses MDCK cells with high susceptibility to infection, including 'MDCK.5F1' cells (ATCC CRL 12042).

The cells used in the methods of the invention are preferably cells which are suitable for producing an influenza vaccine that can be used for administration to humans. Such cells must be derived from a cell bank system which is approved for vaccine manufacture and registered with a national control authority, and must be within the maximum number of passages permitted for vaccine production (see reference 33 for a summary). Examples of suitable cells which have been approved for vaccine manufacture include MDCK cells (like MDCK 33016; see reference 27), CHO cells, Vero cells, and PER.C6 cells. The methods of the invention may not use 293T cells as these cells are not approved for vaccine manufacture.

It is possible to use a mixture of more than one cell type to practise the methods of the present invention. However, it is preferred that the methods of the invention are practised with a single cell type *e.g.* with monoclonal cells. Preferably, the cells used in the methods of the present invention are from a single cell line. Furthermore, the same cell line may be used for reassorting the virus and for any subsequent propagation of the virus.

Preferably, the cells are cultured in the absence of serum, to avoid a common source of contaminants. Various serum-free media for eukaryotic cell culture are known to the person skilled in the art (e.g. Iscove's medium, ultra CHO medium (BioWhittaker), EX-CELL (JRH Biosciences)). Furthermore, protein-free media may be used (e.g. PF-CHO (JRH Biosciences)). Otherwise, the cells for replication can also be cultured in the customary serum-containing media (e.g. MEM or DMEM medium with 0.5% to 10% of fetal calf serum).

The cells may be in adherent culture or in suspension.

Virus preparation

In one embodiment, the invention provides a method for producing influenza viruses comprising steps of (a) infecting a culture host with a reassortant virus of the invention; (b) culturing the host from step (a) to produce the virus; and optionally (c) purifying the virus obtained in step (b).

The culture host may be cells or embryonated hen eggs. Where cells are used as a culture host in this aspect of the invention, it is known that cell culture conditions (*e.g.* temperature, cell density, pH value, *etc.*) are variable over a wide range subject to the cell line and the virus employed and can be adapted to the requirements of the application. The following information therefore merely represents guidelines.

As mentioned above, cells are preferably cultured in serum-free or protein-free media.

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 the context of the present invention, a culture system is 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. It can be advantageous to adjust the pH value of the medium during multiplication of 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. Culturing of cells preferably occurs at a temperature between 30 and 40°C. When culturing the infected cells (step ii), the cells are preferably cultured at a temperature of between 30 °C and 36°C or between 32 °C and 34 °C or at 33°C. This is particularly preferred, as it has been shown that incubation of infected cells in this temperature range results in production of a virus that results in improved efficacy when formulated into a vaccine [34].

Oxygen partial pressure can be adjusted during culturing before infection preferably at a value between 25% and 95% and especially at a value between 35% and 60%. The values for the oxygen partial pressure stated in the context of the invention are based on saturation of air. Infection of cells occurs at a cell density of preferably about 8-25x10^5 cells/mL in the batch system or preferably about 5-20x10^6 cells/mL in the perfusion system. The cells can be infected with a viral dose (MOI value, "multiplicity of infection"; corresponds to the number of virus units per cell at the time of infection) between 10^{-8} and 10, preferably between 0.0001 and 0.5.

Virus may be grown on cells in adherent culture or in suspension. Microcarrier cultures can be used. In some embodiments, the cells may thus be adapted for growth in suspension.

The methods according to the invention also include harvesting and isolation of viruses or the proteins generated by them. During isolation of viruses or proteins, the cells are separated from the culture medium by standard methods like separation, filtration or ultrafiltration. The viruses or the proteins are then concentrated according to methods sufficiently known to those skilled in the art, like gradient centrifugation, filtration, precipitation, chromatography, etc., and then purified. It is also preferred according to the invention that the viruses are inactivated during or after purification. Virus inactivation can occur, for example, by β-propiolactone or formaldehyde at any point within the purification process.

The culture host may be eggs. The current standard method for influenza virus growth for vaccines uses embryonated SPF hen eggs, with virus being purified from the egg contents (allantoic fluid). It is also possible to passage a virus through eggs and subsequently propagate it in cell culture and vice versa.

Vaccine

The invention utilises virus produced according to the method to produce vaccines.

Vaccines (particularly for influenza virus) are generally based either on live virus or on inactivated virus. Inactivated vaccines may be based on whole virions, 'split' virions, or on purified surface antigens. Antigens can also be presented in the form of virosomes. The invention can be used for manufacturing any of these types of vaccine.

Where an inactivated virus is used, the vaccine may comprise whole virion, split virion, or purified surface antigens (for influenza, including hemagglutinin and, usually, also including neuraminidase). Chemical means for inactivating a virus include treatment with an effective amount of one or more of the following agents: detergents, formaldehyde, β-propiolactone, methylene blue, psoralen, carboxyfullerene (C60), binary ethylamine, acetyl ethyleneimine, or combinations thereof. Non-chemical methods of viral inactivation are known in the art, such as for example UV light or gamma irradiation.

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

Split virions are obtained by treating purified virions with detergents (*e.g.* ethyl ether, polysorbate 80, deoxycholate, tri-*N*-butyl phosphate, Triton X-100, Triton N101, cetyltrimethylammonium bromide, Tergitol NP9, *etc.*) to produce subvirion preparations, including the 'Tween-ether' splitting process. Methods of splitting influenza viruses, for example are well known in the art *e.g.* see refs. 35-40, *etc.* Splitting of the virus is typically carried out by disrupting or fragmenting whole virus, whether infectious or non-infectious with a disrupting concentration of a splitting agent. The disruption results in a full or partial solubilisation of the virus proteins, altering the integrity of the virus. Preferred splitting agents are non-ionic and ionic (*e.g.* cationic) surfactants *e.g.* alkylglycosides, alkylthioglycosides, acyl sugars, sulphobetaines, betains, polyoxyethylenealkylethers, N,N-dialkyl-Glucamides, Hecameg, alkylphenoxy-polyethoxyethanols, NP9, quaternary ammonium compounds, sarcosyl, CTABs (cetyl trimethyl ammonium bromides), tri-*N*-butyl phosphate, Cetavlon, myristyltrimethylammonium salts, lipofectin, lipofectamine, and DOT-MA, the octyl- or nonylphenoxy polyoxyethanols (*e.g.* the Triton surfactants, such as Triton X-100 or Triton N101), polyoxyethylene sorbitan esters (the Tween surfactants), polyoxyethylene ethers, polyoxyethlene esters, *etc.* One useful splitting procedure uses the consecutive effects of sodium deoxycholate and

formaldehyde, and splitting can take place during initial virion purification (e.g. in a sucrose density gradient solution). Thus a splitting process can involve clarification of the virion-containing material (to remove non-virion material), concentration of the harvested virions (*e.g.* using an adsorption method, such as $CaHPO_4$ adsorption), separation of whole virions from non-virion material, splitting of virions using a splitting agent in a density gradient centrifugation step (*e.g.* using a sucrose gradient that contains a splitting agent such as sodium deoxycholate), and then filtration (*e.g.* ultrafiltration) to remove undesired materials. Split virions can usefully be resuspended in sodium phosphate-buffered isotonic sodium chloride solution. Examples of split influenza vaccines are the BEGRIVAC™, FLUARIX™, FLUZONE™ and FLUSHIELD™ products.

Purified influenza virus surface antigen vaccines comprise the surface antigens hemagglutinin and, typically, also neuraminidase. Processes for preparing these proteins in purified form are well known in the art. The FLUVIRIN™, AGRIPPAL™ and INFLUVAC™ products are influenza subunit vaccines.

Another form of inactivated antigen is the virosome [41] (nucleic acid free viral-like liposomal particles). Virosomes can be prepared by solubilization of virus with a detergent followed by removal of the nucleocapsid and reconstitution of the membrane containing the viral glycoproteins. An alternative method for preparing virosomes involves adding viral membrane glycoproteins to excess amounts of phospholipids, to give liposomes with viral proteins in their membrane.

The methods of the invention may also be used to produce live vaccines. Such vaccines are usually prepared by purifying virions from virion-containing fluids. For example, the fluids may be clarified by centrifugation, and stabilized with buffer (*e.g.* containing sucrose, potassium phosphate, and monosodium glutamate). Various forms of influenza virus vaccine are currently available (e.g. see chapters 17 & 18 of reference 42). Live virus vaccines include MedImmune's FLUMIST™ product.

The virus may be attenuated. The virus may be temperature-sensitive. The virus may be cold-adapted. These three features are particularly useful when using live virus as an antigen.

HA is the main immunogen in current inactivated influenza vaccines, and vaccine doses are standardised by reference to HA levels, typically measured by SRID. Existing vaccines typically contain about 15μg of HA per strain, although lower doses can be used *e.g.* for children, or in pandemic situations, or when using an adjuvant. Fractional doses such as ½ (*i.e.* 7.5μg HA per strain), ¼ and $^1/_8$ have been used, as have higher doses (*e.g.* 3x or 9x doses [43,44]). Thus vaccines may include between 0.1 and 150μg of HA per influenza strain, preferably between 0.1 and 50μg *e.g.* 0.1-20μg, 0.1-15μg, 0.1-10μg, 0.1-7.5μg, 0.5-5μg, *etc.* Particular doses include *e.g.* about 45, about 30, about 15, about 10, about 7.5, about 5, about 3.8, about 3.75, about 1.9, about 1.5, *etc.* per strain.

For live vaccines, dosing is measured by median tissue culture infectious dose ($TCID_{50}$) rather than HA content, and a $TCID_{50}$ of between 10^6 and 10^8 (preferably between $10^{6.5}$-$10^{7.5}$) per strain is typical.

Influenza strains used with the invention may have a natural HA as found in a wild-type virus, or a modified HA. For instance, it is known to modify HA to remove determinants (*e.g.* hyper-basic regions around the HA1/HA2 cleavage site) that cause a virus to be highly pathogenic in avian species. The use of reverse genetics facilitates such modifications.

As well as being suitable for immunizing against inter-pandemic strains, the compositions of the invention are particularly useful for immunizing against pandemic or potentially-pandemic strains. The invention is suitable for vaccinating humans as well as non-human animals.

Other strains whose antigens can usefully be included in the compositions are strains which are resistant to antiviral therapy (*e.g.* resistant to oseltamivir [45] and/or zanamivir), including resistant pandemic strains [46].

Compositions of the invention may include antigen(s) from one or more (*e.g.* 1, 2, 3, 4 or more) influenza virus strains, including influenza A virus and/or influenza B virus provided that at least one influenza strain is a reassortant influenza strain of the invention. Compositions wherein at least two, at least three or all of the antigens are from reassortant influenza strains of the invention are also envisioned. Where a vaccine includes more than one strain of influenza, the different strains are typically grown separately and are mixed after the viruses have been harvested and antigens have been prepared. Thus a process of the invention may include the step of mixing antigens from more than one influenza strain. A trivalent vaccine is typical, including antigens from two influenza A virus strains and one influenza B virus strain. A tetravalent vaccine is also useful [47], including antigens from two influenza A virus strains and two influenza B virus strains, or three influenza A virus strains and one influenza B virus strain.

Pharmaceutical compositions

Vaccine compositions manufactured according to the invention are pharmaceutically acceptable. They usually include components in addition to the antigens *e.g.* they typically include one or more pharmaceutical carrier(s) and/or excipient(s). As described below, adjuvants may also be included. A thorough discussion of such components is available in reference 48.

Vaccine compositions will generally be in aqueous form. However, some vaccines may be in dry form, *e.g.* in the form of injectable solids or dried or polymerized preparations on a patch.

Vaccine compositions may include preservatives such as thiomersal or 2-phenoxyethanol. It is preferred, however, that the vaccine should be substantially free from (*i.e.* less than 5μg/ml) mercurial material *e.g.* thiomersal-free [39,49]. Vaccines containing no mercury are more preferred. An α-tocopherol succinate can be included as an alternative to mercurial compounds [39]. Preservative-free vaccines are particularly preferred.

To control tonicity, it is preferred to include a physiological salt, such as a sodium salt. Sodium chloride (NaCl) is preferred, which may be present at between 1 and 20 mg/ml. Other salts that may

be present include potassium chloride, potassium dihydrogen phosphate, disodium phosphate dehydrate, magnesium chloride, calcium chloride, *etc.*

Vaccine compositions will generally have an osmolality of between 200 mOsm/kg and 400 mOsm/kg, preferably between 240-360 mOsm/kg, and will more preferably fall within the range of 290-310 mOsm/kg. Osmolality has previously been reported not to have an impact on pain caused by vaccination [50], but keeping osmolality in this range is nevertheless preferred.

Vaccine compositions may include one or more buffers. Typical buffers include: a phosphate buffer; a Tris buffer; a borate buffer; a succinate buffer; a histidine buffer (particularly with an aluminum hydroxide adjuvant); or a citrate buffer. Buffers will typically be included in the 5-20mM range.

The pH of a vaccine composition will generally be between 5.0 and 8.1, and more typically between 6.0 and 8.0 *e.g.* 6.5 and 7.5, or between 7.0 and 7.8. A process of the invention may therefore include a step of adjusting the pH of the bulk vaccine prior to packaging.

The vaccine composition is preferably sterile. The vaccine composition is preferably non-pyrogenic *e.g.* containing <1 EU (endotoxin unit, a standard measure) per dose, and preferably <0.1 EU per dose. The vaccine composition is preferably gluten-free.

Vaccine compositions of the invention may include detergent *e.g.* a polyoxyethylene sorbitan ester surfactant (known as 'Tweens'), an octoxynol (such as octoxynol-9 (Triton X-100) or t-octylphenoxypolyethoxyethanol), a cetyl trimethyl ammonium bromide ('CTAB'), or sodium deoxycholate, particularly for a split or surface antigen vaccine. The detergent may be present only at trace amounts. Thus the vaccine may include less than 1mg/ml of each of octoxynol-10 and polysorbate 80. Other residual components in trace amounts could be antibiotics (*e.g.* neomycin, kanamycin, polymyxin B).

A vaccine composition may include material for a single immunisation, or may include material for multiple immunisations (*i.e.* a 'multidose' kit). The inclusion of a preservative is preferred in multidose arrangements. As an alternative (or in addition) to including a preservative in multidose compositions, the compositions may be contained in a container having an aseptic adaptor for removal of material.

Influenza vaccines are typically administered in a dosage volume of about 0.5ml, although a half dose (*i.e.* about 0.25ml) may be administered to children.

Compositions and kits are preferably stored at between 2°C and 8°C. They should not be frozen. They should ideally be kept out of direct light.

Host cell DNA

Where virus has been isolated and/or grown on a cell line, it is standard practice to minimize the amount of residual cell line DNA in the final vaccine, in order to minimize any potential oncogenic activity of the DNA.

Thus a vaccine composition prepared according to the invention preferably contains less than 10ng (preferably less than 1ng, and more preferably less than 100pg) of residual host cell DNA per dose, although trace amounts of host cell DNA may be present.

It is preferred that the average length of any residual host cell DNA is less than 500bp *e.g.* less than 400bp, less than 300bp, less than 200bp, less than 100bp, *etc.*

Contaminating DNA can be removed during vaccine preparation using standard purification procedures *e.g.* chromatography, *etc.* Removal of residual host cell DNA can be enhanced by nuclease treatment *e.g.* by using a DNase. A convenient method for reducing host cell DNA contamination is disclosed in references 51 & 52, involving a two-step treatment, first using a DNase (*e.g.* Benzonase), which may be used during viral growth, and then a cationic detergent (*e.g.* CTAB), which may be used during virion disruption. Treatment with an alkylating agent, such as β-propiolactone, can also be used to remove host cell DNA, and advantageously may also be used to inactivate virions [53].

Adjuvants

Compositions of the invention may advantageously include an adjuvant, which can function to enhance the immune responses (humoral and/or cellular) elicited in a subject who receives the composition. Preferred adjuvants comprise oil-in-water emulsions. Various such adjuvants 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 5μm in diameter, and ideally have a sub-micron diameter, with these small sizes being achieved with a microfluidiser to provide stable emulsions. Droplets with a size less than 220nm are preferred as they can be subjected to filter sterilization.

The emulsion can comprise 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 can be used *e.g.* obtained from the jojoba bean. 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 practice of this invention. 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 terpenoids known as squalene, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, which is particularly preferred herein. Squalane, the saturated analog to squalene, is also a preferred oil. Fish oils, including squalene and squalane, are readily available from commercial sources or may be obtained by methods known in the art. Another preferred oil is α-tocopherol (see below).

Mixtures of oils can be used.

Surfactants can be classified by their 'HLB' (hydrophile/lipophile balance). Preferred surfactants of the invention have a HLB of at least 10, preferably at least 15, and more preferably at least 16. The invention can be used with 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-octylphenoxypolyethoxyethanol) being of particular interest; (octylphenoxy)polyethoxyethanol (IGEPAL CA-630/NP-40); phospholipids such as phosphatidylcholine (lecithin); nonylphenol ethoxylates, such as the Tergitol™ NP series; 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. Preferred 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 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 useful combination comprises laureth 9 plus a polyoxyethylene sorbitan ester and/or an octoxynol.

Preferred 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 %, preferably 0.1 to 10 % and in particular 0.1 to 1% or about 0.5%.

Where the vaccine contains a split virus, it is preferred that it contains free surfactant in the aqueous phase. This is advantageous as the free surfactant can exert a 'splitting effect' on the antigen, thereby disrupting any unsplit virions and/or virion aggregates that might otherwise be present. This can improve the safety of split virus vaccines [54].

Preferred emulsions have an average droplets size of <1μm *e.g.* ≤750nm, ≤500nm, ≤400nm, ≤300nm, ≤250nm, ≤220nm, ≤200nm, or smaller. These droplet sizes can conveniently be achieved by techniques such as microfluidisation.

Specific oil-in-water emulsion adjuvants useful with the invention include, but are not limited to:

- A submicron emulsion of squalene, Tween 80, and Span 85. The composition of the emulsion by volume can be about 5% squalene, about 0.5% polysorbate 80 and about 0.5% Span 85. In weight terms, these ratios become 4.3% squalene, 0.5% polysorbate 80 and 0.48% Span 85. This adjuvant is known as 'MF59' [55-57], as described in more detail in Chapter 10 of ref. 58 and chapter 12 of ref. 59. The MF59 emulsion advantageously includes citrate ions *e.g.* 10mM sodium citrate buffer.

- An emulsion comprising squalene, a tocopherol, and polysorbate 80. The emulsion may include phosphate buffered saline. These emulsions may have by volume from 2 to 10% squalene, from 2 to 10% tocopherol and from 0.3 to 3% polysorbate 80, and the weight ratio of squalene:tocopherol is preferably <1 (e.g. 0.90) as this can provide a more stable emulsion. Squalene and polysorbate 80 may be present volume ratio of about 5:2 or at a weight ratio of about 11:5. Thus the three components (squalene, tocopherol, polysorbate 80) may be present at a weight ratio of 1068:1186:485 or around 55:61:25. One such emulsion ('AS03') can be made by dissolving Tween 80 in PBS to give a 2% solution, then mixing 90ml of this solution with a mixture of (5g of DL α tocopherol and 5ml squalene), then microfluidising the mixture. The resulting emulsion may have submicron oil droplets e.g. with an average diameter of between 100 and 250nm, preferably about 180nm. The emulsion may also include a 3-de-O-acylated monophosphoryl lipid A (3d MPL). Another useful emulsion of this type may comprise, per human dose, 0.5-10 mg squalene, 0.5-11 mg tocopherol, and 0.1-4 mg polysorbate 80 [60] *e.g.* in the ratios discussed above.

- An emulsion of squalene, a tocopherol, and a Triton detergent (*e.g.* Triton X-100). The emulsion may also include a 3d-MPL (see below). The emulsion may contain a phosphate buffer.

- An emulsion comprising a polysorbate (*e.g.* polysorbate 80), a Triton detergent (*e.g.* Triton X-100) and a tocopherol (*e.g.* an α-tocopherol succinate). The emulsion may include these three components at a mass ratio of about 75:11:10 (*e.g.* 750μg/ml polysorbate 80, 110μg/ml Triton X-100 and 100μg/ml α-tocopherol succinate), and these concentrations should include any contribution of these components from antigens. The emulsion may also include squalene. The emulsion may also include a 3d-MPL (see below). The aqueous phase may contain a phosphate buffer.

- An emulsion of squalane, polysorbate 80 and poloxamer 401 ("Pluronic™ L121"). The emulsion can be formulated in phosphate buffered saline, pH 7.4. This emulsion is a useful

delivery vehicle for muramyl dipeptides, and has been used with threonyl-MDP in the "SAF-1" adjuvant [61] (0.05-1% Thr-MDP, 5% squalane, 2.5% Pluronic L121 and 0.2% polysorbate 80). It can also be used without the Thr-MDP, as in the "AF" adjuvant [62] (5% squalane, 1.25% Pluronic L121 and 0.2% polysorbate 80). Microfluidisation is preferred.

- An emulsion comprising squalene, an aqueous solvent, a polyoxyethylene alkyl ether hydrophilic nonionic surfactant (*e.g.* polyoxyethylene (12) cetostearyl ether) and a hydrophobic nonionic surfactant (*e.g.* a sorbitan ester or mannide ester, such as sorbitan monoleate or 'Span 80'). The emulsion is preferably thermoreversible and/or has at least 90% of the oil droplets (by volume) with a size less than 200 nm [63]. The emulsion may also include one or more of: alditol; a cryoprotective agent (*e.g.* a sugar, such as dodecylmaltoside and/or sucrose); and/or an alkylpolyglycoside. The emulsion may include a TLR4 agonist [64]. Such emulsions may be lyophilized.

- An emulsion of squalene, poloxamer 105 and Abil-Care [65]. The final concentration (weight) of these components in adjuvanted vaccines are 5% squalene, 4% poloxamer 105 (pluronic polyol) and 2% Abil-Care 85 (Bis-PEG/PPG-16/16 PEG/PPG-16/16 dimethicone; caprylic/capric triglyceride).

- An emulsion having from 0.5-50% of an oil, 0.1-10% of a phospholipid, and 0.05-5% of a non-ionic surfactant. As described in reference 66, preferred phospholipid components are phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid, sphingomyelin and cardiolipin. Submicron droplet sizes are advantageous.

- A submicron oil-in-water emulsion of a non-metabolisable oil (such as light mineral oil) and at least one surfactant (such as lecithin, Tween 80 or Span 80). Additives may be included, such as QuilA saponin, cholesterol, a saponin-lipophile conjugate (such as GPI-0100, described in reference 67, produced by addition of aliphatic amine to desacylsaponin via the carboxyl group of glucuronic acid), dimethyidioctadecylammonium bromide and/or N,N-dioctadecyl-N,N-bis (2-hydroxyethyl)propanediamine.

- An emulsion in which a saponin (*e.g.* QuilA or QS21) and a sterol (*e.g.* a cholesterol) are associated as helical micelles [68].

- An emulsion comprising a mineral oil, a non-ionic lipophilic ethoxylated fatty alcohol, and a non-ionic hydrophilic surfactant (*e.g.* an ethoxylated fatty alcohol and/or polyoxyethylene-polyoxypropylene block copolymer) [69].

- An emulsion comprising a mineral oil, a non-ionic hydrophilic ethoxylated fatty alcohol, and a non-ionic lipophilic surfactant (*e.g.* an ethoxylated fatty alcohol and/or polyoxyethylene-polyoxypropylene block copolymer) [69].

In some embodiments an emulsion may be mixed with antigen extemporaneously, at the time of delivery, and thus the adjuvant and antigen may be kept separately in a packaged or distributed vaccine, ready for final formulation at the time of use. In other embodiments an emulsion is mixed with antigen during manufacture, and thus the composition is packaged in a liquid adjuvanted form. The antigen will generally be in an aqueous form, such that the vaccine is finally prepared by mixing two liquids. The volume ratio of the two liquids for mixing can vary (*e.g.* between 5:1 and 1:5) but is generally about 1:1. Where concentrations of components are given in the above descriptions of specific emulsions, these concentrations are typically for an undiluted composition, and the concentration after mixing with an antigen solution will thus decrease.

Packaging of vaccine compositions

Suitable containers for compositions of the invention (or kit components) include vials, syringes (*e.g.* disposable syringes), nasal sprays, *etc.* These containers should be sterile.

Where a composition/component is located in a vial, the vial is preferably made of a glass or plastic material. The vial is preferably sterilized before the composition is added to it. To avoid problems with latex-sensitive patients, vials are preferably sealed with a latex-free stopper, and the absence of latex in all packaging material is preferred. The vial may include a single dose of vaccine, or it may include more than one dose (a 'multidose' vial) *e.g.* 10 doses. Preferred vials are made of colourless glass.

A vial can have a cap (*e.g.* a Luer lock) adapted such that a pre-filled syringe can be inserted into the cap, the contents of the syringe can be expelled into the vial (*e.g.* to reconstitute lyophilised material therein), and the contents of the vial can be removed back into the syringe. After removal of the syringe from the vial, a needle can then be attached and the composition can be administered to a patient. The cap is preferably located inside a seal or cover, such that the seal or cover has to be removed before the cap can be accessed. A vial may have a cap that permits aseptic removal of its contents, particularly for multidose vials.

Where a component is packaged into a syringe, the syringe may have a needle attached to it. If a needle is not attached, a separate needle may be supplied with the syringe for assembly and use. Such a needle may be sheathed. Safety needles are preferred. 1-inch 23-gauge, 1-inch 25-gauge and 5/8-inch 25-gauge needles are typical. Syringes may be provided with peel-off labels on which the lot number, influenza season and expiration date of the contents may be printed, to facilitate record keeping. The plunger in the syringe preferably has a stopper to prevent the plunger from being accidentally removed during aspiration. The syringes may have a latex rubber cap and/or plunger. Disposable syringes contain a single dose of vaccine. The syringe will generally have a tip cap to seal the tip prior to attachment of a needle, and the tip cap is preferably made of a butyl rubber. If the syringe and needle are packaged separately then the needle is preferably fitted with a butyl rubber shield. Preferred syringes are those marketed under the trade name "Tip-Lok"™.

Containers may be marked to show a half-dose volume *e.g.* to facilitate delivery to children. For instance, a syringe containing a 0.5ml dose may have a mark showing a 0.25ml volume.

Where a glass container (*e.g.* a syringe or a vial) is used, then it is preferred to use a container made from a borosilicate glass rather than from a soda lime glass.

A kit or composition may be packaged (*e.g.* in the same box) with a leaflet including details of the vaccine *e.g.* instructions for administration, details of the antigens within the vaccine, *etc.* The instructions may also contain warnings *e.g.* to keep a solution of adrenaline readily available in case of anaphylactic reaction following vaccination, *etc.*

Methods of treatment, and administration of the vaccine

The invention provides a vaccine manufactured according to the invention. These vaccine compositions are suitable for administration to human or non-human animal subjects, such as pigs or birds, and the invention provides a method of raising an immune response in a subject, comprising the step of administering a composition of the invention to the subject. The invention also provides a composition of the invention for use as a medicament, and provides the use of a composition of the invention for the manufacture of a medicament for raising an immune response in a subject.

The immune response raised by these methods and uses will generally include an antibody response, preferably a protective antibody response. Methods for assessing antibody responses, neutralising capability and protection after influenza virus vaccination are well known in the art. Human studies have shown that antibody titers against hemagglutinin of human influenza virus are correlated with protection (a serum sample hemagglutination-inhibition titer of about 30–40 gives around 50% protection from infection by a homologous virus) [70]. Antibody responses are typically measured by hemagglutination inhibition, by microneutralisation, by single radial immunodiffusion (SRID), and/or by single radial hemolysis (SRH). These assay techniques are well known in the art.

Compositions of the invention can be administered in various ways. The most preferred immunisation route is by intramuscular injection (*e.g.* into the arm or leg), but other available routes include subcutaneous injection, intranasal [71-73], oral [74], intradermal [75,76], transcutaneous, transdermal [77], *etc.*

Vaccines prepared according to the invention may be used to treat both children and adults. Influenza vaccines are currently recommended for use in pediatric 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 (*e.g.* an oseltamivir or zanamivir compound; see below) 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.

Preferred compositions of the invention satisfy 1, 2 or 3 of the CPMP criteria for efficacy. In adults (18-60 years), these criteria are: (1) ≥70% seroprotection; (2) ≥40% seroconversion; and/or (3) a GMT increase of ≥2.5-fold. In elderly (>60 years), these criteria are: (1) ≥60% seroprotection; (2) ≥30% seroconversion; and/or (3) a GMT increase of ≥2-fold. These criteria are based on open label studies with at least 50 patients.

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, *etc.* Administration of more than one dose (typically two doses) is particularly useful in immunologically naïve patients *e.g.* for people who have never received an influenza vaccine before, or for vaccinating against a new HA subtype (as 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.*).

Vaccines produced by the invention may be administered to patients at substantially the same time as (*e.g.* during the same medical consultation or visit to a healthcare professional or vaccination centre) other vaccines *e.g.* at substantially the same time as a measles vaccine, a mumps vaccine, a rubella vaccine, a MMR vaccine, a varicella vaccine, a MMRV vaccine, a diphtheria vaccine, a tetanus vaccine, a pertussis vaccine, a DTP vaccine, a conjugated *H.influenzae* type b vaccine, an inactivated poliovirus vaccine, a hepatitis B virus vaccine, a meningococcal conjugate vaccine (such as a tetravalent A-C-W135-Y vaccine), a respiratory syncytial virus vaccine, a pneumococcal conjugate vaccine, *etc.* Administration at substantially the same time as a pneumococcal vaccine and/or a meningococcal vaccine is particularly useful in elderly patients.

Similarly, vaccines of the invention may be administered to patients at substantially the same time as (*e.g.* during the same medical consultation or visit to a healthcare professional) an antiviral compound, and in particular an antiviral compound active against influenza virus (*e.g.* oseltamivir and/or zanamivir). These antivirals include neuraminidase inhibitors, such as a (3R,4R,5S)-4-acetylamino-5-amino-3(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid or 5-(acetylamino)-4-[(aminoiminomethyl)-amino]-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galactonon-2-enonic acid, including esters thereof (*e.g.* the ethyl esters) and salts thereof (*e.g.* the phosphate salts). A preferred antiviral is (3R,4R,5S)-4-acetylamino-5-amino-3(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid, ethyl ester, phosphate (1:1), also known as oseltamivir phosphate (TAMIFLU™).

General

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

The term "about" in relation to a numerical value *x* is optional and means, for example, *x*±10%.

The preferred pairwise alignment algorithm for use with the invention is the Needleman-Wunsch global alignment algorithm [78], using default parameters (*e.g.* with Gap opening penalty = 10.0, and with Gap extension penalty = 0.5, using the EBLOSUM62 scoring matrix). This algorithm is conveniently implemented in the *needle* tool in the EMBOSS package [79].

Unless specifically stated, a process comprising a step of mixing two or more components does not require any specific order of mixing. Thus components can be mixed in any order. Where there are three components then two components can be combined with each other, and then the combination may be combined with the third component, *etc.*

The various steps of the methods may be carried out at the same or different times, in the same or different geographical locations, *e.g.* countries, and by the same or different people or entities.

Where animal (and particularly bovine) materials are used in the culture of cells, they should be obtained from sources that are free from transmissible spongiform encephalopathies (TSEs), and in particular free from bovine spongiform encephalopathy (BSE). Overall, it is preferred to culture cells in the total absence of animal-derived materials.

Where a compound is administered to the body as part of a composition then that compound may alternatively be replaced by a suitable prodrug.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 80. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in reference 81.

References to a percentage sequence identity between two nucleic acid sequences mean that, when aligned, that percentage of bases are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 80. A preferred alignment program is

GCG Gap (Genetics Computer Group, Wisconsin, Suite Version 10.1), preferably using default parameters, which are as follows: open gap = 3; extend gap = 1.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Backbone-derived viruses outperform wt A/Brisbane/10/10 virus in growth and HA yield. (A) HA yield as measured by HA ELISA. The y-axis shows the HA yield (μg/mL) (B) The fold increase in HA yield by ELISA was calculated by normalizing the HA ELISA values to those of the A/Brisbane/10/10 WT virus. The y-axis shows the fold increase in HA yield. (C) HA titers using 0.5% guinea pig RBCs. The y-axis shows the log2 HA titer. (D) Viral titers 60h post-infection as determined by FFA assay. The y-axis shows the FFU/mL.

Bars represent the mean plus SEM of three independent experiments. Statistical significance was determined using one-way ANOVA. The mean value of each group was compared to WT virus using Dunnett's multiple comparison test. * = P<0.05, ** = P< 0.01, *** = P<0.001; The white bars represent the results with wt A/Brisbane/10/10, the dotted column shows the results with the PR8X backbone; the hatched column shows the results with the #19 column and the grey column shows the results with the #21 backbone.

Figure 2 compares the HA yield of different reassortant influenza B strains in MDCK cells relative to the wild-type (WT) or reverse genetics-derived (RG) B/Brisbane/60/08 strain. The viral segments of the tested influenza B viruses are shown in Table 1. The y-axis indicates the HA yield in μg/mL.

Figure 3 compares the HA yield of different reassortant influenza B strains in MDCK cells relative to the wild-type (WT) or reverse genetics-derived (RG) B/Panama/45/90 strain. The viral segments of the tested influenza B viruses are shown in Table 1. The y-axis indicates the HA yield in μg/mL.

Figure 4 (A) Schematic diagram and sequence alignment of chimeric HA constructs. The wild type A/Brisbane/10/10 HA (WT Bris) is shown in white. The terminal regions of HA, non-coding regions (NCR), signal peptide (SP), transmembrane region (TM) and cytoplasmic domain (CT), from two laboratory adapted strains of H1N1, PR8X (dotted fields) and 105p30 (hatched fields), are fused to the A/Brisbane/10/10 ectodomain to produce the chimeric HA segments shown. (B) Sequence alignment of the terminal regions of A/Brisbane/10/10 (Bris), PR8X and 105p30 HA. Dashes represent nucleotides conserved among the strains. The 3'NCR is separated from the signal peptide sequence by the solid bar. For brevity, the ectodomain sequence is omitted (../..). The transmembrane region is separated from the cytoplasmic tail by the dashed line. The stop codon is underlined and followed by the 5'NCR. (C) Schematic diagram and sequence alignment of chimeric NA constructs. The wild type A/Brisbane/10/10 NA (WT Bris) is shown in white. The terminal regions of NA, non-coding regions (NCR), cytoplasmic domain (CT), and transmembrane region (TM) from PR8X (gray) and 105 (slanted lines), are grafted into the A/Brisbane/10/10 ectodomain to produce the chimeric NA segments shown. (D) Sequence alignment of the terminal regions of A/Brisbane/10/10 (Bris), PR8X and 105 NA. Dashes represent nucleotides conserved among the strains. The

cytoplasmic tail is separated from the 3'NCR by the solid bar and from the transmembrane region by the dashed line. For brevity, the ectodomain sequence is omitted (../..). The stop codon is underlined and followed by the 5'NCR.

Figure 5. PR8X(term) HA/NA segments enhance HA yield over PR8X(term) HA or NA only. MDCK 33016PF cells are infected at an MOI of 0.001 with viruses with the PR8X backbone using the indicated HA/NA gene segment combinations. (A) Fold increase as measured by HA ELISA and compared to the yield using WT A/Brisbane/10/10 HA and NA segments. The y-axis shows the fold increase in HA yield. (B) HA titer as determined using 0.5% red blood cells from guinea pigs. The y-axis shows the log2 HA titer. (C) Virus titers 60 h post infection as determined by FFA assay. The y-axis shows the FFU/mL.

Bars represent the mean plus SEM of three independent experiments. Statistical significance was determined using one-way ANOVA. The mean value of each group is compared to Bris HA/NA using Dunnett's multiple comparison test. * = $P<0.05$, ** = $P< 0.01$.

Figure 6. Chimeric HA/NA segments enhance HA yield with all three optimized backbones. MDCK 33016PF cells are infected at an MOI of 0.001 with viruses derived from the three optimised backbones using HA and NA gene segments with the terminal regions from A/Brisbane/10/10 (Bris) (white columns), PR8X (hatched columns) and 105p30 (grey columns). Upper panels (A, B and C) show the fold increase in HA yield as measured by HA ELISA and compared to the yield using WT HA/NA segments (Bris). The y-axis shows fold increase in HA titer. Middle panels (D, E and F) show HA titers 60 h post infection as determined by HA assay. The y-axis shows log2 HA titer. Lower panels (G, H and I) show virus titers 60 h post infection as determined by FFA assay. The y-axis shows FFU/mL.

Bars represent the mean plus SEM of three independent experiments. Statistical significance was determined using one-way ANOVA. The mean value of each group was compared to Bris HA/NA using Dunnett's multiple comparison test. * = $P<0.05$, ** = $P< 0.01$.

Figure 7. Chimeric HA and NA segments enhance HA yield with #21 backbone. MDCK 33016PF cells were infected at an MOI of 0.001 with the X187 working seed (A/Victoria/210/2009 classical reassortant, white columns) and viruses derived from the #21 backbone with the indicated HA and NA gene segment combinations: terminal regions from WT A/Victoria/210/2009 (Vic, grey columns), PR8X (dotted columns) or 105 (hatched columns). (A) shows virus titers 60 h post infection as determined by FFA assay. The y-axis indicates FFU/mL (B) shows HA yield 60 h post infection as determined by HA ELISA. The y-axis indicates HA yield (μg/mL).

Bars represent the mean plus SEM of two independent experiments. Statistical significance was determined using one-way ANOVA. The mean value of each group was compared to Bris HA/NA using Dunnett's multiple comparison test. * = $P<0.05$.

Figure 8. Enhanced HA content of #21 backbone-derived viruses with chimeric HA/NA segments. (A) HA yield of large scale cultures (60mL) of MDCK 33016PF cells, infected at an MOI of 0.001 with #21 derived viruses containing HA and NA gene segments with the terminal regions from A/Brisbane/10/10 (Bris) (white columns), PR8X (hatched columns) and 105p30 (grey columns). HA yield is measured by HPLC after virus is concentrated and purified by sucrose-gradient density centrifugation. The y-axis shows µg HA/mL culture (B) Deglycosylation of HA (dHA) is performed using PNGase and viruses are subsequently separated by SDS-PAGE and viral proteins stained using SYPRO-Ruby. (C) HA content of #21 with the indicated HA and NA segments as assessed by gel densitometry assay and HPLC/BCA assay. The HA content is calculated from gel densitometry and from HPLC by dividing values from (A) over the total protein concentration in the fractions, as determined by a BCA assay. The columns show the results with HA and NA gene segments with the terminal regions from A/Brisbane/10/10 (Bris) (white columns), PR8X (hatched columns) and 105p30 (grey columns).

The y-axis shows % HA content. HA content values were compared to those of Bris(term) HA and NA (WT control), which were assigned a value of 1.

Bars represent the mean plus SEM of two independent experiments. Statistical significance is determined using one-way ANOVA. The mean value of each group was compared to Bris HA/NA using Dunnett's multiple comparison test. * = $P<0.05$, ** = $P<0.001$.

MODES FOR CARRYING OUT THE INVENTION

Materials and Methods

Cells, viruses and plasmids

293T cells and suspension MDCK 33016PF cells are maintained as previously described [82].

The eight segments from PR8X and 105p30, the PB1 segment from A/California/07/09 and the HA/NA segments from A/Brisbane/10/10 are cloned in plasmid pKS10 for virus rescue as previously described [82]. HA terminal region chimeras are generated using overlap PCR and cloned into pKS10 as previously described [82]. Overlap PCR and Quikchange (Agilent) mutagenesis are used to generate the NA terminal region chimeras. All plasmids are sequence verified before use in rescue experiments.

Virus growth in MDCK cells

10 mL suspension cultures of MDCK 33016PF cultures (1 x 10^6 cells/ml) are inoculated with virus at a multiplicity of infection (MOI) of 0.001 and incubated in TubeSpin™ Bioreactor 50 (TPP). Samples are taken at 0 and 60 hours post-infection and frozen at -80 °C until processed. Analogous methods are used for preparations of 60 mL of cultures grown in 125 ml shake flasks. Viral titers are determined using a previously described focus-formation assay [83] with slight modifications.

Infectious foci are detected using an Alexa Fluor® 488-conjugated goat anti-mouse IgG (Invitrogen), and quantified with a BioSpot™ Analyzer (CTL).

HA ELISA

384w plates (Costar) are coated O/N with Galanthus Nivalis (GNA) lectin (Sigma). Plates are washed four times with wash buffer (PBS+ 0.05% Tween20) and blocked with 10 mM Tris-HCl+ 150 mM NaCl+3% Sucrose+ 1%BSA, pH 7.68 (blocking buffer) for 1 hr at room temperature. Three-fold serial dilutions of the samples containing a final concentration of 1% Zwittergent 3-14 (Sigma) are prepared, added in duplicate to the plates, and incubated at 37°C for 30 minutes in a shaker. Biotinylated-IgG purified from pooled sheep antisera (NIBSC cat# 11/110) raised against A/California/07/09 (antigenically similar to A/Brisbane/10/10) are added and further incubated at 37°C for 30 minutes in a shaker. Plates are then washed four times with wash buffer and incubated with Streptavidin-Alkaline phosphatase (KPL) in wash buffer at 37°C for 30 minutes in a shaker. Plates are washed four times with wash buffer and developed using 1mg/ml p-Nitrophenyl Phosphate pNPP (Sigma) in DEA buffer phosphatase substrate (KPL). Plates are read after 40-50 min incubation in the dark at 405 nm using an InfiniteTM 200 PRO plate reader (Tecan). Data are analyzed using GraphPad Prism software.

Hemagglutination inhibition assay

The Haemagglutination Inhibition Assay (HAI) is performed using ferret antisera FR-359 raised against A/California/07/09 (IRR) and a 0.75% suspension of chicken erythrocytes (Lampire Biologicals).

The hemagglutination inhibition assay (HAI) is performed as described in the World Health Organization Manual for the Laboratory Diagnosis and Virological Surveillance of Influenza. Ferret antisera FR-359 raised against A/California/07/09 (IRR) and a 0.75% suspension of chicken erythrocytes (Lampire Biologicals) prepared in phosphate-buffered saline (PBS) are used.

Sucrose density gradient separation

40 mL of the harvested medum is concentrated ~ 16 fold by centrifugal ultrafiltration (Vivaspin 20 with 300 kD MWCO, Sartorius-Stedim Biotech) and viruses are purified. A hemagglutination assay with 0.5% guinea pig red blood cells (Cleveland Scientific) is performed to identify the fractions with the highest virion content, which are then pooled. The protein content of the pooled fractions is determined using a BCA assay (Pierce) following the manufacturer's directions.

Reversed-phase HPLC (RP-HPLC)

Purified virions are analyzed by HPLC. The HA1 concentration is quantified using purified HA1 (a HA maturational cleavage fragment) from A/California/07/09 reagent (NIBSC cat # 09/146 and 09/174) and prepared using identical methods.

SDS-PAGE and PNGaseF deglycosylation assay

Equal volumes from pooled virus-containing fractions are deglycosylated following the protocol of reference 3 with minor modifications. Samples are separated using 4-12 % Nu-PAGE precast gels (Invitrogen), stained overnight by shaking at room temperature using SYPRO-Ruby stain (Sigma) and destained by shaking in 10% methanol for 30 mins at room temperature. Gels are scanned using a Chemidoc XRS Imager (BioRad) and analyzed using ImageJ software.

Results

Three optimized backbones outperform the current vaccine seed virus for growth and HA yield in MDCK cell cultures.

To overcome the limitations of using egg-derived high-growth reassortants as seed viruses for manufacturing influenza vaccines, three MDCK cell-optimized backbones (PR8-X, #19 and #21) are developed. PR8X contains all backbone segments from the cell-adapted PR8X strain. The #19 backbone contains PB1, PB2 and NP from the cell-adapted 105p30 strain, and the remaining backbone segments from PR8X. The #21 backbone contains an A/California/07/09-like PB1 and the remaining backbone segments from PR8X. Figure 1 shows the data compiled from three independent experiments that compare the HA yield (Fig. 1A and B) and growth (Fig.1C) of the WT virus A/Brisbane/10/10 with reassortant influenza viruses comprising these three optimized backbones (PR8X, #19 and #21) and the A/Brisbane/10/10 HA/NA segments. All reassortant influenza viruses display better performance relative to the WT A/Brisbane/10/10 virus. The virus with the #21 backbone produces the highest HA yield increase by ELISA (7.5-fold more than wild type, $P < 0.001$) and has the highest hemagglutination (HA) (~10-fold more, $P < 0.001$) and viral titers (~50-fold more than WT, $P < 0.05$).

Growth characteristics of reassortant influenza B viruses

Reassortant influenza B viruses are produced by reverse genetics which contain the HA and NA proteins from various influenza strains and the other viral segments from B/Brisbane/60/08 and/or B/Panama/45/90. As a control the corresponding wild-type influenza B strain is used. These viruses are cultured either in embryonated chicken eggs or in MDCK cells. The following influenza B strains are used:

Table 1

	Backbone segments						**Antigenic determinants**	
combo #	**PA**	**PB1**	**PB2**	**NP**	**NS**	**M**	**HA**	**NA**
1 (WT)	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane
2	Panama	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane
3	Brisbane	Panama	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane

4	Brisbane	Brisbane	Panama	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane
5	Brisbane	Brisbane	Brisbane	Panama	Brisbane	Brisbane	Brisbane	Brisbane
6	Panama	Panama	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane
7	Panama	Brisbane	Panama	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane
8	Panama	Brisbane	Brisbane	Panama	Brisbane	Brisbane	Brisbane	Brisbane
9	Brisbane	Panama	Panama	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane
10	Brisbane	Panama	Brisbane	Panama	Brisbane	Brisbane	Brisbane	Brisbane
11	Brisbane	Brisbane	Panama	Panama	Brisbane	Brisbane	Brisbane	Brisbane
12	Panama	Panama	Panama	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane
13	Panama	Panama	Brisbane	Panama	Brisbane	Brisbane	Brisbane	Brisbane
14	Panama	Brisbane	Panama	Panama	Brisbane	Brisbane	Brisbane	Brisbane
15	Brisbane	Panama	Panama	Panama	Brisbane	Brisbane	Brisbane	Brisbane
16	Panama	Panama	Panama	Panama	Brisbane	Brisbane	Brisbane	Brisbane
17	Panama	Panama	Panama	Panama	Panama	Panama	Brisbane	Brisbane
20	Brisbane	Panama	Panama	Panama	Panama	Panama	Panama	Panama
21	Panama	Brisbane	Panama	Panama	Panama	Panama	Panama	Panama
22	Panama	Panama	Brisbane	Panama	Panama	Panama	Panama	Panama
23	Panama	Panama	Panama	Brisbane	Panama	Panama	Panama	Panama
24	Brisbane	Brisbane	Panama	Panama	Panama	Panama	Panama	Panama
25	Brisbane	Panama	Brisbane	Panama	Panama	Panama	Panama	Panama
26	Brisbane	Panama	Panama	Brisbane	Panama	Panama	Panama	Panama
27	Panama	Brisbane	Brisbane	Panama	Panama	Panama	Panama	Panama
28	Panama	Brisbane	Panama	Brisbane	Panama	Panama	Panama	Panama
29	Panama	Panama	Brisbane	Brisbane	Panama	Panama	Panama	Panama
30	Brisbane	Brisbane	Brisbane	Panama	Panama	Panama	Panama	Panama
31	Brisbane	Brisbane	Panama	Brisbane	Panama	Panama	Panama	Panama
32	Brisbane	Panama	Brisbane	Brisbane	Panama	Panama	Panama	Panama
33	Panama	Brisbane	Brisbane	Brisbane	Panama	Panama	Panama	Panama
34	Brisbane	Brisbane	Brisbane	Brisbane	Panama	Panama	Panama	Panama
35	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane	Brisbane	Panama	Panama

The results indicate that reassortant viruses #2, #9, #30, #31, #32, #33, #34 and #35 grow equally well or even better in the culture host (see Figures 2 and 3) than the corresponding wild-type strain. Most of these strains comprise the NP segment from B/Brisbane/60/08 and some (in particular those which grew best) further contain the PB2 segment from B/Brisbane/60/08. All of these viruses also contain viral segments from the B/Victoria/2/87-like strain and the B/Yamagata/16/88-like strain at a ratio 7:1, 6:2, 4:4, 3:4 or 1:7.

Chimeric HA and NA segments with terminal regions from cell-adapted strains

Chimeric HA and NA segments are constructed that combine the non-antigenic terminal regions from HA (NCRs, signal peptide, transmembrane and cytoplasmic domains) and NA (NCRs, cytoplasmic and transmembrane domains) from PR8X and 105p30 with the ectodomain of the A/Brisbane/10/10 HA and NA segments, respectively. Figure 4 shows a diagram of the constructs and a sequence alignment of the terminal regions of HA (panels A, B) and NA (panels C, D).

PR8X(term) HA and NA constructs significantly enhance HA yield with the PR8X backbone

Reassortant influenza viruses are rescued which contain the PR8X backbone in combination with either the A/Brisbane/10/10 (H1N1) wt HA and NA segments, or chimeric HA and NA segments which comprise the ectodomain from A/Brisbane/10/10 and the other domains from PR8X (PR8X(term)). The growth and HA yield from the different rescued viruses is compared.

HA yield (Fig. 5A), as measured by HA ELISA, is 4-fold higher for the virus with PR8X(term) HA and NA segments than for the virus with WT HA and NA segments ($P < 0.01$). Virus with the PR8X(term) HA segment and WT NA segment yields a 3-fold increase in HA compared to the virus with WT HA and NA ($P < 0.05$). Virus with PR8X(term) HA and NA segments has 2-fold higher HA titers ($P<0.05$) and 4-fold higher viral titers than the virus with WT HA and NA segments (Figs. 5B, C). Overall, these data show that viruses with chimeric PR8X(term) HA and NA segments yield more HA than viruses containing only chimeric PR8X(term) HA or NA segments.

Chimeric HA and NA constructs enhance HA yield with all three optimized backbones

The inventors next tested whether the PR8X(term) or 105p30(term) HA/NA segments can enhance growth and HA yield of the resulting viruses in all three of the optimized backbones (Fig. 1A.). HA yield, as measured by ELISA and normalized to the yield from WT HA and NA segments, increase ~4-fold with PR8X(term) HA and NA segments and ~5-fold ($P<0.05$) with 105(term) HA and NA segments using the PR8X backbone (Fig. 6A). HA yield increases correlate with increases in HA titer and viral titers using the PR8X(term) and 105(term) HA and NA constructs (Figs. 6D, G). With the #19 backbone, HA yield is ~2.5-fold higher ($P < 0.05$) with the PR8X(term) HA and NA segments and ~3-fold higher ($P < 0.05$) with the 105(term) HA and NA segments over virus with WT HA and NA segments (Fig. 6B). HA yield increases are not associated with increases in viral titers or HA titers (Figs. 6E, H).

When using the #21 backbone, the inventors find significant increases with PR8X(term) and 105(term) HA and NA segments in HA yield, ~2.5-fold ($P < 0.01$) and ~3-fold ($P < 0.01$) respectively, HA titers (2-fold ($P<0.05$)) and viral titers over virus containing WT HA and NA segments (Figs. 6C, F, I). Overall, these data show that using chimeric HA and NA segments with terminal regions derived from cell-adapted strains increase HA yield independent of the backbone used.

The inventors confirm that these results are not limited to a specific vaccine strain, by preparing a reassortant influenza virus which comprises the #21 backbone, the HA and NA ectodomain from

A/Victoria/210/2009, and the terminal regions from WT A/Victoria/210/2009, PR8X or 105p30. The results (Figure 7) show that reassortants which comprise chimeric HA or NA segments give better HA yields.

Sequence analyses of the viruses recovered from all backbones with WT or chimeric HA and NA segments confirmed their sequence identity with the plasmids used in virus rescue. To confirm that viruses with chimeric HA and NA segments maintain their correct antigenicity, a hemagglutination inhibition (HAI) assay is performed using ferret antisera raised against A/California/07/2009, which is antigenically similar to WT A/Brisbane/10/10. Table 2 shows, as expected, that the viruses with the chimeric HA and NA segments are antigenically indistinguishable (within 2-fold in an HAI assay) from the reference antigen that contains the WT HA and NA segments.

Table 2: Antigenic analysis of viruses derived from the three optimized backbones (Values represent the geometric mean of HI titers from duplicate experiments)

Antigen	Ferret Sera FR-359 (H1N1)
A/Brisbane/10/10	2560
PR8X+Bris(term) HA/NA	1920
PR8X+PR8X(term) HA/NA	1920
PR8X+105(term) HA/NA	1920
#19+Bris(term) HA/NA	1280
#19+PR8X(term) HA/NA	1280
#19+105(term) HA/NA	2560
#21+Bris(term) HA/NA	2560
#21+PR8X(term) HA/NA	1920
#21+105(term) HA/NA	1280
IVR165 (H3N2)	10>

Increased HA content of viruses containing chimeric HA/NA segments

To verify further that the results observed using unpurified cell culture supernatants reflect HA yield from purified viruses, the inventors performed additional characterizations of viruses derived from the #21 backbone, which produce the highest amounts of HA (Fig. 1). To this end, large-scale amplifications (60 mL) of these viruses are performed and viruses purified using sucrose density-gradient centrifugation, as described in the methods. HA1 yield (normalized to the original culture volume of 60mL) is determined using HPLC. Compared to viruses with wt HA/NA segments, viruses with the chimeric PR8X(term) and 105p30(term) HA/NA segments have ~1.8 fold increase (11.3 ug/mL vs 6.2 ug/mL) and a ~2.2 fold increase (13.6 ug/mL vs 6.2 ug/mL) in HA yield, respectively (Fig 8A).

The HA content in these purified preparations is determined by using either gel densitometry or a combination of HPLC measurement of HA and total protein measurement by BCA assay. For gel

densitometry determination, the pooled fractions are treated with PNGaseF, resolved by SDS-PAGE, and then stained with SYPRO-Ruby to permit accurate determination of NP, HA1, M, and HA2 by densitometry. Fig. 8B shows the positions of these bands on the stained gel, and Fig. 8C shows that viruses with the PR8X(term) and 105(term) HA and NA segments had increases of 14% ($P<0.05$) and 32% ($P<0.01$), respectively, compared to viruses containing the WT HA and NA segments.

To quantitate HA1 content using the HPLC data, the HA1 values obtained by HPLC (Fig. 8A) are expressed as a fraction of the total protein content (as measured by the BCA assay) of the pooled fractions. The results in Fig. 8C show that viruses with PR8X(term) and 105(term) HA and NA segments had increased HA content of 29% and 46 %, respectively, compared to WT HA and NA containing viruses.

In conclusion, these data show that the productivity of three optimized backbones for virus rescue can be enhanced by modifying the terminal regions of the HA and NA segments with those from cell-adapted strains.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

SEQUENCES

SEQ ID NO: 1 (PA, PR8-X)

MEDFVRQCFNPMIVELAEKTMKEYGEDLKIETNKFAAICTHLEVCFMYSDFHFINEQGESIIVELGDPNALLKHRFE
IIEGRDRTMAWTVVNSICNTTGAEKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKANKIKSEKTHIHIFSFTGEE
MATKADYTLDEESRARIKTRLFTIRQEMASRGLWDSFRQSERGEETIEERFEITGTMRKLADQSLPPNFSSLENFRA
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DVGDLKQYDSDEPELRSLASWIQNEFNKACELTDSSWIELDEIGEDVAPIEHIASMRRNYFTSEVSHCRATEYIMKG
VYINTALLNASCAAMDDFQLIPMISKCRTKEGRRKTNLYGFIIKGRSHLRNDTDVVNFVSMEFSLTDPRLEPHKWEK
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LINDPWVLLNASWFNSFLTHALS

SEQ ID NO: 2 (PB1, PR8-X)

MDVNPTLLFLKVPTQNAISTTFPYTGDPPYSHGTGTGYTMDTVNRTHQYSEKGRWTTNTETGAPQLNPIDGPLPEDN
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NLFEKFFPSSSYRRPVGISSMVEAMVSRARIDARIDFESGRIKKEEFTEIMKICSTIEELRRQK

SEQ ID NO: 3 (PB2, PR8-X)

MERIKELRNLMSQSRTREILTKTTVDHMAIIKKYTSGRQEKNPALRMKWMMAMKYPITADKRITEMIPERNEQGQTL
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SAKEAQDVIMEVVFPNEVGARILTSESQLTITKEKKEELQDCKISPLMVAYMLERELVRKTRFLPVAGGTSSVYIEV
LHLTQGTCWEQMYTPGGEVRNDDVDQSLIIAARNIVRRAAVSADPLASLLEMCHSTQIGGIRMVDILRQNPTEEQAV

DICKAAMGLRISSSFSFGGFTFKRTSGSSVKREEEVLTGNLQTLKIRVHEGYEEFTMVGRRATAILRKATRRLIQLI
VSGRDEQSIAEAIIVAMVFSQEDCMIKAVRGDLNFVNRANQRLNPMHQLLRHFQKDARVLFQNWGVEPIDNVMGMIG
ILPDMTPSIEMSMRGVRISKMGVDEYSSTERVVVSIDRFLRIRDQRGNVLLSPEEVSETQGTEKLTITYSSSMMWEI
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SEQ ID NO: 4 (NP, PR8-X)

MASQGTKRSYEQMETDGERQNATEIRASVGKMIGGIGRFYIQMCTELKLSDYEGRLIQNSLTIERMVLSAFDERRNK
YLEEHPSAGKDPKKTGGPIYRRVNGKWMRELILYDKEEIRRIWRQANNGDDATAGLTHMMIWHSNLNDATYQRTRAL
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TAAQKAMMDQVRESRNPGNAEFEDLTFLARSALILRGSVAHKSCLPACVYGPAVASGYDFEREGYSLVGIDPFRLLQ
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SEQ ID NO: 5 (M, PR8-X)

MSLLTEVETYVLSIIPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRPILSPLTKGILGFVFTLTVPSERGLQRR
RFVQNALNGNGDPNNMDKAVKLYRKLKREITFHGAKEISLSYSAGALASCMGLIYNRMGAVTTEVAFGLVCATCEQI
ADSQHRSHRQMVTTTNPLIRHENRMVLASTTAKAMEQMAGSSEQAAEAMEVASQARQMVQAMRTIGTHPSSSAGLKN
DLLENLQAYQKRMGVQMQRFK

SEQ ID NO: 6 (NS, PR8-X)

MDPNTVSSFQVDCFLWHVRKRVADQELGDAPFLDRLRRDQKSLRGRGSTLGLDIKTATRAGKQIVERILKEESDEAL
KMTMASVPASRYLTDMTLEEMSRDWSMLIPKQKVAGPLCIRMDQAIMDKNIILKANFSVIFDRLETLILLRAFTEEG
AIVGEISPLPSLPGHTAEDVKNAVGVLIGGLEWNDNTVRVSETLQRFAWRSSNENGRPPLTPKQKREMAGTIRSEV

SEQ ID NO: 7 (HA, PR8-X)

MKANLLVLLCALAAADADTICIGYHTNNSTDTVDTVLEKNVTVTHSVNLLEDSHNGKLCRLKGIAPLQLGKCNIAGW
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NAINGITNKVNTVIEKMNIQFTAVGKEFNKLEKRMENLNKKVDDGFLDIWTYNAELLVLLENERTLEFHDSNVKNLY
EKVKSQLKNNAKEIGNGCFEFYHKCDNECMESVRNGTYDYPKYSEESKLNREKVDGVKLESMGIYQILAIYSTVASS
LVLLVSLGAISFWMCSNGSLQCRICI

SEQ ID NO: 8 (NA, PR8-X)

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SEQ ID NO: 9 (PA, PR8-X)

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SEQ ID NO: 10 (PB1, PR8-X)

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CCCGTTCCAAAGCTGGACTGCTGGTCTCCGACGGAGGCCCAAATTTATACAACATTAGAAATCTCCACATTCCTGAA
GTCTGCCTAAAATGGGAATTGATGGATGAGGATTACCAGGGGCGTTTATGCAACCCACTGAACCCATTTGTCAGCCA
TAAAGAAATTGAATCAATGAACAATGCAGTGATGATGCCAGCACATGGTCCAGCCAAAAACATGGAGTATGATGCTG
TTGCAACAACACACTCCTGGATCCCCAAAAGAAATCGATCCATCTTGAATACAAGTCAAAGAGGAGTACTTGAGGAT
GAACAAATGTACCAAAGGTGCTGCAATTTATTTGAAAAATTCTTCCCCAGCAGTTCATACAGAAGACCAGTCGGGAT
ATCCAGTATGGTGGAGGCTATGGTTTCCAGAGCCCGAATTGATGCACGGATTGATTTCGAATCTGGAAGGATAAAGA
AAGAAGAGTTCACTGAGATCATGAAGATCTGTTCCACCATTGAAGAGCTCAGACGGCAAAAATAGTGAATTTAGCTT
GTCCTTCATGAAAAAATGCCTTGTTTCTACT

SEQ ID NO: 11 (PB2, PR8-X)

AGCGAAAGCAGGTCAATTATATTCAATATGGAAAGAATAAAAGAACTAAGAAATCTAATGTCGCAGTCTCGCACCCG
CGAGATACTCACAAAAACCACCGTGGACCATATGGCCATAATCAAGAAGTACACATCAGGAAGACAGGAGAAGAACC
CAGCACTTAGGATGAAATGGATGATGGCAATGAAATATCCAATTACAGCAGACAAGAGGATAACGGAAATGATTCCT
GAGAGAAATGAGCAAGGACAAACTTTATGGAGTAAAATGAATGATGCCGGATCAGACCGAGTGATGGTATCACCTCT
GGCTGTGACATGGTGGAATAGGAATGGACCAATAACAAATACAGTTCATTATCCAAAAATCTACAAAACTTATTTTG
AAAGAGTAGAAAGGCTAAAGCATGGAACCTTTGGCCCTGTCCATTTTAGAAACCAAGTCAAAATACGTCGGAGAGTT
GACATAAATCCTGGTCATGCAGATCTCAGTGCCAAGGAGGCACAGGATGTAATCATGGAAGTTGTTTTCCCTAACGA
AGTGGGAGCCAGGATACTAACATCGGAATCGCAACTAACGATAACCAAAGAGAAGAAAGAAGAACTCCAGGATTGCA
AAATTTCTCCTTTGATGGTTGCATACATGTTGGAGAGAGAACTGGTCCGCAAAACGAGATTCCTCCCAGTGGCTGGT
GGAACAAGCAGTGTGTACATTGAAGTGTTGCATTTGACTCAAGGAACATGCTGGGAACAGATGTATACTCCAGGAGG

GGAAGTGAGGAATGATGATGTTGATCAAAGCTTGATTATTGCTGCTAGGAACATAGTGAGAAGAGCTGCAGTATCAG
CAGATCCACTAGCATCTTTATTGGAGATGTGCCACAGCACACAGATTGGTGGAATTAGGATGGTAGACATCCTTAGG
CAGAACCCAACAGAAGAGCAAGCCGTGGATATATGCAAGGCTGCAATGGGACTGAGAATTAGCTCATCCTTCAGTTT
TGGTGGATTCACATTTAAGAGAACAAGCGGATCATCAGTCAAGAGAGAGGAAGAGGTGCTTACGGGAAATCTTCAAA
CATTGAAGATAAGAGTGCATGAGGGATATGAAGAGTTCACAATGGTTGGGAGAAGAGCAACAGCCATACTCAGAAAA
GCAACCAGGAGATTGATTCAGCTGATAGTGAGTGGGAGAGACGAACAGTCGATTGCCGAAGCAATAATTGTGGCCAT
GGTATTTTCACAAGAGGATTGTATGATAAAAGCAGTCAGAGGTGATCTGAATTTCGTCAATAGGGCGAATCAGCGAT
TGAATCCTATGCATCAACTTTTAAGACATTTTCAGAAGGATGCGAGAGTGCTTTTTCAAAATTGGGGAGTTGAACCT
ATCGACAATGTGATGGGAATGATTGGGATATTGCCCGACATGACTCCAAGCATCGAGATGTCAATGAGAGGAGTGAG
AATCAGCAAAATGGGTGTAGATGAGTACTCCAGCACGGAGAGGGTAGTGGTGAGCATTGACCGTTTTTTGAGAATCC
GGGACCAACGAGGAAATGTACTACTGTCTCCCGAGGAGGTCAGTGAAACACAGGGAACAGAGAAACTGACAATAACT
TACTCATCGTCAATGATGTGGGAGATTAATGGTCCTGAATCAGTATTGGTCAATACCTATCAATGGATCATCAGAAA
CTGGGAAACTGTTAAAATTCAGTGGTCCCAGAACCCTACAATGCTATACAATAAAATGGAATTTGAACCATTTCAGT
CTTTAGTACCTAAGGCCATTAGAGGCCAATACAGTGGGTTTGTAAGAACTCTGTTCCAACAAATGAGGGATGTGCTT
GGGACATTTGATACCGCACAGATAATAAAACTTCTTCCCTTCGCAGCCGCTCCACCAAAGCAAAGTAGAATGCAGTT
CTCCTCATTTACTGTGAATGTGAGGGGATCAGGAATGAGAATACTTGTAAGGGGCAATTCTCCTGTATTCAACTATA
ACAAGGCCACGAAGAGACTCACAGTTCTCGGAAAGGATGCTGGCACTTTAACTGAAGACCCAGATGAAGGCACAGCT
GGAGTGGAGTCCGCTGTTCTGAGGGGATTCCTCATTCTGGGCAAAGAAGACAAGAGATATGGGCCAGCACTAAGCAT
CAATGAACTGAGCAACCTTGCGAAAGGAGAGAAGGCTAATGTGCTAATTGGGCAAGGAGACGTGGTGTTGGTAATGA
AACGGAAACGGGACTCTAGCATACTTACTGACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAGTGT
CGAATAGTTTAAAAACGACCTTGTTTCTACT

SEQ ID NO: 12 (NP, PR8-X)

AGCAAAAGCAGGGTAGATAATCACTCACTGAGTGACATCAAAATCATGGCGTCTCAAGGCACCAAACGATCTTACGA
ACAGATGGAGACTGATGGAGAACGCCAGAATGCCACTGAAATCAGAGCATCCGTCGGAAAAATGATTGGTGGAATTG
GACGATTCTACATCCAAATGTGCACCGAACTCAAACTCAGTGATTATGAGGGACGGTTGATCCAAAACAGCTTAACA
ATAGAGAGAATGGTGCTCTCTGCTTTTGACGAAAGGAGAAATAAATACCTTGAAGAACATCCCAGTGCGGGAAAAGA
TCCTAAGAAAACTGGAGGACCTATATACAGGAGAGTAAACGGAAAGTGGATGAGAGAACTCATCCTTTATGACAAAG
AAGAAATAAGGCGAATCTGGCGCCAAGCTAATAATGGTGACGATGCAACGGCTGGTCTGACTCACATGATGATCTGG
CATTCCAATTTGAATGATGCAACTTATCAGAGGACAAGAGCTCTTGTTCGCACCGGAATGGATCCCAGGATGTGCTC
TCTGATGCAAGGTTCAACTCTCCCTAGGAGGTCTGGAGCCGCAGGTGCTGCAGTCAAAGGAGTTGGAACAATGGTGA
TGGAATTGGTCAGAATGATCAAACGTGGGATCAATGATCGGAACTTCTGGAGGGGTGAGAATGGACGAAAAACAAGA
ATTGCTTATGAAAGAATGTGCAACATTCTCAAAGGGAAATTTCAAACTGCTGCACAAAAAGCAATGATGGATCAAGT
GAGAGAGAGCCGGAACCCAGGGAATGCTGAGTTCGAAGATCTCACTTTTCTAGCACGGTCTGCACTCATATTGAGAG
GGTCGGTTGCTCACAAGTCCTGCCTGCCTGCCTGTGTGTATGGACCTGCCGTAGCCAGTGGGTACGACTTTGAAAGG
GAGGGATACTCTCTAGTCGGAATAGACCCTTTCAGACTGCTTCAAAACAGCCAAGTGTACAGCCTAATCAGACCAAA
TGAGAATCCAGCACACAAGAGTCAACTGGTGTGGATGGCATGCCATTCTGCCGCATTTGAAGATCTAAGAGTATTAA
GCTTCATCAAAGGGACGAAGGTGCTCCCAAGAGGGAAGCTTTCCACTAGAGGAGTTCAAATTGCTTCCAATGAAAAT
ATGGAGACTATGGAATCAAGTACACTTGAACTGAGAAGCAGGTACTGGGCCATAAGGACCAGAAGTGGAGGAAACAC
CAATCAACAGAGGGCATCTGCGGGCCAAATCAGCATACAACCTACGTTCTCAGTACAGAGAAATCTCCCTTTTGACA
GAACAACCATTATGGCAGCATTCAATGGGAATACAGAGGGGAGAACATCTGACATGAGGACCGAAATCATAAGGATG
ATGGAAAGTGCAAGACCAGAAGATGTGTCTTTCCAGGGGCGGGGAGTCTTCGAGCTCTCGGACGAAAAGGCAGCGAG
CCCGATCGTGCCTTCCTTTGACATGAGTAATGAAGGATCTTATTTCTTCGGAGACAATGCAGAGGAGTACGACAATT
AAAGAAAAATACCCTTGTTTCTACT

SEQ ID NO: 13 (M, PR8-X)

AGCAAAAGCAGGTAGATATTGAAAGATGAGTCTTCTAACCGAGGTCGAAACGTACGTACTCTCTATCATCCCGTCAG
GCCCCCTCAAAGCCGAGATCGCACAGAGACTTGAAGATGTCTTTGCAGGGAAGAACACCGATCTTGAGGTTCTCATG
GAATGGCTAAAGACAAGACCAATCCTGTCACCTCTGACTAAGGGGATTTTAGGATTTGTGTTCACGCTCACCGTGCC
CAGTGAGCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCTTAATGGGAACGGGGATCCAAATAACATGGACA
AAGCAGTTAAACTGTATAGGAAGCTCAAGAGGGAGATAACATTCCATGGGGCCAAAGAAATCTCACTCAGTTATTCT
GCTGGTGCACTTGCCAGTTGTATGGGCCTCATATACAACAGGATGGGGGCTGTGACCACTGAAGTGGCATTTGGCCT
GGTATGTGCAACCTGTGAACAGATTGCTGACTCCCAGCATCGGTCTCATAGGCAAATGGTGACAACAACCAATCCAC
TAATCAGACATGAGAACAGAATGGTTTTAGCCAGCACTACAGCTAAGGCTATGGAGCAAATGGCTGGATCGAGTGAG
CAAGCAGCAGAGGCCATGGAGGTTGCTAGTCAGGCTAGACAAATGGTGCAAGCGATGAGAACCATTGGGACTCATCC
TAGCTCCAGTGCTGGTCTGAAAAATGATCTTCTTGAAAATTTGCAGGCCTATCAGAAACGAATGGGGGTGCAGATGC
AACGGTTCAAGTGATCCTCTCACTATTGCCGCAAATATCATTGGGATCTTGCACTTGACATTGTGGATTCTTGATCG
TCTTTTTTTCAAATGCATTTACCGTCGCTTTAAATACGGACTGAAAGGAGGGCCTTCTACGGAAGGAGTGCCAAAGT
CTATGAGGGAAGAATATCGAAAGGAACAGCAGAGTGCTGTGGATGCTGACGATGGTCATTTTGTCAGCATAGAGCTG
GAGTAAAAAACTACCTTGTTTCTACT

SEQ ID NO: 14 (NS, PR8-X)

AGCAAAAGCAGGGTGACAAAAACATAATGGATCCAAACACTGTGTCAAGCTTTCAGGTAGATTGCTTTCTTTGGCAT
GTCCGCAAACGAGTTGCAGACCAAGAACTAGGTGATGCCCCATTCCTTGATCGGCTTCGCCGAGATCAGAAATCCCT
AAGAGGAAGGGGCAGTACTCTCGGTCTGGACATCAAGACAGCCACACGTGCTGGAAAGCAGATAGTGGAGCGGATTC
TGAAAGAAGAATCCGATGAGGCACTTAAAATGACCATGGCCTCTGTACCTGCGTCGCGTTACCTAACTGACATGACT
CTTGAGGAAATGTCAAGGGACTGGTCCATGCTCATACCCAAGCAGAAAGTGGCAGGCCCTCTTTGTATCAGAATGGA
CCAGGCGATCATGGATAAGAACATCATACTGAAAGCGAACTTCAGTGTGATTTTTGACCGGCTGGAGACTCTAATAT
TGCTAAGGGCTTTCACCGAAGAGGGAGCAATTGTTGGCGAAATTTCACCATTGCCTTCTCTTCCAGGACATACTGCT
GAGGATGTCAAAAATGCAGTTGGAGTCCTCATCGGAGGACTTGAATGGAATGATAACACAGTTCGAGTCTCTGAAAC
TCTACAGAGATTCGCTTGGAGAAGCAGTAATGAGAATGGGAGACCTCCACTCACTCCAAAACAGAAACGAGAAATGG
CGGGAACAATTAGGTCAGAAGTTTGAAGAAATAAGATGGTTGATTGAAGAAGTGAGACACAAACTGAAGATAACAGA
GAATAGTTTTGAGCAAATAACATTTATGCAAGCCTTACATCTATTGCTTGAAGTGGAGCAAGAGATAAGAACTTTCT
CGTTTCAGCTTATTTAGTACTAAAAAACACCCTTGTTTCTACT

SEQ ID NO: 15 (HA, PR8-X)

AGCAAAAGCAGGGGAAAATAAAAACAACCAAAATGAAGGCAAACCTACTGGTCCTGTTATGTGCACTTGCAGCTGCA
GATGCAGACACAATATGTATAGGCTACCATACGAACAATTCAACCGACACTGTTGACACAGTACTCGAGAAGAATGT
GACAGTGACACACTCTGTTAACCTGCTCGAAGACAGCCACAACGGAAAACTATGTAGATTAAAAGGAATAGCCCCAC
TACAATTGGGGAAATGTAACATCGCCGGATGGCTCTTGGGAAACCCAGAATGCGACCCACTGCTTCCAGTGAGATCA
TGGTCCTACATTGTAGAAACACCAAACTCTGAGAATGGAATATGTTATCCAGGAGATTTCATCGACTATGAGGAGCT
GAGGGAGCAATTGAGCTCAGTGTCATCATTCGAAAGATTCGAAATATTTCCCAAAGAAAGCTCATGGCCCAACCACA
ACACAAACGGAGTAACGGCAGCATGCTCCCATGAGGGGAAAAGCAGTTTTTACAGAAATTTGCTATGGCTGACGGAG
AAGGAGGGCTCATACCCAAAGCTGAAAAATTCTTATGTGAACAAAAAAGGGAAAGAAGTCCTTGTACTGTGGGGTAT
TCATCACCCGCCTAACAGTAAGGAACAACAGAATCTCTATCAGAATGAAAATGCTTATGTCTCTGTAGTGACTTCAA
ATTATAACAGGAGATTTACCCCGGAAATAGCAGAAAGACCCAAAGTAAGAGATCAAGCTGGGAGGATGAACTATTAC
TGGACCTTGCTAAAACCCGGAGACACAATAATATTTGAGGCAAATGGAAATCTAATAGCACCAATGTATGCTTTCGC
ACTGAGTAGAGGCTTTGGGTCCGGCATCATCACCTCAAACGCATCAATGCATGAGTGTAACACGAAGTGTCAAACAC
CCCTGGGAGCTATAAACAGCAGTCTCCCTTACCAGAATATACACCCAGTCACAATAGGAGAGTGCCCAAAATACGTC
AGGAGTGCCAAATTGAGGATGGTTACAGGACTAAGGAACATTCCGTCCATTCAATCCAGAGGTCTATTTGGAGCCAT
TGCCGGTTTTATTGAAGGGGGATGGACTGGAATGATAGATGGATGGTATGGTTATCATCATCAGAATGAACAGGGAT
CAGGCTATGCAGCGGATCAAAAAAGCACACAAAATGCCATTAACGGGATTACAAACAAGGTGAACACTGTTATCGAG
AAAATGAACATTCAATTCACAGCTGTGGGTAAAGAATTCAACAAATTAGAAAAAAGGATGGAAAATTTAAATAAAAA
AGTTGATGATGGATTTCTGGACATTTGGACATATAATGCAGAATTGTTAGTTCTACTGGAAAATGAAAGGACTCTGG
AATTCCATGACTCAAATGTGAAGAATCTGTATGAGAAAGTAAAAAGCCAATTAAAGAATAATGCCAAAGAAATCGGA
AATGGATGTTTTGAGTTCTACCACAAGTGTGACAATGAATGCATGGAAAGTGTAAGAAATGGGACTTATGATTATCC
CAAATATTCAGAAGAGTCAAAGTTGAACAGGGAAAAGGTAGATGGAGTGAAATTGGAATCAATGGGGATCTATCAGA
TTCTGGCGATCTACTCAACTGTCGCCAGTTCACTGGTGCTTTTGGTCTCCCTGGGGGCAATCAGTTTCTGGATGTGT
TCTAATGGATCTTTGCAGTGCAGAATATGCATCTGAGATTAGAATTTCAGAGATATGAGGAAAAACACCCTTGTTTC
TACT

SEQ ID NO: 16 (NA, PR8-X)

AGCAAAAGCAGGGGTTTAAAATGAATCCAAATCAGAAAATAATAACCATTGGATCAATCTGTCTGGTAGTCGGACTA
ATTAGCCTAATATTGCAAATAGGGAATATAATCTCAATATGGATTAGCCATTCAATTCAAACTGGAAGTCAAAACCA
TACTGGAATATGCAACCAAAACATCATTACCTATAAAAATAGCACCTGGGTAAAGGACACAACTTCAGTGATATTAA
CCGGCAATTCATCTCTTTGTCCCATCCGTGGGTGGGCTATATACAGCAAAGACAATAGCATAAGAATTGGTTCCAAA
GGAGACGTTTTTGTCATAAGAGAGCCCTTTATTTCATGTTCTCACTTGGAATGCAGGACCTTTTTTCTGACCCAAGG
TGCCTTACTGAATGACAAGCATTCAAGTGGGACTGTTAAGGACAGAAGCCCTTATAGGGCCTTAATGAGCTGCCCTG
TCGGTGAAGCTCCGTCCCCGTACAATTCAAGATTTGAATCGGTTGCTTGGTCAGCAAGTGCATGTCATGATGGCATG
GGCTGGCTAACAATCGGAATTTCAGGTCCAGATAATGGAGCAGTGGCTGTATTAAAATACAACGGCATAATAACTGA
AACCATAAAAAGTTGGAGGAAGAAAATATTGAGGACACAAGAGTCTGAATGTGCCTGTGTAAATGGTTCATGTTTTA
CTATAATGACTGATGGCCCGAGTGATGGGCTGGCCTCGTACAAAATTTTCAAGATCGAAAAGGGGAAGGTTACTAAA
TCAATAGAGTTGAATGCACCTAATTCTCACTATGAGGAATGTTCCTGTTACCCTGATACCGACAAAGTGATGTGTGT
GTGCAGAGACAATTGGCATGGTTCGAACCGGCCATGGGTGTCTTTCGATCAAAACCTGGATTATCAAATAGGATACA
TCTGCAGTGGGGTTTTCGGTGACAACCCGCGTCCCGAAGATGGAACAGGCAGCTGTGGTCCAGTGTATGTTGATGGA
GCAAACGGAGTAAAGGGATTTTCATATAGGTATGGTAATGGTGTTTGGATAGGAAGGACCAAAAGTCACAGTTCCAG
ACATGGGTTTGAGATGATTTGGGATCCTAATGGATGGACAGAGACTGATAGTAAGTTCTCTGTGAGGCAAGATGTTG
TGGCAATGACTGATTGGTCAGGGTATAGCGGAAGTTTCGTTCAACATCCTGAGCTGACAGGGCTAGACTGTATGAGG
CCGTGCTTCTGGGTTGAATTAATCAGGGGACGACCTAAAGAAAAAACAATCTGGACTAGTGCGAGCAGCATTTCTTT
TTGTGGCGTGAATAGTGATACTGTAGATTGGTCTTGGCCAGACGGTGCTGAGTTGCCATTCAGCATTGACAAGTAGT
CTGTTCAAAAAACTCCTTGTTTCTACT

SEQ ID NO: 17 (PA, A/California/07/09)

MEDFVRQCFNPMIVELAEKAMKEYGEDPKIETNKFAAICTHLEVCFMYSDFHFIDERGESIIVESGDPNALLKHRFE
IIEGRDRIMAWTVVNSICNTTGVEKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKANKIKSEKTHIHIFSFTGEE
MATKADYTLDEESRARIKTRLFTIRQEMASRSLWDSFRQSERGEETIEEKFEITGTMRKLADQSLPPNFPSLENFRA
YVDGFEPNGCIEGKLSQMSKEVNAKIEPFLRTTPRPLRLPDGPLCHQRSKFLLMDALKLSIEDPSHEGEGIPLYDAI
KCMKTFFGWKEPNIVKPHEKGINPNYLMAWKQVLAELQDIENEEKIPRTKNMKRTSQLKWALGENMAPEKVDFDDCK
DVGDLKQYDSDEPEPRSLASWVQNEFNKACELTDSSWIELDEIGEDVAPIEHIASMRRNYFTAEVSHCRATEYIMKG
VYINTALLNASCAAMDDFQLIPMISKCRTKEGRRKTNLYGFIIKGRSHLRNDTDVVNFVSMEFSLTDPRLEPHKWEK
YCVLEIGDMLLRTAIGQVSRPMFLYVRTNGTSKIKMKWGMEMRRCLLQSLQQIESMIEAESSVKEKDMTKEFFENKS
ETWPIGESPRGVEEGSIGKVCRTLLAKSVFNSLYASPQLEGFSAESRKLLLIVQALRDNLEPGTFDLGGLYEAIEEC
LINDPWVLLNASWFNSFLTHALK

SEQ ID NO: 18 (PB1, A/California/07/09)

MDVNPTLLFLKIPAQNAISTTFPYTGDPPYSHGTGTGYTMDTVNRTHQYSEKGKWTTNTETGAPQLNPIDGPLPEDN
EPSGYAQTDCVLEAMAFLEESHPGIFENSCLETMEVVQQTRVDKLTQGRQTYDWTLNRNQPAATALANTIEVFRSNG
LTANESGRLIDFLKDVMESMNKEEIEITTHFQRKRRVRDNMTKKMVTQRTIGKKKQRLNKRGYLIRALTLNTMTKDA
ERGKLKRRAIATPGMQIRGFVYFVETLARSICEKLEQSGLPVGGNEKKAKLANVVRKMMTNSQDTEISFTITGDNTK
WNENQNPRMFLAMITYITRNQPEWFRNILSMAPIMFSNKMARLGKGYMFESKRMKIRTQIPAEMLASIDLKYFNEST
KKKIEKIRPLLIDGTASLSPGMMMGMFNMLSTVLGVSILNLGQKKYTKTIYWWDGLQSSDDFALIVNAPNHEGIQAG
VDRFYRTCKLVGINMSKKKSYINKTGTFEFTSFFYRYGFVANFSMELPSFGVSGVNESADMSIGVTVIKNNMINNDL
GPATAQMALQLFIKDYRYTYRCHRGDTQIQTRRSFELKKLWDQTQSKVGLLVSDGGPNLYNIRNLHIPEVCLKWELM
DDDYRGRLCNPLNPFVSHKEIDSVNNAVVMPAHGPAKSMEYDAVATTHSWIPKRNRSILNTSQRGILEDEQMYQKCC
NLFEKFFPSSSYRRPVGISSMVEAMVSRARIDARVDFESGRIKKEEFSEIMKICSTIEELRRQK

SEQ ID NO: 19 (PB2, A/California/07/09)

MERIKELRDLMSQSRTREILTKTTVDHMAIIKKYTSGRQEKNPALRMKWMMAMRYPITADKRIMDMIPERNEQGQTL
WSKTNDAGSDRVMVSPLAVTWWNRNGPTTSTVHYPKVYKTYFEKVERLKHGTFGPVHFRNQVKIRRRVDTNPGHADL
SAKEAQDVIMEVVFPNEVGARILTSESQLAITKEKKEELQDCKIAPLMVAYMLERELVRKTRFLPVAGGTGSVYIEV
LHLTQGTCWEQMYTPGGEVRNDDVDQSLIIAARNIVRRAAVSADPLASLLEMCHSTQIGGVRMVDILRQNPTEEQAV
DICKAAIGLRISSSFSFGGFTFKRTSGSSVKKEEEVLTGNLQTLKIRVHEGYEEFTMVGRRATAILRKATRRLIQLI
VSGRDEQSIAEAIIVAMVFSQEDCMIKAVRGDLNFVNRANQRLNPMHQLLRHFQKDAKVLFQNWGIESIDNVMGMIG
ILPDMTPSTEMSLRGIRVSKMGVDEYSSTERVVVSIDRFLRVRDQRGNVLLSPEEVSETQGTEKLTITYSSSMMWEI
NGPESVLVNTYQWIIRNWEIVKIQWSQDPTMLYNKMEFEPFQSLVPKATRSRYSGFVRTLFQQMRDVLGTFDTVQII
KLLPFAAAPPEQSRMQFSSLTVNVRGSGLRILVRGNSPVFNYNKATKRLTVLGKDAGALTEDPDEGTSGVESAVLRG
FLILGKEDKRYGPALSINELSNLAKGEKANVLIGQGDVVLVMKRKRDSSILTDSQTATKRIRMAIN

SEQ ID NO: 20 (NP, A/California/07/09)

MASQGTKRSYEQMETGGERQDATEIRASVGRMIGGIGRFYIQMCTELKLSDYDGRLIQNSITIERMVLSAFDERRNK
YLEEHPSAGKDPKKTGGPIYRRVDGKWMRELILYDKEEIRRVWRQANNGEDATAGLTHIMIWHSNLNDATYQRTRAL
VRTGMDPRMCSLMQGSTLPRRSGAAGAAVKGVGTIAMELIRMIKRGINDRNFWRGENGRRTRVAYERMCNILKGKFQ
TAAQRAMMDQVRESRNPGNAEIEDLIFLARSALILRGSVAHKSCLPACVYGLAVASGHDFEREGYSLVGIDPFKLLQ
NSQVVSLMRPN

SEQ ID NO: 21 (M1, A/California/07/09)

MSLLTEVETYVLSIIPSGPLKAEIAQRLESVFAGKNTDLEALMEWLKTRPILSPLTKGILGFVFTLTVPSERGLQRR
RFVQNALNGNGDPNNMDRAVKLYKKLKREITFHGAKEVSLSYSTGALASCMGLIYNRMGTVTTEAAFGLVCATCEQI
ADSQHRSHRQMATTTNPLIRHENRMVLASTTAKAMEQMAGSSEQAAEAMEVANQTRQMVHAMRTIGTHPSSSAGLKD
DLLENLQAYQKRMGVQMQRFK

SEQ ID NO: 22 (NS1, A/California/07/09)

MDSNTMSSFQVDCFLWHIRKRFADNGLGDAPFLDRLRRDQKSLKGRGNTLGLDIETATLVGKQIVEWILKEESSETL
RMTIASVPTSRYLSDMTLEEMSRDWFMLMPRQKIIGPLCVRLDQAIMEKNIVLKANFSVIFNRLETLILLRAFTEEG
AIVGEISPLPSLPGHTYEDVKNAVGVLIGGLEWNGNTVRVSENIQRFAWRNCDENGRPSLPPEQK

SEQ ID NO: 23 (PA, A/California/07/09)

ATGGAAGACTTTGTGCGACAATGCTTCAATCCAATGATCGTCGAGCTTGCGGAAAAGGCAATGAAAGAATATGGGGA
AGATCCGAAAATCGAAACTAACAAGTTTGCTGCAATATGCACACATTTGGAAGTTTGTTTCATGTATTCGGATTTCC
ATTTCATCGACGAACGGGGTGAATCAATAATTGTAGAATCTGGTGACCCGAATGCACTATTGAAGCACCGATTTGAG
ATAATTGAAGGAAGAGACCGAATCATGGCCTGGACAGTGGTGAACAGTATATGTAACACAACAGGGGTAGAGAAGCC
TAAATTTCTTCCTGATTTGTATGATTACAAAGAGAACCGGTTCATTGAAATTGGAGTAACACGGAGGGAAGTCCACA
TATATTACCTAGAGAAAGCCAACAAAATAAAATCTGAGAAGACACACATTCACATCTTTTCATTCACTGGAGAGGAG
ATGGCCACCAAAGCGGACTACACCCTTGACGAAGAGAGCAGGGCAAGAATCAAAACTAGGCTTTTCACTATAAGACA
AGAAATGGCCAGTAGGAGTCTATGGGATTCCTTTCGTCAGTCCGAAAGAGGCGAAGAGACAATTGAAGAAAAATTTG
AGATTACAGGAACTATGCGCAAGCTTGCCGACCAAAGTCTCCCACCGAACTTCCCCAGCCTTGAAAACTTTAGAGCC
TATGTAGATGGATTCGAGCCGAACGGCTGCATTGAGGGCAAGCTTTCCCAAATGTCAAAAGAAGTGAACGCCAAAAT
TGAACCATTCTTGAGGACGACACCACGCCCCCTCAGATTGCCTGATGGGCCTCTTTGCCATCAGCGGTCAAAGTTCC
TGCTGATGGATGCTCTGAAATTAAGTATTGAAGACCCGAGTCACGAGGGGGAGGGAATACCACTATATGATGCAATC
AAATGCATGAAGACATTCTTTGGCTGGAAAGAGCCTAACATAGTCAAACCACATGAGAAAGGCATAAATCCCAATTA
CCTCATGGCTTGGAAGCAGGTGCTAGCAGAGCTACAGGACATTGAAAATGAAGAGAAGATCCCAAGGACAAAGAACA
TGAAGAGAACAAGCCAATTGAAGTGGGCACTCGGTGAAAATATGGCACCAGAAAAAGTAGACTTTGATGACTGCAAA
GATGTTGGAGACCTTAAACAGTATGACAGTGATGAGCCAGAGCCCAGATCTCTAGCAAGCTGGGTCCAAAATGAATT
CAATAAGGCATGTGAATTGACTGATTCAAGCTGGATAGAACTTGATGAAATAGGAGAAGATGTTGCCCCGATTGAAC
ATATCGCAAGCATGAGGAGGAACTATTTTACAGCAGAAGTGTCCCACTGCAGGGCTACTGAATACATAATGAAGGGA
GTGTACATAAATACGGCCTTGCTCAATGCATCCTGTGCAGCCATGGATGACTTTCAGCTGATCCCAATGATAAGCAA
ATGTAGGACCAAAGAAGGAAGACGGAAAACAAACCTGTATGGGTTCATTATAAAAGGAAGGTCTCATTTGAGAAATG
ATACTGATGTGGTGAACTTTGTAAGTATGGAGTTCTCACTCACTGACCCGAGACTGGAGCCACACAAATGGGAAAAA
TACTGTGTTCTTGAAATAGGAGACATGCTCTTGAGGACTGCGATAGGCCAAGTGTCGAGGCCCATGTTCCTATATGT
GAGAACCAATGGAACCTCCAAGATCAAGATGAAATGGGGCATGGAAATGAGGCGCTGCCTTCTTCAGTCTCTTCAGC
AGATTGAGAGCATGATTGAGGCCGAGTCTTCTGTCAAAGAGAAAGACATGACCAAGGAATTCTTTGAAAACAAATCG
GAAACATGGCCAATCGGAGAGTCACCCAGGGGAGTGGAGGAAGGCTCTATTGGGAAAGTGTGCAGGACCTTACTGGC
AAAATCTGTATTCAACAGTCTATATGCGTCTCCACAACTTGAGGGGTTTTCGGCTGAATCTAGAAAATTGCTTCTCA
TTGTTCAGGCACTTAGGGACAACCTGGAACCTGGAACCTTCGATCTTGGGGGGCTATATGAAGCAATCGAGGAGTGC
CTGATTAATGATCCCTGGGTTTTGCTTAATGCATCTTGGTTCAACTCCTTCCTCACACATGCACTGAAGTAG

SEQ ID NO: 24 (PB1, A/California/07/09)

AGCGAAAGCAGGCAAACCATTTGAATGGATGTCAATCCGACTCTACTTTTCCTAAAAATTCCAGCGCAAAATGCCAT
AAGCACCACATTCCCTTATACTGGAGATCCTCCATACAGCCATGGAACAGGAACAGGATACACCATGGACACAGTAA
ACAGAACACACCAATACTCAGAAAAGGGAAAGTGGACGACAAACACAGAGACTGGTGCACCCCAGCTCAACCCGATT
GATGGACCACTACCTGAGGATAATGAACCAAGTGGGTATGCACAAACAGACTGTGTTCTAGAGGCTATGGCTTTCCT
TGAAGAATCCCACCCAGGAATATTTGAGAATTCATGCCTTGAAACAATGGAAGTTGTTCAACAAACAAGGGTAGATA
AACTAACTCAAGGTCGCCAGACTTATGATTGGACATTAAACAGAAATCAACCGGCAGCAACTGCATTGGCCAACACC
ATAGAAGTCTTTAGATCGAATGGCCTAACAGCTAATGAGTCAGGAAGGCTAATAGATTTCTTAAAGGATGTAATGGA
ATCAATGAACAAAGAGGAAATAGAGATAACAACCCACTTTCAAAGAAAAAGGAGAGTAAGAGACAACATGACCAAGA
AGATGGTCACGCAAAGAACAATAGGGAAGAAAAAACAAAGACTGAATAAGAGAGGCTATCTAATAAGAGCACTGACA
TTAAATACGATGACCAAAGATGCAGAGAGAGGCAAGTTAAAAAGAAGGGCTATCGCAACACCTGGGATGCAGATTAG
AGGTTTCGTATACTTTGTTGAAACTTTAGCTAGGAGCATTTGCGAAAAGCTTGAACAGTCTGGGCTCCCAGTAGGGG
GCAATGAAAAGAAGGCCAAACTGGCAAATGTTGTGAGAAAGATGATGACTAATTCACAAGACACAGAGATTTCTTTC
ACAATCACTGGGGACAACACTAAGTGGAATGAAAATCAAAATCCTCGAATGTTCCTGGCGATGATTACATATATCAC
CAGAAATCAACCCGAGTGGTTCAGAAACATCCTGAGCATGGCACCCATAATGTTCTCAAACAAAATGGCAAGACTAG
GGAAAGGGTACATGTTCGAGAGTAAAAGAATGAAGATTCGAACACAAATACCAGCAGAAATGCTAGCAAGCATTGAC
CTGAAGTACTTCAATGAATCAACAAAGAAGAAAATTGAGAAAATAAGGCCTCTTCTAATAGATGGCACAGCATCACT
GAGTCCTGGGATGATGATGGGCATGTTCAACATGCTAAGTACGGTCTTGGGAGTCTCGATACTGAATCTTGGACAAA
AGAAATACACCAAGACAATATACTGGTGGGATGGGCTCCAATCATCCGACGATTTTGCTCTCATAGTGAATGCACCA
AACCATGAGGGAATACAAGCAGGAGTGGACAGATTCTACAGGACCTGCAAGTTAGTGGGAATCAACATGAGCAAAAA
GAAGTCCTATATAAATAAGACAGGGACATTTGAATTCACAAGCTTTTTTTATCGCTATGGATTTGTGGCTAATTTTA
GCATGGAGCTACCCAGCTTTGGAGTGTCTGGAGTAAATGAATCAGCTGACATGAGTATTGGAGTAACAGTGATAAAG
AACAACATGATAAACAATGACCTTGGACCTGCAACGGCCCAGATGGCTCTTCAATTGTTCATCAAAGACTACAGATA
CACATATAGGTGCCATAGGGGAGACACACAAATTCAGACAAGAAGATCATTTGAGTTAAAGAAGCTGTGGGATCAAA
CCCAATCAAAGGTAGGGCTATTAGTATCAGATGGAGGACCAAACTTATACAATATACGGAATCTTCACATTCCTGAA
GTCTGCTTAAAATGGGAGCTAATGGATGATGATTATCGGGGAAGACTTTGTAATCCCCTGAATCCCTTTGTCAGTCA
TAAAGAGATTGATTCTGTAAACAATGCTGTGGTAATGCCAGCCCATGGTCCAGCCAAAAGCATGGAATATGATGCCG
TTGCAACTACACATTCCTGGATTCCCAAGAGGAATCGTTCTATTCTCAACACAAGCCAAAGGGGAATTCTTGAGGAT
GAACAGATGTACCAGAAGTGCTGCAATCTATTCGAGAAATTTTTCCCTAGCAGTTCATATAGGAGACCGGTTGGAAT
TTCTAGCATGGTGGAGGCCATGGTGTCTAGGGCCCGGATTGATGCCAGGGTCGACTTCGAGTCTGGACGGATCAAGA

AAGAAGAGTTCTCTGAGATCATGAAGATCTGTTCCACCATTGAAGAACTCAGACGGCAAAAATAATGAATTTAACTT
GTCCTTCATGAAAAAATGCCTTGTTTCTACT

SEQ ID NO: 25 (PB2, A/California/07/09)

ATGGAGAGAATAAAAGAACTGAGAGATCTAATGTCGCAGTCCCGCACTCGCGAGATACTCACTAAGACCACTGTGGA
CCATATGGCCATAATCAAAAAGTACACATCAGGAAGGCAAGAGAAGAACCCCGCACTCAGAATGAAGTGGATGATGG
CAATGAGATACCCAATTACAGCAGACAAGAGAATAATGGACATGATTCCAGAGAGGAATGAACAAGGACAAACCCTC
TGGAGCAAAACAAACGATGCTGGATCAGACCGAGTGATGGTATCACCTCTGGCCGTAACATGGTGGAATAGGAATGG
CCCAACAACAAGTACAGTTCATTACCCTAAGGTATATAAAACTTATTTCGAAAAGGTCGAAAGGTTGAAACATGGTA
CCTTCGGCCCTGTCCACTTCAGAAATCAAGTTAAAATAAGGAGGAGAGTTGATACAAACCCTGGCCATGCAGATCTC
AGTGCCAAGGAGGCACAGGATGTGATTATGGAAGTTGTTTTCCCAAATGAAGTGGGGGCAAGAATACTGACATCAGA
GTCACAGCTGGCAATAACAAAAGAGAAGAAAGAAGAGCTCCAGGATTGTAAAATTGCTCCCTTGATGGTGGCGTACA
TGCTAGAAAGAGAATTGGTCCGTAAAACAAGGTTTCTCCCAGTAGCCGGCGGAACAGGCAGTGTTTATATTGAAGTG
TTGCACTTAACCCAAGGGACGTGCTGGGAGCAGATGTACACTCCAGGAGGAGAAGTGAGAAATGATGATGTTGACCA
AAGTTTGATTATCGCTGCTAGAAACATAGTAAGAAGAGCAGCAGTGTCAGCAGACCCATTAGCATCTCTCTTGGAAA
TGTGCCACAGCACACAGATTGGAGGAGTAAGGATGGTGGACATCCTTAGACAGAATCCAACTGAGGAACAAGCCGTA
GACATATGCAAGGCAGCAATAGGGTTGAGGATTAGCTCATCTTTCAGTTTTGGTGGGTTCACTTTCAAAAGGACAAG
CGGATCATCAGTCAAGAAAGAAGAAGAAGTGCTAACGGGCAACCTCCAAACACTGAAAATAAGAGTACATGAAGGGT
ATGAAGAATTCACAATGGTTGGGAGAAGAGCAACAGCTATTCTCAGAAAGGCAACCAGGAGATTGATCCAGTTGATA
GTAAGCGGGAGAGACGAGCAGTCAATTGCTGAGGCAATAATTGTGGCCATGGTATTCTCACAGGAGGATTGCATGAT
CAAGGCAGTTAGGGGCGATCTGAACTTTGTCAATAGGGCAAACCAGCGACTGAACCCCATGCACCAACTCTTGAGGC
ATTTCCAAAAAGATGCAAAAGTGCTTTTCCAGAACTGGGGAATTGAATCCATCGACAATGTGATGGGAATGATCGGA
ATACTGCCCGACATGACCCCAAGCACGGAGATGTCGCTGAGAGGGATAAGAGTCAGCAAAATGGGAGTAGATGAATA
CTCCAGCACGGAGAGAGTGGTAGTGAGTATTGACCGATTTTTAAGGGTTAGAGATCAAAGAGGGAACGTACTATTGT
CTCCCGAAGAAGTCAGTGAAACGCAAGGAACTGAGAAGTTGACAATAACTTATTCGTCATCAATGATGTGGGAGATC
AATGGCCCTGAGTCAGTGCTAGTCAACACTTATCAATGGATAATCAGGAACTGGGAAATTGTGAAAATTCAATGGTC
ACAAGATCCCACAATGTTATACAACAAAATGGAATTTGAACCATTTCAGTCTCTTGTCCCTAAGGCAACCAGAAGCC
GGTACAGTGGATTCGTAAGGACACTGTTCCAGCAAATGCGGGATGTGCTTGGGACATTTGACACTGTCCAAATAATA
AAACTTCTCCCCTTTGCTGCTGCCCCACCAGAACAGAGTAGGATGCAATTTTCCTCATTGACTGTGAATGTGAGAGG
ATCAGGGTTGAGGATACTGGTAAGAGGCAATTCTCCAGTATTCAATTACAACAAGGCAACCAAACGACTTACAGTTC
TTGGAAAGGATGCAGGTGCATTGACTGAAGATCCAGATGAAGGCACATCTGGGGTGGAGTCTGCTGTCCTGAGAGGA
TTTCTCATTTTGGGCAAAGAAGACAAGAGATATGGCCCAGCATTAAGCATCAATGAACTGAGCAATCTTGCAAAAGG
AGAGAAGGCTAATGTGCTAATTGGGCAAGGGGACGTAGTGTTGGTAATGAAACGAAAACGGGACTCTAGCATACTTA
CTGACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAG

SEQ ID NO: 26 (NP, A/California/07/09)

ATGGCGTCTCAAGGCACCAAACGATCATATGAACAAATGGAGACTGGTGGGGAGCGCCAGGATGCCACAGAAATCAG
AGCATCTGTCGGAAGAATGATTGGTGGAATCGGGAGATTCTACATCCAAATGTGCACTGAACTCAAACTCAGTGATT
ATGATGGACGACTAATCCAGAATAGCATAACAATAGAGAGGATGGTGCTTTCTGCTTTTGATGAGAGAAGAAATAAA
TACCTAGAAGAGCATCCCAGTGCTGGGAAGGACCCTAAGAAAACAGGAGGACCCATATATAGAAGAGTAGACGGAAA
GTGGATGAGAGAACTCATCCTTTATGACAAAGAAGAAATAAGGAGAGTTTGGCGCCAAGCAAACAATGGCGAAGATG
CAACAGCAGGTCTTACTCATATCATGATTTGGCATTCCAACCTGAATGATGCCACATATCAGAGAACAAGAGCGCTT
GTTCGCACCGGAATGGATCCCAGAATGTGCTCTCTAATGCAAGGTTCAACACTTCCCAGAAGGTCTGGTGCCGCAGG
TGCTGCGGTGAAAGGAGTTGGAACAATAGCAATGGAGTTAATCAGAATGATCAAACGTGGAATCAATGACCGAAATT
TCTGGAGGGGTGAAAATGGACGAAGGACAAGGGTTGCTTATGAAAGAATGTGCAATATCCTCAAAGGAAAATTTCAA
ACAGCTGCCCAGAGGGCAATGATGGATCAAGTAAGAGAAAGTCGAAACCCAGGAAACGCTGAGATTGAAGACCTCAT
TTTCCTGGCACGGTCAGCACTCATTCTGAGGGGATCAGTTGCACATAAATCCTGCCTGCCTGCTTGTGTGTATGGGC
TTGCAGTAGCAAGTGGGCATGACTTTGAAAGGGAAGGGTACTCACTGGTCGGGATAGACCCATTCAAATTACTCCAA
AACAGCCAAGTGGTCAGCCTGATGAGACCAAATG

SEQ ID NO: 27 (M, A/California/07/09)

ATGAGTCTTCTAACCGAGGTCGAAACGTACGTTCTTTCTATCATCCCGTCAGGCCCCCTCAAAGCCGAGATCGCGCA
GAGACTGGAAAGTGTCTTTGCAGGAAAGAACACAGATCTTGAGGCTCTCATGGAATGGCTAAAGACAAGACCAATCT
TGTCACCTCTGACTAAGGGAATTTTAGGATTTGTGTTCACGCTCACCGTGCCCAGTGAGCGAGGACTGCAGCGTAGA
CGCTTTGTCCAAAATGCCCTAAATGGGAATGGGGACCCGAACAACATGGATAGAGCAGTTAAACTATACAAGAAGCT
CAAAAGAGAAATAACGTTCCATGGGGCCAAGGAGGTGTCACTAAGCTATTCAACTGGTGCACTTGCCAGTTGCATGG
GCCTCATATACAACAGGATGGGAACAGTGACCACAGAAGCTGCTTTTGGTCTAGTGTGTGCCACTTGTGAACAGATT
GCTGATTCACAGCATCGGTCTCACAGACAGATGGCTACTACCACCAATCCACTAATCAGGCATGAAAACAGAATGGT
GCTGGCTAGCACTACGGCAAAGGCTATGGAACAGATGGCTGGATCGAGTGAACAGGCAGCGGAGGCCATGGAGGTTG
CTAATCAGACTAGGCAGATGGTACATGCAATGAGAACTATTGGGACTCATCCTAGCTCCAGTGCTGGTCTGAAAGAT

GACCTTCTTGAAAATTTGCAGGCCTACCAGAAGCGAATGGGAGTGCAGATGCAGCGATTCAAGTGATCCTCTCGTCA
TTGCAGCAAATATCATTGGGATCTTGCACCTGATATTGTGGATTACTGATCGTCTTTTTTTCAAATGTATTTATCGT
CGCTTTAAATACGGTTTGAAAAGAGGGCCTTCTACGGAAGGAGTGCCTGAGTCCATGAGGGAAGAATATCAACAGGA
ACAGCAGAGTGCTGTGGATGTTGACGATGGTCATTTTGTCAACATAGAGCTAGAGTAA

SEQ ID NO: 28 (NS, A/California/07/09)

ATGGACTCCAACACCATGTCAAGCTTTCAGGTAGACTGTTTCCTTTGGCATATCCGCAAGCGATTTGCAGACAATGG
ATTGGGTGATGCCCCATTCCTTGATCGGCTCCGCCGAGATCAAAAGTCCTTAAAAGGAAGAGGCAACACCCTTGGCC
TCGATATCGAAACAGCCACTCTTGTTGGGAAACAAATCGTGGAATGGATCTTGAAAGAGGAATCCAGCGAGACACTT
AGAATGACAATTGCATCTGTACCTACTTCGCGCTACCTTTCTGACATGACCCTCGAGGAAATGTCACGAGACTGGTT
CATGCTCATGCCTAGGCAAAAGATAATAGGCCCTCTTTGCGTGCGATTGGACCAGGCGATCATGGAAAAGAACATAG
TACTGAAAGCGAACTTCAGTGTAATCTTTAACCGATTAGAGACCTTGATACTACTAAGGGCTTTCACTGAGGAGGGA
GCAATAGTTGGAGAAATTTCACCATTACCTTCTCTTCCAGGACATACTTATGAGGATGTCAAAAATGCAGTTGGGGT
CCTCATCGGAGGACTTGAATGGAATGGTAACACGGTTCGAGTCTCTGAAAATATACAGAGATTCGCTTGGAGAAACT
GTGATGAGAATGGGAGACCTTCACTACCTCCAGAGCAGAAATGAAAAGTGGCGAGAGCAATTGGGACAGAAATTTGA
GGAAATAAGGTGGTTAATTGAAGAAATGCGGCACAGATTGAAAGCGACAGAGAATAGTTTCGAACAAATAACATTTA
TGCAAGCCTTACAACTACTGCTTGAAGTAGAACAAGAGATAAGAGCTTTCTCGTTTCAGCTTATTTAATGATAAAAA
ACACCCTTGTTTCTACTG

SEQ ID NO: 29 (A/Texas/1/77 PB1)

MDVNPTLLFLKIPAQNAISTTFPYTGDPPYSHGTGTGYTMDTVNRTHQYSEKGKWTTNTETGAPQLNPIDGPLPEDN
EPSGYAQTDCVLEAMAFLEESHPGIFENSCLETMEVVQQTRVDRLTQGRQTYDWTLNRNQPAATALANTIEVFRSNG
LTANESGRLIDFLKDVMESMDKEEIEITTHFQRKRRVRDNMTKKMVTQRTIGKKKQRVNKRSYLIRALTLNTMTKDA
ERGKLKRRAIATPGMQIRGFVYFVETLARSICEKLEQSGLPVGGNEKKAKLANVVRKMMTNSQDTELSFTITGDNTK
WNENQNPRMFLAMITYITKNQPEWFRNILSIAPIMFSNKMARLGKGYMFESKRMKLRTQIPAEMLASIDLKYFNEST
RKKIEKIRPLLIDGTASLSPGMMMGMFNMLSTVLGVSILNLGQKKYTKTTYWWDGLQSSDDFALIVNAPNHEGIQAG
VDRFYRTCKLVGINMSKKKSYINRTGTFEFTSFFYRYGFVANFSMELPSFGVSGINESADMSIGVTVIKNNMINNDL
GPATAQMALQLFIKDYRYTYRCHRGDTQIQTRRSFELKKLWEQTRSKAGLLVSDGGPNLYNIRNLHIPEVCLKWELM
DEDYQGRLCNPLNPFVSHKEIESVNNAVVMPAHGPAKSMEYDAVATTHSWIPKRNRSILNTSQRGILEDEQMYQKCC
NLFEKFFPSSSYRRPVGISSMVEAMVSRARIDARIDFESGRIKKEEFSEIMKICSTIEELRRQKQ

SEQ ID NO: 30 (A/Puerto Rico/8/34 PA)

MEDFVRQCFNPMIVELAEKTMKEYGEDLKIETNKFAAICTHLEVCFMYSDFHFINEQGESIIVELGDPNALLKHRFE
IIEGRDRTMAWTVVNSICNTTGAEKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKANKIKSEKTHIHIFSFTGEE
MATKADYTLDEESRARIKTRLFTIRQEMASRGLWDSFRQSERGEETIEERFEITGTMRKLADQSLPPNFSSLENFRA
YVDGFEPNGYIEGKLSQMSKEVNARIEPFLKTTPRPLRLPNGPPCSQRSKFLLMDALKLSIEDPSHEGEGIPLYDAI
KCMRTFFGWKEPNVVKPHEKGINPNYLLSWKQVLAELQDIENEEKIPKTKNMKKTSQLKWALGENMAPEKVDFDDCK
DVGDLKQYDSDEPELRSLASWIQNEFNKACELTDSSWIELDEIGEDVAPIEHIASMRRNYFTSEVSHCRATEYIMKG
VYINTALLNASCAAMDDFQLIPMISKCRTKEGRRKTNLYGFIIKGRSHLRNDTDVVNFVSMEFSLTDPRLEPHKWEK
YCVLEIGDMLIRSAIGQVSRPMFLYVRTNGTSKIKMKWGMEMRRCLLQSLQQIESMIEAESSVKEKDMTKEFFENKS
ETWPIGESPKGVEESSIGKVCRTLLAKSVFNSLYASPQLEGFSAESRKLLLIVQALRDNLEPGTFDLGGLYEAIEEC
LINDPWVLLNASWFNSFLTHALS

SEQ ID NO: 31 (A/Puerto Rico/8/34 NP)

MASQGTKRSYEQMETDGERQNATEIRASVGKMIGGIGRFYIQMCTELKLSDYEGRLIQNSLTIERMVLSAFDERRNK
YLEEHPSAGKDPKKTGGPIYRRVNGKWMRELILYDKEEIRRIWRQANNGDDATAGLTHMMIWHSNLNDATYQRTRAL
VRTGMDPRMCSLMQGSTLPRRSGAAGAAVKGVGTMVMELVRMIKRGINDRNFWRGENGRKTRIAYERMCNILKGKFQ
TAAQKAMMDQVRESRDPGNAEFEDLTFLARSALILRGSVAHKSCLPACVYGPAVASGYDFEREGYSLVGIDPFRLLQ
NSQVYSLIRPNENPAHKS

SEQ ID NO: 32 (A/Puerto Rico/8/34 M)

MSLLTEVETYVLSIIPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRPILSPLTKGILGFVFTLTVPSERGLQRR
RFVQNALNGNGDPNNMDKAVKLYRKLKREITFHGAKEISLSYSAGALASCMGLIYNRMGAVTTEVAFGLVCATCEQI
ADSQHRSHRQMVTTTNPLIRHENRMVLASTTAKAMEQMAGSSEQAAEAMEVASQARQMVQAMRTIGTHPSSSAGLKN
DLLENLQAYQKRMGVQMQRFK

SEQ ID NO: 33 (PB2, A/New Caledonia/20/1999)

AATATGGAAAGAATAAAAGAGCTAAGGAATCTGATGTCACAATCTCGCACTCGCGAGATACTTACAAAAACTACTGT
AGACCACATGGCCATAATCAAGAAATACACATCAGGAAGACAGGAGAAAAACCCATCACTTAGAATGAAATGGATGA

TGGCAATGAAATACCCAATTACAGCAGATAAAAGGATAACGGAAATGATTCCTGAAAGAAATGAGCAAGGACAGACA
TTATGGAGTAAAGTGAATGATGCCGGATCAGACCGAGTGATGATATCACCCCTGGCTGTGACATGGTGGAACAGAAA
TGGACCAGTGGCAAGTACTATTCACTATCCAAAAATCTACAAAACTTACTTTGAAAAGGTTGAAAGGTTAAAACATG
GAACCTTTGGCCCTGTACACTTTAGAAACCAAGTCAAAATACGCCGAAGAGTCGACATAAATCCTGGTCATGCAGAC
CTCAGCGCCAAGGAGGCACAGGATGTAATTATGGAAGTTGTTTTCCCTAATGAAGTGGGAGCCAGAATACTAACATC
AGAATCGCAATTAACGATAACCAAGGAGAAAAAAGAAGAACTCCAGAATTGCAAAATTTCCCCTTTGATGGTTGCAT
ACATGTTAGAGAGGGAACTTGTCCGCAAAACGAGATTTCTCCCGGTTGCTGGTGGAACAAGCAGTGTGTACATTGAA
GTTTTGCATTTAACACAGGGGACATGCTGGGAGCAGATGTACACTCCAGGTGGGGAGGTGAGGAATGATGATGTTGA
TCAAAGCCTAATTATTGCTGCTAGGAACATAGTGAGAAGAGCTGCAGTATCAGCAGATCCACTAGCATCTTTATTAG
AAATGTGCCATAGCACACAGATTGGTGGGACAAGGATGGTGGATATTCTCAGGCAAAATCCAACAGAAGAACAAGCT
GTGGATATATGCAAAGCAGCAATGGGGCTGAGAATCAGTTCATCCTTCAGTTTTGGCGGATTCACATTTAAGAGAAC
AAGTGGATCATCAGTCAAAAGGGAGGAAGAAGTGCTCACGGGCAATCTGCAAACATTGAAGCTAACTGTGCATGAGG
GATATGAAGAGTTCACAATGGTTGGGAAAAGGGCAACAGCTATACTCAGAAAAGCAACCAGGAGATTGATTCAACTA
ATAGTGAGTGGAAGAGACGAACAGTCAATAGTCGAAGCAATAGTTGTAGCAATGGTATTCTCACAAGAAGATTGCAT
GGTAAAAGCAGTTAGAGGTGATCTGAATTTCGTTAATAGAGCGAATCAGCGGTTGAATCCCATGCATCAACTTTTGA
GACATTTTCAGAAGGATGCTAAAGTACTTTTCTTAAATTGGGGAATTGAACCTATCGACAATGTGATGGGAATGATT
GGGATATTACCTGATATGACTCCAAGTACCGAGATGTCAATGAGAGGAGTGAGAGTCAGCAAAATGGGTGTAGATGA
ATACTCCAATGCTGAAAGGGTAGTGGTGAGCATTGACCGTTTTTTGAGAGTCCGGGACCAAAGAGGAAATGTACTAC
TGTCTCCAGAGGAAGTCAGTGAAACACAGGGAACAGAGAAACTGACAATAACTTACTCTTCATCAATGATGTGGGAG
ATTAATGGCCCTGAGTCAGTGTTGATCAATACCTATCAGTGGATCATCAGAAACTGGGAGACTGTTAAAATTCAGTG
GTCTCAGAACCCTACAATGCTATACAATAAAATGGAATTCGAGCCATTTCAGTCTCTAGTCCCTAAGGCCATTAGAG
GCCAATACAGTGGGTTTGTTAGAACTCTATTTCAACAAATGAGGGATGTGCTTGGGACCTTTGACACAACTCAGATA
ATAAAACTTCTTCCCTTTGCAGCCGCTCCACCAAAGCAAAGTAGAATGCAATTCTCATCATTGACTGTGAATGTGAG
GGGATCAGGAATGAGAATACTTGTAAGGGGTAATTCTCCAGTATTCAACTACAACAAGACCACTAAGAGACTCACAG
TCCTCGGAAAGGATGCTGGCACTTTAACTGAAGACCCAGATGAAGGCACAGCTGGAGTGGAATCTGCTGTTCTAAGG
GGATTCCTCATTCTAGGCAAAGAAGATAGAAGATATGGGCCAGCATTAAGCATCAATGAATTGAGCAACCTTGCGAA
AGGGGAAAAAGCTAATGTGCTAATTGGGCAAGGGGACGTAGTGTTGGTAATGAAACGAAAACGGGACTCTAGCATAC
TTACTGACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAATTTCGAATAATTTAAA

SEQ ID NO: 34 (encodes the same amino acid sequence as SEQ ID NO: 33)

ATGGAACGCATTAAAGAACTGCGCAACCTGATGAGCCAGAGCCGCACCCGCGAAATTCTGACCAAAACCACCGTGGA
TCATATGGCGATTATTAAAAAATATACCAGCGGCCGCCAGGAAAAAAACCCGAGCCTGCGCATGAAATGGATGATGG
CGATGAAATATCCGATTACCGCGGATAAACGCATTACCGAAATGATTCCGGAACGCAACGAACAGGGCCAGACCCTG
TGGAGCAAAGTGAACGATGCGGGCAGCGATCGCGTGATGATTAGCCCGCTGGCGGTGACCTGGTGGAACCGCAACGG
CCCGGTGGCGAGCACCATTCATTATCCGAAAATTTATAAAACCTATTTTGAAAAAGTGGAACGCCTGAAACATGGCA
CCTTTGGCCCGGTGCATTTTCGCAACCAGGTGAAAATTCGCCGCCGCGTGGATATTAACCCGGGCCATGCGGATCTG
AGCGCGAAAGAAGCGCAGGATGTGATTATGGAAGTGGTGTTTCCGAACGAAGTGGGCGCGCGCATTCTGACCAGCGA
AAGCCAGCTGACCATTACCAAAGAAAAAAAAGAAGAACTGCAGAACTGCAAAATTAGCCCGCTGATGGTGGCGTATA
TGCTGGAACGCGAACTGGTGCGCAAAACCCGCTTTCTGCCGGTGGCGGGCGGCACCAGCAGCGTGTATATTGAAGTG
CTGCATCTGACCCAGGGCACCTGCTGGGAACAGATGTATACCCCGGGCGGCGAAGTGCGCAACGATGATGTGGATCA
GAGCCTGATTATTGCGGCGCGCAACATTGTGCGCCGCGCGGCGGTGAGCGCGGATCCGCTGGCGAGCCTGCTGGAAA
TGTGCCATAGCACCCAGATTGGCGGCACCCGCATGGTGGATATTCTGCGCCAGAACCCGACCGAAGAACAGGCGGTG
GATATTTGCAAAGCGGCGATGGGCCTGCGCATTAGCAGCAGCTTTAGCTTTGGCGGCTTTACCTTTAAACGCACCAG
CGGCAGCAGCGTGAAACGCGAAGAAGAAGTGCTGACCGGCAACCTGCAGACCCTGAAACTGACCGTGCATGAAGGCT
ATGAAGAATTTACCATGGTGGGCAAACGCGCGACCGCGATTCTGCGCAAAGCGACCCGCCGCCTGATTCAGCTGATT
GTGAGCGGCCGCGATGAACAGAGCATTGTGGAAGCGATTGTGGTGGCGATGGTGTTTAGCCAGGAAGATTGCATGGT
GAAAGCGGTGCGCGGCGATCTGAACTTTGTGAACCGCGCGAACCAGCGCCTGAACCCGATGCATCAGCTGCTGCGCC
ATTTTCAGAAAGATGCGAAAGTGCTGTTTCTGAACTGGGGCATTGAACCGATTGATAACGTGATGGGCATGATTGGC
ATTCTGCCGGATATGACCCCGAGCACCGAAATGAGCATGCGCGGCGTGCGCGTGAGCAAAATGGGCGTGGATGAATA
TAGCAACGCGGAACGCGTGGTGGTGAGCATTGATCGCTTTCTGCGCGTGCGCGATCAGCGCGGCAACGTGCTGCTGA
GCCCGGAAGAAGTGAGCGAAACCCAGGGCACCGAAAAACTGACCATTACCTATAGCAGCAGCATGATGTGGGAAATT
AACGGCCCGGAAAGCGTGCTGATTAACACCTATCAGTGGATTATTCGCAACTGGGAAACCGTGAAAATTCAGTGGAG
CCAGAACCCGACCATGCTGTATAACAAAATGGAATTTGAACCGTTTCAGAGCCTGGTGCCGAAAGCGATTCGCGGCC
AGTATAGCGGCTTTGTGCGCACCCTGTTTCAGCAGATGCGCGATGTGCTGGGCACCTTTGATACCACCCAGATTATT
AAACTGCTGCCGTTTGCGGCGGCGCCGCCGAAACAGAGCCGCATGCAGTTTAGCAGCCTGACCGTGAACGTGCGCGG
CAGCGGCATGCGCATTCTGGTGCGCGGCAACAGCCCGGTGTTTAACTATAACAAAACCACCAAACGCCTGACCGTGC
TGGGCAAAGATGCGGGCACCCTGACCGAAGATCCGGATGAAGGCACCGCGGGCGTGGAAAGCGCGGTGCTGCGCGGC
TTTCTGATTCTGGGCAAAGAAGATCGCCGCTATGGCCCGGCGCTGAGCATTAACGAACTGAGCAACCTGGCGAAAGG
CGAAAAAGCGAACGTGCTGATTGGCCAGGGCGATGTGGTGCTGGTGATGAAACGCAAACGCGATAGCAGCATTCTGA
CCGATAGCCAGACCGCGACCAAACGCATTCGCATGGCGATTAAC

SEQ ID NO: 35 (PA, A/New Caledonia/20/1999)

GATTCGAAATGGAAGATTTTGTGCGACAATGCTTCAATCCGATGATTGTCGAGCTTGCGGAAAAGGCAATGAAAGAG
TATGGAGAGGACCTGAAAATCGAAACAAACAAATTTGCAGCAATATGCACTCACTTGGAAGTATGCTTCATGTATTC
AGATTTTCATTTCATCAATGAGCAAGGCGAATCAATAATAGTAGAGCCTGAGGACCCAAATGCACTTTTAAAGCACA
GATTTGAGATAATAGAGGGACGAGATCGTACAATGGCATGGACAGTTGTAAACAGTATTTGCAACACCACAGGAGCT
GAGAAACCAAAGTTTCTGCCAGATCTGTATGATTACAAAGAGAATAGATTCATCGAGATTGGAGTGACAAGGAGGGA
AGTTCACATATACTATCTGGAAAAGGCCAACAAAATTAAATCTGAGAAGACACACATTCACATTTTCTCATTCACTG
GCGAAGAAATGGCCACAAAGGCCGATTACACTCTCGATGAAGAAAGCAGGGCTAGGATTAAAACCAGACTATTCACC
ATAAGACAAGAAATGGCAAGCAGAGGTCTTTGGGACTCCTTTCGTCAGTCCGAAAGAGGCGAAGAAACAATTGAAGA
AAGATTTGAAATCACAGGGACAATGCGCAGGCTCGCTGACCAAAGCCTTCCGCCGAACTTCTCCTGCATTGAGAATT
TTAGAGCCTATGTGGATGGATTTGAACCGAACGGCTACATTGAGGGCAAGCTTTCTCAAATGTCCAAAGAAGTAAAT
GCTAGAATTGAGCCTTTTTTGAAAACAACACCACGACCAATTAGACTTCCGGATGGGCCTCCTTGTTTTCAGCGGTC
AAAATTCCTGCTGATGGATTCTTTAAAATTAAGCATTGAGGATCCAAATCATGAAGGAGAGGGAATACCACTATATG
ATGCAATCAAGTGTATGAGAACATTCTTTGGATGGAAAGAACCCTCTGTTGTCAAGCCACACGGGAAGGGAATAAAT
CCGAATTATCTGCTGTCATGGAAGCAGGTATTGGAAGAGCTGCAGGACATTGAGAGTGAGGAGAAGATTCCAAGAAC
AAAAAACATGAAAAAAACGAGTCAGCTAAAGTGGGCACTTGGTGAGAACATGGCACCAGAGAAGGTGGATTTTGATG
ACTGTAAAGATATAAGCGATTTGAAGCAATATGATAGTGACGAACCTGAATTAAGGTCATTTTCAAGTTGGATCCAG
AATGAGTTCAACAAGGCATGCGAGCTGACCGATTCAATCTGGATAGAGCTCGATGAGATTGGAGAAGATGTGGCCCC
GATTGAACACATTGCAAGCATGAGAAGAAATTACTTCACAGCTGAGGTGTCCCATTGCAGAGCCACAGAATATATAA
TGAAGGGGGTATACATTAATACTGCTTTGCTTAATGCATCCTGTGCAGCAATGGATGATTTCCAACTAATTCCCATG
ATAAGCAAATGTAGAACTAAAGAGGGAAGGAGAAAGACCAATTTGTACGGCTTCATCGTAAAAGGAAGATCTCACTT
AAGGAATGACACCGATGTGGTAAACTTTGTGAGCATGGAGTTTTCCCTCACTGACCCAAGACTTGAGCCACACAAAT
GGGAGAAGTACTGTGTTCTTGAGATAGGAGATATGCTTCTAAGGAGTGCAATAGGCCAAGTGTCAAGGCCCATGTTC
TTGTATGTAAGGACAAATGGAACCTCAAAAATTAAAATGAAATGGGGAATGGAGATGAGGCGTTGCCTCCTCCAATC
CCTTCAACAAATAGAGAGCATGATTGAAGCTGAGTCCTCCGTCAAGGAGAAAGACATGACAAAAGAGTTTTTTGAGA
ATAGATCAGAAACATGGCCCATTGGAGAGTCACCAAAAGGAGTGGAAGAAGGTTCCATTGGGAAAGTATGCAGGACA
CTATTGGCTAAGTCAGTATTCAATAGTCTGTATGCATCTCCACAATTAGAAGGATTTTCAGCTGAGTCAAGAAAGTT
GCTCCTCATTGTTCAGGCTCTTAGGGACAATCTGGAACCTGGGACCTTTGATCTTGGGGGGCTATATGAAGCAATTG
AGGAGTGCCTGATTAATGATCCCTGGGTTTTGCTTAATGCTTCTTGGTTCAACTCCTTCCTAACACATGCATTGAGA
TAGCTGGGGCAATGCTACTATTTACTATCCATACTGTCCAAAAAA

SEQ ID NO: 36 (PB1, A/New Caledonia/20/1999)

AATGGATGTCAATCCGACATTACTTTTCTTAAAAGTGCCAGCACAAAATGCTATAAGCACAACTTTTCCTTATACTG
GTGACCCTCCTTACAGCCATGGGACAGGAACAGGGTACACCATGGATACAGTCAACAGGACACATCAGTACTCAGAA
AGAGGAAGATGGACAAAAAATACCGAAACTGGAGCACCGCAACTCAACCCAATTGATGGGCCACTACCAAAAGACAA
TGAACCAAGTGGCTATGCCCAAACAGATTGTGTATTAGAAGCAATGGCTTTCCTTGAGGAATCCCATCCTGGTATTT
TTGAAAACTCTTGTATTGAAACAATGGAGGTTGTTCAGCAAACAAGGGTGGACAAACTGACACAAGGCAGACAGACC
TATGACTGGACTCTAAATAGGAACCAGCCTGCTGCCACAGCATTGGCCAACACTATAGAAGTGTTCAGATCAAACGG
CCTCATAGCAAATGAATCTGGGAGGCTAATAGACTTCCTTAAAGATGTAATGGAGTCGATGGACAGAGACGAAGTAG
AGATCACAACTCATTTTCAAAGAAAGAGGAGAGTGAGAGACAATGTAACTAAAAAAATGGTGACCCAAAGAACAATA
GGCAAAAAGAAACATAAATTAGACAAAAGAAGTTACCTAATTAGGGCATTAACCCTGAACACAATGACCAAAGATGC
TGAGAGGGGGAAACTAAAACGCAGAGCAATTGCAACCCCAGGAATGCAAATAAGGGGGTTTGTATACTTTGTTGAGA
CACTGGCAAGAAGCATATGTGAAAAGCTTGAACAATCAGGGTTGCCAGTTGGAGGAAATGAAAAGAAAGCAAAGTTA
GCAAATGTTGTAAGGAAGATGATGACCAACTCCCAGGACACTGAAATTTCTTTCACCATCACTGGAGATAACACAAA
ATGGAACGAAAATCAAAACCCTAGAATGTTCTTGGCCATGATCACATATATAACCAAAAATCAGCCTGAATGGTTCA
GAAATATTCTAAGTATTGCTCCAATAATGTTTTCAAACAAAATGGCGAGACTAGGTAAGGGGTACATGTTTGAAAGC
AAGAGTATGAAACTGAGAACTCAAATACCTGCAGAGATGCTAGCCAACATAGATTTGAAATATTTCAATGATTCAAC
TAAAAAGAAAATTGAAAAAATCCGGCCATTATTAATAGATGGAACTGCATCATTGAGTCCTGGAATGATGATGGGCA
TGTTCAATATGTTAAGCACCGTCTTGGGCGTCTCCATTCTGAATCTTGGGCAAAAGAGATACACCAAGACTACTTAC
TGGTGGGATGGTCTTCAATCGTCTGATGATTTTGCTCTGATTGTGAATGCACCCAACTATGCAGGAATTCAAGCTGG
AGTTGACAGGTTTTATCGAACCTGTAAGCTGCTCGGAATTAATATGAGCAAAAAGAAGTCTTACATAAACAGAACAG
GTACCTTTGAGTTCACGAGCTTTTTCTATCGTTATGGGTTTGTTGCCAATTTCAGCATGGAGCTTCCTAGTTTTGGG
GTGTCTGGGGTCAATGAATCTGCAGACATGAGTATTGGAGTCACTGTCATCAAAAACAATATGATAAACAATGACCT
TGGCCCAGCAACTGCTCAAATGGCCCTTCAGTTATTTATAAAAGATTACAGGTACACGTATCGATGCCACAGAGGTG
ACACACAAATACAAACCCGGAGATCATTTGAGATAAAGAAACTATGGGACCAAACCCGCTCCAAAGCTGGGCTGTTG
GTCTCTGATGGAGGCCCCAATTTATATAACATTAGAAATCTCCATATTCCTGAAGTCTGCTTGAAATGGGAGTTGAT
GGATGAGGATTACCAGGGGCGTTTATGCAACCCATTGAACCCGTTTGTCAGTCATAAAGAGATTGAATCAGTGAACA
ATGCAGTGATGATGCCGGCACATGGTCCAGCCAAAAATATGGAGTATGACGCTGTTGCAACAACACACTCCTGGGTT
CCCAAAAGGAATCGATCCATTTTGAATACGAGCCAAAGGGGGATACTTGAGGATGAGCAAATGTATCAGAGGTGCTG
CAATTTATTTGAAAAATTCTTCCCAAGTAGCTCATACAGAAGACCAGTTGGAATATCCAGTATGGTAGAGGCTATGG

TTTCCAGAGCCCGAATTGATGCACGGATTGATTTCGAATCTGGAAGGATAAAAAAAGAGGAATTCGCTGAGATCATG
AAGACCTGTTCCACCATTGAAGACCTCAGACGGCAAAAATAGGGAATTTGGCTTGTCCTTCATGAAAA

SEQ ID NO: 37 (NP, A/New Caledonia/20/1999)

ATCACTCACTGAGTGACATCAAAGTCATGGCGTCCCAAGGCACCAAACGGTCTTACGAACAGATGGAGACTGATGGG
GAACGCCAGAATGCAACTGAAATCAGAGCATCCGTCGGAAGAATGATTGGTGGAATTGGGCGATTCTACATCCAAAT
GTGCACCGAGCTTAAACTCAATGATTATGAGGGACGACTGATCCAGAACAGCTTGACAATAGAGAGAATGGTGCTCT
CTGCTTTTGATGAGAGGAGGAATAAATATCTGGAAGAACATCCCAGCGCGGGGAAAGATCCTAAGAAAACTGGAGGA
CCCATATACAAGAGAGTAGATGGAAAGTGGGTGAGGGAACTCGTCCTTTATGACAAAGAAGAAATAAGGCGGATTTG
GCGCCAAGCCAACAATGGTGATGATGCAACGGCTGGTTTGACTCACATTATGATCTGGCATTCTAATTTGAATGATA
CAACTTACCAGAGGACAAGAGCTCTTGTCCGCACCGGAATGGATCCCAGGATGTGCTCTTTGATGCAAGGTTCAACT
CTCCCTAGAAGATCTGGAGCAGCAGGCGCTGCAGTCAAAGGAGTTGGGACAATGGTGTTGGAGTTAATCAGGATGAT
CAAACGTGGGATCAATGACCGAAACTTCTGGAGGGGTGAGAATGGAAGAAAAACAAGGATTGCTTATGAGAGAATGT
GCAACATTCTCAAAGGAAAATTTCAAACAGCTGCACAAAAAGCAATGATGGATCAAGTGAGAGAAAGCCGGAACCCA
GGAAATGCTGAGATCGAAGATCTCACTTTTCTGGCACGGTCTGCACTCATATTAAGAGGGTCAGTTGCTCACAAGTC
TTGCCTGCCTGCCTGTGTGTATGGACCAGCCGTAGCCAGTGGGTACGACTTCGAAAAAGAGGGATACTCTTTGGTAG
GGGTAGACCCTTTTAAACTGCTTCAAACCAGTCAGGTATACAGCCTAATCAGACCAAACGAGAATCCCGCACACAAG
AGTCAGTTGGTGTGGATGGCATGCAATTCTGCTGCATTTGAAGATCTAAGAGTGTCAAGCTTCATCAGAGGGACAAG
AGTACTTCCAAGGGGGAAGCTCTCCACTAGAGGAGTACAAATTGCTTCAAATGAAAACATGGATGCTATTGTATCAA
GTACTCTTGAACTGAGAAGCAGATACTGGGCCATAAGAACCAGAAGTGGAGGGAACACTAATCAACAAAGGGCCTCT
GCGGGCCAAATCAGCACACAACCTACGTTTTCTGTGCAGAGAAACCTCCCATTTGACAAAACAACCATCATGGCAGC
ATTCACTGGGAATACGGAGGGAAGAACATCAGACATGAGGGCAGAAATCATAAAGATGATGGAAAGTGCAAGACCAG
AAGAAGTGTCCTTCCAGGGGCGGGGAGTCTTTGAGCTCTCGGACGAAAGGGCAACGAACCCGATCGTGCCCTCCTTT
GACATGAGTAATGAAGGATCTTATTTCTTCGGAGACAATGCAGAGGAGTACGACAATTAATGAA

SEQ ID NO: 38 (M, A/New Caledonia/20/1999)

GATGAGTCTTCTAACCGAGGTCGAAACGTACGTTCTCTCTATCGTCCCGTCAGGCCCCCTCAAAGCCGAGATCGCAC
AGAGACTTGAAAATGTCTTTGCTGGAAAGAATACCGATCTTGAGGCTCTCATGGAATGGCTAAAGACAAGACCAATC
CTGTCACCTCTGACTAAGGGGATTTTAGGATTTGTGTTCACGCTCACCGTGCCCAGTGAGCGAGGACTGCAGCGTAG
ACGCTTTGTCCAAAATGCCCTTAATGGGAATGGGGATCCAAATAATATGGACAGAGCAGTTAAACTGTATCGAAAGC
TTAAGAGGGAGATAACATTCCATGGGGCCAAAGAAATAGCACTCAGTTATTCTGCTGGTGCACTTGCCAGTTGTATG
GGACTCATATACAACAGGATGGGGGCTGTGACCACCGAATCAGCATTTGGCCTTATATGCGCAACCTGTGAACAGAT
TGCCGACTCCCAGCATAAGTCTCATAGGCAAATGGTAACAACAACCAACCCATTAATAAGACATGAGAACAGAATGG
TTCTGGCCAGCACTACAGCTAAGGCTATGGAGCAAATGGCTGGATCGAGTGAACAAGCAGCTGAGGCCATGGAGGTT
GCTAGTCAGGCCAGGCAGATGGTGCAGGCAATGAGAGCCATTGGGACTCATCCTAGCTCTAGCACTGGTCTGAAAAA
TGATCTCCTTGAAAATTTGCAGGCCTATCAGAAACGAATGGGGGTGCAGATGCAACGATTCAAGTGATCCTCTTGTT
GTTGCCGCAAGTATAATTGGGATTGTGCACCTGATATTGTGGATTATTGATCGCCTTTTTTCCAAAAGCATTTATCG
TATCTTTAAACACGGTTTAAAAAGAGGGCCTTCTACGGAAGGAGTACCAGAGTCTATGAGGGAAGAATATCGAGAGG
AACAGCAGAATGCTGTGGATGCTGACGATGGTCATTTTGTCAGCATAGAGCTAGAGTAAA

SEQ ID NO: 39 (NS, A/New Caledonia/20/1999)

ATGGATTCCCACACTGTGTCAAGCTTTCAGGTAGATTGCTTCCTTTGGCATGTCCGCAAACAAGTTGCAGACCAAGA
TCTAGGCGATGCCCCATTCCTTGATCGGCTTCGCCGAGATCAGAAGTCTCTAAAGGGAAGAGGCAGCACTCTCGGTC
TGAACATCGAAACAGCCACTTGTGTTGGAAAGCAAATAGTAGAGAGGATTCTGAAAGAAGAATCCGATGAGGCATTT
AAAATGACCATGGCCTCCGCACTTGCTTCGCGGTACCTAACTGACATGACTATTGAAGAAATGTCAAGGGACTGGTT
CATGCTCATGCCCAAGCAGAAAGTGGCTGGCCCTCTTTGTGTCAGAATGGACCAGGCGATAATGGATAAGAACATCA
TACTGAAAGCGAATTTCAGTGTGATTTTTGACCGGTTGGAGAATCTGACATTACTAAGGGCTTTCACCGAAGAGGGA
GCAATTGTTGGCGAAATTTCACCATTGCCTTCTCTTCCAGGACATACTAATGAGGATGTCAAAAATGCAATTGGGGT
CCTCATCGGGGGACTTGAATGGAATGATAACACAGTTCGAGTCTCTGAAACTCTACAGAGATTCGCTTGGAGAAGCA
GTAATGAGACTGGGGGACCTCCATTCACTCCAACACAGAAACGGAAAATGGCGGGAACAATTAGGTCAGAAGTTTGA
AGAAATAAGATGGCTGATTGAAGAAGTGAGGCATAAATTGAAGACGACAGAGAATAGTTTTGAGCAAATAACATTTA
TGCAAGCATTACAGCTATTGTTTGAAGTGGAACAAGAGATTAGAACGTTTTCGTTTCAGCTTATTTAATGATAA

SEQ ID NO: 40 (HA, A/New Caledonia/20/1999)

CCAAAATGAAAGCAAAACTACTGGTCCTGTTATGTACATTTACAGCTACATATGCAGACACAATATGTATAGGCTAC
CATGCCAACAACTCAACCGACACTGTTGACACAGTACTTGAGAAGAATGTGACAGTGACACACTCTGTCAACCTACT
TGAGGACAGTCACAATGGAAAACTATGTCTACTAAAAGGAATAGCCCCACTACAATTGGGTAATTGCAGCGTTGCCG
GATGGATCTTAGGAAACCCAGAATGCGAATTACTGATTTCCAAGGAATCATGGTCCTACATTGTAGAAACACCAAAT
CCTGAGAATGGAACATGTTACCCAGGGTATTTCGCCGACTATGAGGAACTGAGGGAGCAATTGAGTTCAGTATCTTC
ATTTGAGAGATTCGAAATATTCCCCAAAGAAAGCTCATGGCCCAACCACACCGTAACCGGAGTATCAGCATCATGCT

CCCATAATGGGAAAAGCAGTTTTTACAGAAATTTGCTATGGCTGACGGGGAAGAATGGTTTGTACCCAAACCTGAGC
AAGTCCTATGTAAACAACAAAGAGAAAGAAGTCCTTGTACTATGGGGTGTTCATCACCCGCCTAACATAGGGAACCA
AAGGGCCCTCTATCATACAGAAAATGCTTATGTCTCTGTAGTGTCTTCACATTATAGCAGAAGATTCACCCCAGAAA
TAGCCAAAAGACCCAAAGTAAGAGATCAGGAAGGAAGAATCAACTACTACTGGACTCTGCTGGAACCTGGGGATACA
ATAATATTTGAGGCAAATGGAAATCTAATAGCGCCATGGTATGCTTTTGCACTGAGTAGAGGCTTTGGATCAGGAAT
CATCACCTCAAATGCACCAATGGATGAATGTGATGCGAAGTGTCAAACACCTCAGGGAGCTATAAACAGCAGTCTTC
CTTTCCAGAATGTACACCCAGTCACAATAGGAGAGTGTCCAAAGTATGTCAGGAGTGCAAAATTAAGGATGGTTACA
GGACTAAGGAACATCCCATCCATTCAATCCAGAGGTTTGTTTGGAGCCATTGCCGGTTTCATTGAAGGGGGGTGGAC
TGGAATGGTAGATGGGTGGTATGGTTATCATCATCAGAATGAGCAAGGATCTGGCTATGCTGCAGATCAAAAAAGTA
CACAAAATGCCATTAACGGGATTACAAACAAGGTGAATTCTGTAATTGAGAAAATGAACACTCAATTCACAGCTGTG
GGCAAAGAATTCAACAAATTGGAAAGAAGGATGGAAAACTTAAATAAAAAAGTTGATGATGGGTTTCTAGACATTTG
GACATATAATGCAGAATTGTTGGTTCTACTGGAAAATGAAAGGACTTTGGATTTCCATGACTCCAATGTGAAGAATC
TGTATGAGAAAGTAAAAAGCCAATTAAAGAATAATGCCAAAGAAATAGGAAACGGGTGTTTTGAATTCTATCACAAG
TGTAACAATGAATGCATGGAGAGTGTGAAAAATGGAACTTATGACTATCCAAAATATTCCGAAGAATCAAAGTTAAA
CAGGGAGAAAATTGATGGAGTGAAATTGGAATCAATGGGAGTCTATCAGATTCTGGCGATCTACTCAACTGTCGCCA
GTTCCCTGGTTCTTTTGGTCTCCCTGGGGGCAATCAGCTTCTGGATGTGTTCCAATGGGTCTTTGCAGTGTAGAATA
TGCATCTGAGACCAGAATTTCAGAAATATAAGAA

SEQ ID NO: 41 (NA, A/New Caledonia/20/1999)

AATGAATCCAAATCAAAAAATAATAACCATTGGATCAATCAGTATAGCAATCGGAATAATTAGTCTAATGTTGCAAA
TAGGAAATATTATTTCAATATGGGCTAGTCACTCAATCCAAACTGGAAGTCAAAACCACACTGGAGTATGCAACCAA
AGAATCATCACATATGAAAACAGCACCTGGGTGAATCACACATATGTTAATATTAACAACACTAATGTTGTTGCTGG
AAAGGACAAAACTTCAGTGACATTGGCCGGCAATTCATCTCTTTGTTCTATCAGTGGATGGGCTATATACACAAAAG
ACAACAGCATAAGAATTGGCTCCAAAGGAGATGTTTTTGTCATAAGAGAACCTTTCATATCATGTTCTCACTTGGAA
TGCAGAACCTTTTTTCTGACCCAAGGTGCTCTATTAAATGACAAACATTCAAATGGGACCGTTAAGGACAGAAGTCC
TTATAGGGCCTTAATGAGCTGTCCTCTAGGTGAAGCTCCGTCCCCATACAATTCAAAGTTTGAATCAGTTGCATGGT
CAGCAAGCGCATGCCATGATGGCATGGGCTGGTTAACAATCGGAATTTCTGGTCCAGACAATGGAGCTGTGGCTGTA
CTAAAATACAACGGCATAATAACTGAAACCATAAAAAGTTGGAAAAAGCGAATATTAAGAACACAAGAGTCTGAATG
TGTCTGTGTGAACGGGTCATGTTTCACCATAATGACCGATGGCCCGAGTAATGGGGCCGCCTCGTACAAAATCTTCA
AGATCGAAAAGGGGAAGGTTACTAAATCAATAGAGTTGAATGCACCCAATTTTCATTATGAGGAATGTTCCTGTTAC
CCAGACACTGGCACAGTGATGTGTGTATGCAGGGACAACTGGCATGGTTCAAATCGACCTTGGGTGTCTTTTAATCA
AAACCTGGATTATCAAATAGGATACATCTGCAGTGGGGTGTTCGGTGACAATCCGCGTCCCAAAGATGGAGAGGGCA
GCTGTAATCCAGTGACTGTTGATGGAGCAGACGGAGTAAAGGGGTTTTCATACAAATATGGTAATGGTGTTTGGATA
GGAAGGACTAAAAGTAACAGACTTAGAAAGGGGTTTGAGATGATTTGGGATCCTAATGGATGGACAGATACCGACAG
TGATTTCTCAGTGAAACAGGATGTTGTGGCAATAACTGATTGGTCAGGGTACAGCGGAAGTTTCGTTCAACATCCTG
AGTTAACAGGATTGGACTGTATAAGACCTTGCTTCTGGGTTGAGTTAGTCAGAGGACTGCCTAGAGAAAATACAACA
ATCTGGACTAGTGGGAGCAGCATTTCTTTTTGTGGCGTAAATAGTGATACTGCAAACTGGTCTTGGCCAGACGGTGC
TGAGTTGCCGTTCACCATTGACAAGTAG

SEQ ID NO: 42 (PA, 105p30)

AGCGAAAGCAGGTACTGAtTCgaAaTGGAAGATTTTGTGCGACAATGCTTCAATCCGATGATTGTCGAGCTTGCGGA
AAAGGCAATGAAAGAGTATGGAGAGGACCTGAAAATCGAAACAAACAAATTTGCAGCAATATGCACCCACTTGGAAG
TATGCTTCATGTATTCAGATTTTCATTTCATCAATGAGCAAGGCGAATCAATAATAGTAGAGCCTGAGGACCCAAAT
GCACTTTTAAAACACAGATTTGAGATAATAGAGGGGCGAGATCGTACAATGGCATGGACAGTTGTAAACAGTATTTG
CAACACCACAGGAGCTGAGAAACCAAAGTTTCTGCCAGATCTGTATGATTACAAAGAGAATAGGTTCATCGAAATTG
GAGTGACAAGGAGAGAAGTTCACATATACTATCTGGAAAAGGCCAACAAAATTAAATCTGAGAAGACACATATTCAC
ATTTTCTCATTTACTGGCGAAGAAATGGCCACAAAGGCCGATTACACTCTCGATGAAGAAAGCAGGGCTAGAATTAA
AACCAGACTATTCACCATAAGGCAAGAAATGGCAAGCAGAGGTCTTTGGGACTCCTTTCGTCAGTCCGAAAGAGGCG
AAGAGACAATTGAAGAAAGGTTTGAAATCACAGGGACAATGCGCAGGCTCGCTGATCAAAGCCTTCCGCCGAACTTC
TCCTGCATTGAGAATTTTAGAGCCTATGTGGATGGATTTGAACCGAACGGCTACATTGAGGGCAAGCTTTCTCAAAT
GTCCAAAGAAGTAAATGCTAAAATTGAGCCTTTTTTGAAAACAACACCTCGACCAATTAGACTTCCGAATGGGCCTC
CTTGTTTTCAGCGGTCAAAATTCCTGCTGATGGATTCTTTAAAATTAAGCATTGAGGATCCAAATCATGAAGGGGAG
GGAATACCACTATATGATGCAATCAAGTGTATGAGAACATTCTTTGGATGGAAAGAACCCACTGTTGTCAAGCCACA
CGAGAAGGGAATAAATCCGAATTATCTGCTGTCGTGGAAGCAGGTGTTGGAAGAGCTGCAGGACATTGAGAGTGAGG
AGAAGATTCCAAGAACAAAAAACATGAAAAAAACGAGTCAGTTAAAGTGGGCACTTGGTGAGAACATGGCACCAGAG
AAGGTGGATTTTGATGACTGTAAAGATATAAGCGATTTGAAGCAATATGATAGTGACGAACCTGAATTAAGGTCATT
TTCAAGTTGGATCCAGAATGAGTTCAACAAGGCATGCGAGCTGACCGATTCAATCTGGATAGAGCTCGATGAGATTG
GAGAAGATGTGGCCCCGATTGAACACATTGCAAGCATGAGAAGAAATTACTTCACAGCTGAGGTGTCCCATTGCAGA
GCCACTGAATATATAATGAAAGGGGTATACATTAATACTGCTTTGCTTAATGCATCCTGTGCAGCAATGGATGATTT
CCAACTAATTCCTATGATAAGCAAATGTAGAACTAAAGAGGGAAGGAGAAAGACCAATTTGTACGGCTTCATCATAA
AAGGAAGATCTCACTTAAGGAATGATACCGATGTGGTAAACTTTGTGAGCATGGAGTTTTCCCTCACTGACCCAAGA

CTTGAGCCACACAAATGGGAGAAGTACTGTGTTCTTGAGATAGGAGATATGCTTCTAAGGAGTGCAATAGGCCAAGT
GTCAAGGCCCATGTTCTTGTATGTAAGAACAAATGGAACCTCAAAAATTAAAATGAAATGGGGAATGGAGATGAGGC
GTTGCCTCCTCCAATCCCTCCAACAAATAGAGAGCATGATTGAAGCTGAGTCCTCTGTCAAGGAGAAAGACATGACA
AAAGAGTTTTTTGAGAATAGATCAGAAACATGGCCCATTGGAGAGTCACCAAAAGGAGTGGAAGAAGGTTCCATTGG
GAAAGTATGCAGGACACTATTGGCTAAATCAGTATTCAATAGTCTGTATGCATCTCCACAATTAGAAGGATTTTCAG
CTGAGTCAAGAAAGTTGCTCCTTATTGTTCAGGCTCTTAGGGACAATCTGGAACCTGGGACCTTTGATCTTGGGGGA
CTATATGAAGCAATTGAGGAGTGCCTGATTAATGATCCCTGGGTTTTGCTTAATGCTTCTTGGTTCAACTCCTTCCT
AAAACATGCATTGAGATAGCTGAGGCAATGCTACTATTTGTTATCCATACTGTCCAAAAAAGTA

SEQ ID NO: 43 (PB1, 105p30)

AGCGAAAGCAGGCAAACCATTTGAATGGATGTCAATCCGACATTACTTTTCTTAAAAGTGCCAGCACAAAATGCTAT
AAGCACAACTTTTCCTTATACTGGTGACCCTCCTTACAGCCATGGAACAGGAACAGGATACACCATGGATACAGTCA
ACAGGACACATCAGTACTCAGAAAGAGGAAGATGGACGAAAAATACCGAAACTGGAGCACCGCAACTCAACCCAATT
GATGGGCCACTACCAGAAGACAATGAACCAAGTGGCTATGCCCAAACAGATTGTGTATTAGAGGCAATGGCTTTCCT
TGAAGAATCCCATCCTGGTATTTTTGAAAACTCTTGTATTGAAACAATGGAGGTTGTTCAGCAAACAAGGGTGGACA
AACTGACACAAGGCAGACAAACCTATGACTGGACTCTAAATAGGAACCAGCCTGCTGCCACAGCATTGGCAAACACC
ATAGAAGTATTCAGATCAAATGGCCTCATAGCAAATGAATCTGGAAGGCTAATAGACTTCCTTAAAGATGTAATGGA
GTCGATGGACAGAGACGAAGTAGAGGTCACAACTCATTTTCAAAGAAAGAGGAGAGTGAGAGACAATGTAACTAAAA
AAATGGTGACCCAAAGAACAATAGGAAAAAAGAAACATAAATTAGACAAAAGAAGTTACCTAATTAGGGCATTAACC
CTGAACACAATGACCAAAGATGCTGAGAGGGGGAAACTAAAACGCAGAGCAATTGCAACCCCAGGAATGCAAATAAG
GGGGTTTGTATACTTTGTTGAGACACTGGCAAGAAGCATATGTGAAAAGCTTGAACAATCAGGGTTGCCAGTTGGAG
GAAATGAGAAGAAAGCAAAGTTAGCAAATGTTGTAAGGAAGATGATGACCAACTCCCAGGACACTGAAATTTCTTTT
ACCATCACTGGAGATAACACAAAATGGAACGAAAATCAAAACCCTAGAATGTTCTTGGCCATGATCACATATATAAC
CAAAGATCAGCCTGAATGGTTCAGAAATATTCTAAGTATTGCTCCAATAATGTTTTCAAACAAAATGGCGAGACTAG
GTAGGGGGTATATGTTTGAAAGCAAGAGTATGAAACTGAGAACCCAAATACCTGCAGAGATGCTAGCCAACATAGAT
TTGAAATATTTCAATGATTCAACTAAAAAGAAAATTGAAAAAATTCGACCATTATTAATAGATGGAACTGCATCATT
GAGTCCTGGAATGATGATGGGCATGTTCAATATGTTAAGCACCGTCTTGGGCGTTTCCATTCTGAATCTTGGGCAAA
AAAGATACACCAAGACTACTTACTGGTGGGATGGTCTTCAATCGTCTGATGATTTTGCTTTGATTGTGAATGCACCC
AATTATGCAGGAATTCAAGCTGGAGTTGACAGGTTTTATCGAACCTGTAAGCTGCTCGGAATTAATATGAGCAAAAA
GAAGTCTTACATAAACAGAACAGGTACCTTTGAATTCACGAGCTTTTTCTATCGTTATGGGTTTGTTGCCAATTTCA
GCATGGAGCTTCCTAGTTTTGGGGTGTCTGGGGTCAATGAATCTGCAGACATGAGTATTGGAGTCACTGTCATCAAA
AACAATATGATAAACAATGACCTTGGCCCAGCAACTGCTCAAATGGCCCTTCAGTTATTTATAAAAGATTACAGGTA
CACTTATCGATGCCACAGAGGTGACACACAAATACAAACCCGGAGATCATTTGAAATAAAGAAACTATGGGACCAAA
CCCGCTCCAAAGCTGGGCTGTTGGTCTCTGATGGAGGCCCCAATTTATATAACATTAGGAATCTACATATTCCTGAA
GTCTGCTTGAAATGGGAGTTGATGGATGAGGATTACCAGGGGCGTTTATGCAACCCATTGAACCCGTTTGTCAGCCA
TAAAGAGATTGAATCAGTGAACAATGCAGTGATAATGCCGGCACATGGTCCAGCCAAAAATATGGAGTATGACGCTG
TTGCAACAACACACTCTTGGGTCCCCAAAAGAAATCGATCCATTTTAAACACGAGCCAAAGAGGGATACTTGAAGAT
GAGCAAATGTACCAAAGGTGCTGCAATTTATTTGAAAAATTCTTCCCAAGTAGCTCATACAGAAGACCAGTTGGAAT
ATCCAGTATGGTAGAGGCTATGGTTTCAAGAGCCCGAATTGATGCACGGATTGATTTCGAATCTGGAAGGATAAAGA
AAGAGGAATTCGCTGAGATCATGAAGACCTGTTCCACCATTGAAGACCTCAGACGGCAAAAATAGGGAATTTGGCTT
GTCCTTCATGAAAAAATGCCTTGTTTCTACT

SEQ ID NO: 44 (PB2, 105p30)

AGCGAAAGCAGGTCAATTATATTCAATATGGAAAGAATAAAAGAGCTAAGGAATCTGATGTCACAATCTCGCACTCG
CGAGATACTTACCAAAACTACTGTAGACCACATGGCCATAATAAAGAAATACACATCAGGAAGACAGGAGAAAAACC
CATCACTTAGGATGAAATGGATGATGGCAATGAAATACCCAATTACAGCTGATAAAAGGATAACGGAAATGATTCCT
GAAAGAAATGAGCAAGGACAGACACTATGGAGTAAAGTGAATGATGCCGGATCAGACCGAGTGATGATATCACCCCT
AGCTGTGACATGGTGGAACAGAAATGGACCAGTGGCAAACACTATCCACTATCCAAAAATCTACAAAACTTACTTTG
AAAAGGTTGAAAGGTTAAAACATGGAACCTTTGGCCCTGTACACTTTAGAAACCAAGTCAAAATACGCCGAAGAGTC
GACATAAATCCTGGTCATGCAGACCTCAGCGCCAAGGAGGCACAGGATGTAATTATGGAAGTTGTTTTCCCTAATGA
AGTGGGAGCCAGAATACTAACATCAGAATCGCAATTAACGATAACTAAGGAGAAAAAAGAGGAACTCCAGAATTGCA
AAATTTCCCCTTTGATGGTTGCATACATGTTAGAGAGGGAACTTGTCCGCAAAACAAGATTTCTCCCGGTTGCAGGT
GGAACAAGCAGTGTGTACATTGAAGTTTTGCATTTAACACAGGGGACATGCTGGGAGCAGATGTACACTCCAGGTGG
GGAGGTGAGGAATGATGATGTTGATCAAAGCCTAATTATTGCTGCTAGGAACATAGTGAGAAGAGCTGCAGTATCAG
CAGATCCACTAGCATCTTTATTAGAAATGTGCCATAGCACACAGATTGGTGGAACAAGGATGGTGGATATTCTCAGG
CAAAATCCAACAGAAGAACAAGCTGTGGACATATGCAAAGCAGCAATGGGGCTGAGAATCAGTTCATCCTTCAGTTT
TGGCGGATTCACATTTAAGAGAACAAGTGGATCGTCAGTCAAAAGGGAGGAAGAAGTGCTAACGGGCAATCTGCAAA
CATTGAAGCTAACTGTGCATGAGGGATATGAAGAATTCACAATAGTTGGGAAAAAGGCAACAGCTATACTCAGAAAA
GCAACCAGGAGATTGATTCAACTAATAGTGAGTGGAAGAGACGAACAGTCAATAGTCGAAGCAATAGTTGTAGCAAT
GGTATTCTCACAAGAAGATTGCATGGTAAAAGCGGTTAGAGGTGATCTGAATTTCGTTAATAGAGCGAATCAGCGGT
TGAATCCCATGCATCAACTTTTGAGACATTTTCAGAAGGATGCTAAAGTACTTTTCCTAAATTGGGGAATTGAACAT

ATTGACAATGTGATGGGAATGATTGGGATATTACCTGATATGACTCCAAGTACCGAGATGTCAATGAGAGGAGTGAG
AGTCAGCAAAATGGGTGTAGATGAATACTCCAATGCTGAAAGGGTAGTGGTAAGCATTGACCGTTTTTTGAGGGTCC
GGGACCAAAGAGGAAATGTATTACTGTCTCCAGAGGAAGTCAGTGAAACACAAGGAACAGAGAAACTGACAATAACT
TACTCTTCATCATTGATGTGGGAGATTAATGGCCCTGAGTCAGTGTTGATCAATACCTACCAATGGATCATCAGAAA
CTGGGAGACTGTTAAAATTCAGTGGTCTCAGAACCCTACAATGCTATACAATAAAATGGAATTTGAGCCATTTCAAT
CTCTAGTCCCCAAGGCCATTAGAGGCCAATACAGTGGGTTTGTTAGAACTCTATTTCAACAAATGAGGGATGTGCTC
GGGACCTTTGACACAACTCAGATAATAAAACTTCTTCCCTTTGCAGCCGCTCCACCAAAGCAAAGTAGAATGCAATT
CTCGTCATTAACTGTGAATGTGAGGGGATCAGGAATGAGAATACTTGTAAGGGGTAATTCTCCAGTATTCAACTACA
ACAAGACCACTAAGAGACTCACAATCCTCGGAAAGGATGCTGGCACTTTAACTGAAGACCCAGATGAAGGCACAGCT
GGAGTGGAATCTGCTGTTTTAAGGGGATTCCTCATTCTAGGCAAAGAAGATAGAAGATATGGGCCAGCATTAAGCAT
CAGTGAATTGAGCAACCTTGCGAAAGGGGAGAAAGCTAATGTGCTAATTGGGCAAGGGGATGTAGTGTTGGTAATGA
AACGAAAACGGGACTCTAGCATACTTACTGACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAATTT
CGAATAATTTAAAAACGACCTTGTTTCTACT

SEQ ID NO: 45 (NP, 105p30)

AGCAAAAGCAGGGTAGATAATCACTCACTGAGTGACATCAAAGTCATGGCGTCCCAAGGCACCAAACGGTCTTACGA
ACAGATGGAGACTGATGGGGAACGCCAGAATGCAACTGAAATCAGAGCATCCGTCGGAAGAATGATTGGGGGAATTG
GGCGATTCTACATCCAAATGTGCACCGAGCTTAAGCTCAATGATTATGAGGGACGACTGATCCAGAACAGCTTAACA
ATAGAGAGAATGGTGCTTTCTGCTTTTGATGAGAGGAGAAATAAATATCTGGAAGAACATCCCAGCGCAGGGAAAGA
TCCTAAGAAAACTGGAGGACCCATATACAAGAGAGTAGATGGAAAGTGGGTGAGGGAACTCGTCCTTTATGACAAAG
AAGAAATAAGGCGGATTTGGCGCCAAGCCAACAATGGTGATGATGCAACAGCTGGTTTGACTCACATTATGATCTGG
CATTCTAATTTGAATGATACAACTTACCAGAGGACAAGAGCTCTTGTCCGCACCGGAATGGATCCCAGGATGTGCTC
TTTGATGCAAGGTTCAACTCTCCCTAGAAGATCTGGAGCAGCAGGCGCTGCAGTCAAAGGAGTTGGGACAATGGTAT
TGGAGTTAATCAGGATGATCAAACGTGGGATCAACGACCGAAACTTCTGGAGGGGTGAGAATGGGAGAAAAACAAGG
ATTGCTTATGAGAGAATGTGCAACATTCTCAAAGGAAAATTTCAAACAGCTGCACAAAAAGCAATGATGGATCAAGT
GAGAGAAAGCCGGAACCCAGGAAATGCTGAGATCGAAGATCTCACTTTTCTGGCACGGTCTGCACTCATATTGAGAG
GATCAGTTGCTCACAAGTCTTGCCTGCCTGCTTGTGTGTATGGACCAGCCGTAGCCAGTGGGTATGACTTCGAAAAA
GAGGGATACTCTTTGGTGGGAGTAGACCCTTTCAAACTGCTTCAAACCAGTCAGGTATACAGCCTAATTAGACCAAA
CGAGAATCCCGCACACAAGAGCCAGTTGGTGTGGATGGCATGCAATTCTGCTGCATTTGAAGATCTAAGAGTGTCAA
GCTTCATCAGAGGGACAAGAGTACTTCCAAGGGGGAAGCTCTCCACTAGAGGAGTACAAATTGCTTCAAATGAAAAC
ATGGATGCTATTGTCTCAAGTACTCTTGAACTGAGAAGCAGATACTGGGCCATAAGAACCAGAAGTGGAGGGAACAC
CAATCAACAAAGGGCCTCTGCGGGCCAAATCAGCACACAACCTACGTTTTCTGTGCAGAGAAACCTCCCATTTGACA
AAACAACCATCATGGCAGCATTCACTGGGAATACAGAGGGAAGAACATCAGACATGCGGGCAGAAATCATAAAGATG
ATGGAAAGTGCAAGACCAGAAGAAGTGTCCTTCCAGGGACGGGGAGTCTTTGAGCTCTCGGACGAAAGGGCAACGAA
CCCGATCGTGCCCTCCTTTGACATGAGTAATGAAGGATCTTATTTCTTCGGAGACAATGCAGAGGAGTACGACAATT
AATGAAAAATACCCTTGTTTCTACT

SEQ ID NO: 46 (M, 105p30)

AGCAAAAGCAGGTAGATATTGAAAGATGAGTCTTCTAACCGAGGTCGAAACGTACGTTCTCTCTATCGTCCCATCAG
GCCCCCTCAAAGCCGAGATCGCACAGAGACTTGAAGATGTATTTGCTGGAAAGAATACCGATCTTGAGGCTCTCATG
GAATGGCTAAAGACAAGACCAATCCTGTCACCTCTGACTAAGGGGATTTTAGGATTTGTGTTCACGCTCACCGTGCC
CAGTGAGCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCTTAATGGGAATGGGGATCCAAATAATATGGACA
AGGCTGTCAAACTGTATCGAAAGCTTAAGAGGGAGATAACATTCCATGGGGCCAAAGAAATAGCACTCAGTTATTCT
GCTGGAGCACTTGCCAGTTGTATGGGACTCATATACAACAGGATGGGGGCTGTGACCACCGAATCAGCATTTGGCCT
TATATGTGCAACCTGTGAACAGATTGCCGACTCCCAGCATAAGTCTCATAGGCAAATGGTAACAACAACCAATCCAT
TAATAAGACATGAGAACAGAATGGTTCTGGCCAGCACTACAGCTAAGGCTATGGAGCAAATGGCTGGATCGAGTGAA
CAAGCAGCTGAGGCCATGGAGGTTGCTAGTCAGGCCAGGCAGATGGTGCAGGCAATGAGAGCCATTGGGACTCATCC
TAGCTCTAGCACTGGTCTGAAAAATGATCTCCTTGAAAATTTGCAGGCCTATCAGAAACGAATGGGGGTGCAGATGC
AACGATTCAAGTGATCCTCTTGTTGTTGCCGCAAGTATAATTGGGATTGTGCACCTGATATTGTGGATTATTGATCG
CCTTTTTTCCAAAAGCATTTATCGTATTTTTAAACACGGTTTAAAAAGAGGGCCTTCTACGGAAGGAGTACCGGAGT
CTATGAGGGAAGAATATCGAGAGGAACAGCAGAATGCTGTGGATGCTGACGATGGTCATTTTGTCAGCATAGAGCTA
GAGTAAAAAACTACCTTGTTTCTACT

SEQ ID NO: 47 (NS, 105p30)

AGCAAAAGCAGGGTGGCAAAGACATAATGGATTCCCACACTGTGTCAAGCTTTCAGGTAGATTGTTTCCTTTGGCAT
GTCCGCAAACAAGTTGCAGACCAAGATCTAGGCGATGCCCCCTTCCTTGATCGGCTTCGCCGAGATCAGAAGTCTCT
AAAGGGACGAGGCAACACTCTCGGTCTGAACATCGAAACAGCCACTTGTGTTGGAAAGCAAATAGTAGAGAGGATTC
TGAAAGAAGAATCCGATGAGACATTTAGAATGACCATGGCCTCCGCACTTGCTTCGCGGTACCTAACTGACATGACT
GTTGAAGAAATGTCAAGGGACTGGTTCATGCTCATGCCCAAGCAGAAAGTGGCTGGCCCTCTTTGTGTCAGAATGGA
CCAGGCGATAATGGATAAGAACATCATACTGAAAGCGAACTTCAGTGTGATTTTTGACCGGTTGGAGAATCTGACAT

TACTAAGGGCTTTCACCGAAGAGGGAGCAATTGTTGGCGAAATTTCACCATTGCCTTCTTTTCCAGGACATACTAAT
GAGGATGTCAAAAATGCAATTGGGGTCCTCATCGGGGGACTTGAATGGAATGATAACACAGTTCGAGTCTCTGAAGC
TCTACAGAGATTCGCTTGGAGAAGCAGTAATGAGACTGGGGGACCTCCATTCACTACAACACAGAAACGGAAAATGG
CGGGAACAATTAGGTCAGAAGTTTGAAGAAATAAGATGGCTGATTGAAGAAGTGAGGCATAAATTGAAGACGACAGA
GAGTAGTTTTGAACAAATAACATTTATGCAAGCATTACAGCTATTGTTTGAAGTGGAACAAGAGATTAGAACGTTCT
CGTTTCAGCTTATTTAATGATAAAAACACCCTTGTTTCTACT

SEQ ID NO: 48 (HA, 105p30)

AGCGAAAGCAGGGGAAAATAAAAGCAACCAAAATGAAAGTAAAACTACTGGTTCTGTTATGTACATTTACAGCTACA
TATGCAGACACAATATGTATAGGCTACCATGCCAACAACTCAACCGACACTGTTGACACAGTACTTGAGAAGAATGT
AACAGTGACACACTCTGTCAACCTACTTGAGGACAGTCACAATGGAAAACTATGTCTACTAAAAGGAATAGCCCCAC
TACAATTGGGTAATTGCAGCGTTGCCGGATGGATCTTAGGAAACCCAGAATGCGAATTACTGATTTCCAAGGAATCA
TGGTCCTACATTGTAGAAACACCAAATCCTGAGAATGGAACATGTTACCCAGGGTATTTCGCCGACTATGAGGAACT
GAGGGAGCAATTGAGTTCAGTATCTTCATTTGAAAGGTTCGAAATATTCCCCAAAGAGAGCTCATGGCCCAACCACA
CCGTAACCGGAGTATCAGCATCATGCTCCCATAACGGGAAAAGCAGTTTTTACAGAAATTTGCTATGGCTGACGGGG
AAGAATGGTTTGTACCCAAACCTGAGCAAGTCCTATGCAAACAACAAAGAGAAAGAAGTCCTTGTACTATGGGGTGT
TCATCACCCGCCTAACATAGGGGACCAAAGGGCCCTCTATCATACAGAAAATGCTTATGTCTCTGTAGTGTCTTCAC
ATTATAGCAGAAGATTCACCCCAGAAATAGCCAAAAGACCCAAGGTGAGAGACCAGGAAGGAAGAATCAACTACTAC
TGGACTCTGCTGGAACCCGGGGATACAATAATATTTGAGGCAAATGGAAATCTAATAGCGCCAAGGTATGCTTTCGC
ACTGAGTAGAGGCTTGGGATCAGGAATCATCACCTCAAATGCACCAATGGATGAATGTGATGCAAAGTGTCAAACAC
CTCAGGGAGCTATAAACAGCAGTCTTCCTTTCCAGAATGTACACCCAGTCACAATAGGAGAGTGTCCAAAGTATGTC
AGGAGTGCAAAATTAAGGATGGTTACAGGACTAAGGAACATCCCATCCATTCAATCCAGAGGTTTGTTTGGAGCAAT
TGCCGGTTTCATTGAAGGGGGGTGGACTGGAATGGTAGATGGTTGGTATGGTTATCATCATCAGAATGAGCAAGGAT
CTGGGTATGCTGCAGATCAAAAAAGCACACAAAATGCCATTAACGGGATTACAAACAAGGTGAATTCTGTAATTGAG
AAAATGAACACTCAATTCACAGCTGTGGGCAAAGAATTCAACAAATTGGAAAGAAGGATGGAAAACTTAAATAAAAA
AGTTGATGATGGGTTTCTAGACATTTGGACCTATAATGCAGAATTGTTGGTTCTACTGGAAAATGAAAGGACTTTGG
ATTTCCATGACTCCAACGTGAAGAATCTGTATGAGAAAGTAAAAAGCCAATTAAAGAATAATGCCAAAGAAATAGGA
AACGGGTGTTTTGAATTCTATCACAAGTGTAACGATGAATGCATGGAGAGTGTGAAAAATGGAACTTATGACTATCC
AAAATATTCCGAAGAATCAAAGTTAAACAGAGAGAAAATTGATGGAGTGAAATTGGAATCAATGGGAGTCTATCAGA
TTCTGGCGATCTACTCAACAGTCGCCAGTTCCCTGGTTCTTTTGGTCTCCCTGGGGGCAATCAGCTTCTGGATGTGT
TCCAATGGGTCTTTGCAGTGTAGAATATGCATCTAAGACCAGAATTTCAGAAATATAAGGAAAAACACCCTTGTTTC
TACT

SEQ ID NO: 49 (NA, 105p30)

AGCAAAAGCAGGAGTTTAAAATGAATCCAAATCAAAAAATAATAACCATTGGATCAATCAGTATAGCAATCGGAATA
ATTAGTCTAATGTTGCAAATAGGAAATATTATTTCAATATGGGCTAGTCACTCAATCCAAACTGGAAGTCAAAACCA
CACTGGAATATGCAACCAAAAAATCATCACATATGAAAACAGCACCTGGGTGAATCACACATATGTTAATATTAACA
ACACTAATGTTGTTGCTGGAAAGGACAAAACTTCAGTGACACTGGCCGGCAATTCATCTCTTTGTCCTATCAGTGGA
TGGGCTATATACACAAAAGACAACAGCATAAGAATTGGCTCCAAAGGAGATGTTTTTGTCATAAGAGAACCTTTCAT
ATCATGTTCTCACTTGGAATGCAGAACCTTTTTTCTGACCCAAGGTGCTCTATTAAATGACAAACATTCAAATGGAA
CCGTTAAGGACAGAAGTCCTTATAGGGCCTTAATGAGCTGTCCTCTAGGTGAAGCCCCGTCACCATACAATTCAAAG
TTTGAATCAGTTGCATGGTCAGCAAGCGCATGCCATGATGGCAAGGGCTGGTTAACAATCGGAATTTCTGGTCCAGA
CAATGGAGCTGTGGCTGTACTAAAATACAACGGAATAATAACTGAAACCATAAAAAGTTGGGAAAAGCGAATATTGA
GAACACAAGAGTCTGAATGTGTTTGTGTGAACGGGTCATGTTTCACCATAATGACCGATGGCCCGAGTAATGGGGCC
GCCTCGTACAAAATCTTCAAGATCGAAAAGGGGAAGGTTACTAAATCAACAGAGTTGAATGCACCCAATTTTCATTA
TGAGGAATGTTCCTGTTACCCAGACACTGGCACAGTGATGTGTGTATGCAGGGACAACTGGCATGGTTCAAATCGAC
CTTGGGTATCTTTTAATCAAAACTTGGATTATCAAATAGGATACATCTGCAGTGGAGTGTTCGGTGACAATCCGCGT
CCCAAAGATGGGAAGGGCAGCTGTAATCCAGTGACTGTTGATGGAGCAGACGGAGTTAAGGGGTTTTCATACAAATA
TGGTAATGGTGTTTGGATAGGAAGGACTAAAAGTAACAGACTTAGAAAGGGGTTTGAGATGATTTGGGATCCTAATG
GATGGACAGATACCGACAGTGATTTCTCAGTGAAACAGGATGTTGTGGCAATAACTGATTGGTCAGGGTACAGCGGA
AGTTTCGTCCAACATCCTGAGTTAACAGGATTGGACTGTATAAGACCTTGCTTCTGGGTTGAGTTAGTCAGAGGACT
GCCTAGAGAAAATACAACAATCTGGACTAGTGGGAGCAGCATTTCTTTTTGTGGCGTTGATAGTGATACTGCAAATT
GGTCTTGGCCAGACGGTGCTGAGTTGCCGTTCACCATTGACAAGTAGCTCGTTGAAAAAAACTCCTTGTTTCTACT

SEQ ID NO: 50 (HA, A/Chile/1/1983)

MKAKLLVLLCALSATDADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDNHNGKLCKLKGIAPLQLGKCSIAGW
ILGNPECESLFSKKSWSYIAETPNSENGTCYPGYFADYEELREQLSSVSSFERFEIFPKESSWPKHNVTKGVTAACS
HKGKSSFYRNLLWLTEKNGSYPNLSKSYVNNKEKEVLVLWGVHHPSNIEDQKTIYRKENAYVSVVSSHYNRRFTPEI
AKRPKVRNQEGRINYYWTLLEPGDTIIFEANGNLIAPWYAFALSRGFGSGIITSNASMDECDAKCQTPQGAINSSLP
FQNVHPVTIGECPKYVRSTKLRMVTGLRNIPSIQSRGLFGAIAGFIEGGWTGMIDGWYGYHHQNEQGSGYAADQKST

QNAINGITNKVNSIIEKMNTQFTAVGKEFNKLEKRMENLNKKVDDGFLDIWTYNAELLVLLENERTLDFHDSNVKNL
YEKVKSQLKNNAKEIGNGCFEFYHKCNNECMESVKNGTYDYPKYSEESKLNREKIDGVKLESMGVYQILAIYSTVAS
SLVLLVSLGAISFWMCSNGSLQCRICI

SEQ ID NO: 51 (NA, A/Chile/1/1983)

MNPNQKIITIGSICMTIGIISLILQIGNIISIWVSHSIQTGSQNHTGICNQRIITYENSTWVNQTYVNINNTNVVAG
KDTTSVTLAGNSSLCPIRGWAIYSKDNSIRIGSKGDVFVIREPFISCSHLECRTFFLTQGALLNDKHSNGTVKDRSP
YRALMSCPIGEAPSPYNSRFESVAWSASACHDGMGWLTIGISGPDDGAVAVLKYNGIITETIKSWRKRILRTQESEC
VCVNGSCFTIMTDGPSNGPASYRIFKIEKGKITKSIELDAPNSHYEECSCYPDTGTVMCVCRDNWHGSNRPWVSFNQ
NLDYQIGYICSGVFGDNPRPKDGKGSCDPVTVDGADGVKGFSYRYGNGVWIGRTKSNSSRKGFEMIWDPNGWTDTDS
NFLVKQDVVAMTDWSGYSGSFVQHPELTGLDCMRPCFWVELVRGRPREGTTVWTSGSSISFCGVNSDTANWSWPDGA
ELPFTIDK

SEQ ID NO: 52 (NA, A/California/04/09)

MNPNQKIITIGSVCMTIGMANLILQIGNIISIWISHSIQLGNQNQIETCNQSVITYENNTWVNQTYVNISNTNFAAG
QSVVSVKLAGNSSLCPVSGWAIYSKDNSVRIGSKGDVFVIREPFISCSPLECRTFFLTQGALLNDKHSNGTIKDRSP
YRTLMSCPIGEVPSPYNSRFESVAWSASACHDGINWLTIGISGPDNGAVAVLKYNGIITDTIKSWRNNILRTQESEC
ACVNGSCFTVMTDGPSNGQASYKIFRIEKGKIVKSVEMNAPNYHYEECSCYPDSSEITCVCRDNWHGSNRPWVSFNQ
NLEYQIGYICSGIFGDNPRPNDKTGSCGPVSSNGANGVKGFSFKYGNGVWIGRTKSISSRNGFEMIWDPNGWTGTDN
NFSIKQDIVGINEWSGYSGSFVQHPELTGLDCIRPCFWVELIRGRPKENTIWTSGSSISFCGVNSDTVGWSWPDGAE
LPFTIDK

SEQ ID NO: 53 (PA, A/New Caledonia/20/1999)

MEDFVRQCFNPMIVELAEKAMKEYGEDPKIETNKFAAICTHLEVCFMYSDFHFIDERGESIIVESGDPNALLKHRFE
IIEGRDRIMAWTVVNSICNTTGVEKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKANKIKSEKTHIHIFSFTGEE
MATKADYTLDEESRARIKTRLFTIRQEMASRSLWDSFRQSERGEETIEEKFEITGTMRKLADQSLPPNFPSLENFRA
YVDGFEPNGCIEGKLSQMSKEVNAKIEPFLRTTPRPLRLPDGPLCHQRSKFLLMDALKLSIEDPSHEGEGIPLYDAI
KCMKTFFGWKEPNIVKPHEKGINPNYLMAWKQVLAELQDIENEEKIPRTKNMKRTSQLKWALGENMAPEKVDFDDCK
DVGDLKQYDSDEPEPRSLASWVQNEFNKACELTDSSWIELDEIGEDVAPIEHIASMRRNYFTAEVSHCRATEYIMKG
VYINTALLNASCAAMDDFQLIPMISKCRTKEGRRKTNLYGFIIKGRSHLRNDTDVVNFVSMEFSLTDPRLEPHKWEK
YCVLEIGDMLLRTAIGQVSRPMFLYVRTNGTSKIKMKWGMEMRRCLLQSLQQIESMIEAESSVKEKDMTKEFFENKS
ETWPIGESPRGVEEGSIGKVCRTLLAKSVFNSLYASPQLEGFSAESRKLLLIVQALRDNLEPGTFDLGGLYEAIEEC
LINDPWVLLNASWFNSFLTHALK

SEQ ID NO: 54 (PB1, A/New Caledonia/20/1999)

MDVNPTLLFLKVPAQNAISTTFPYTGDPPYSHGTGTGYTMDTVNRTHQYSERGRWTKNTETGAPQLNPIDGPLPKDN
EPSGYAQTDCVLEAMAFLEESHPGIFENSCIETMEVVQQTRVDKLTQGRQTYDWTLNRNQPAATALANTIEVFRSNG
LIANESGRLIDFLKDVMESMDRDEVEVTTHFQRKRRVRDNVTKKMVTQRTIGKKKHKLDKRSYLIRALTLNTMTKDA
ERGKLKRRAIATPGMQIRGFVYFVETLARSICEKLEQSGLPVGGNEKKAKLANVVRKMMTNSQDTEISFTITGDNTK
WNENQNPRMFLAMITYITKNQPEWFRNILSIAPIMFSNKMARLGKGYMFESKSMKLRTQIPAEMLANIDLKYFNDST
KRKIEKIRPLLIDGTASLSPGMMMGMFNMLSTVLGVSILNLGQKRYTKTTYWWDGLQSSDDFALIVNAPNYAGIQAG
VDRFYRTCKLLGINMSKKKSYINRTGTFEFTSFFYRYGFVANFSMELPSFGVSGVNESADMSIGVTVIKNNMINNDL
GPATAQMALQLFIKDYRYTYRCHRGDTQIQTRRSFEIKKLWDQTRSKAGLLVSDGGPNLYNIRNLHIPEVCLKWELM
DEDYQGRLCNPSNPFVSHKEIESVNNAVMMPAHGPAKNMEYDAVATTHSWVPKRNRSILNTSQRGILEDEQMYQRCC
NLFEKFFPSSSYRRPVGISSMVEAMVSRARIDARIDFESGRIKKEEFAEIMKTCSTIEDLRRQK

SEQ ID NO: 55 (PB2, A/New Caledonia/20/1999)

MERIKELRNLMSQSRTREILTKTTVDHMAIIKKYTSGRQEKNPSLRMKWMMAMKYPITADKRITEMIPERNEQGQTL
WSKVNDAGSDRVMISPLAVTWWNRNGPVASTIHYPKIYKTYFEKVERLKHGTFGPVHFRNQVKIRRRVDINPGHADL
SAKEAQDVIMEVVFPNEVGARILTSESQLTITKEKKEELQNCKISPLMVAYMLERELVRKTRFLPVAGGTSSVYIEV
LHLTQGTCWEQMYTPGGEVRNDDVDQSLIIAARNIVRRAAVSADPLASLLEMCHSTQIGGTRMVDILRQNPTEEQAV
DICKAAMGLRISSSFSFGGFTFKRTSGSSVKREEEVLTGNLQTLKLTVHEGYEEFTMVGKRATAILRKATRRLIQLI
VSGRDEQSIVEAIVVAMVFSQEDCMVKAVRGDLNFVNRANQRLNPMHQLLRHFQKDAKVLFLNWGIEPIDNVMGMIG
ILPDMTPSTEMSMRGVRVSKMGVDEYSNAERVVVSIDRFLRVRDQRGNVLLSPEEVSETQGTEKLTITYSSSMMWEI
NGPESVLINTYQWIIRNWETVKIQWSQNPTMLYNKMEFEPFQSLVPKAIRGQYSGFVRTLFQQMRDVLGTFDTTQII
KLLPFAAAPPKQSRMQFSSLTVNVRGSGMRILVRGNSPVFNYNKTTKRLTVLGKDAGTLTEDPDEGTAGVESAVLRG
FLILGKEDRRYGPALSINELSNLAKGEKANVLIGQGDVVLVMKRKRDSSILTDSQTATKRIRMAIN

SEQ ID NO: 56 (NP, A/New Caledonia/20/1999)

MASQGTKRSYEQMETDGERQNATEIRASVGRMIGGIGRFYIQMCTELKLNDYEGRLIQNSLTIERMVLSAFDERRNK
YLEEHPSAGKDPKKTGGPIYKRVDGKWVRELVLYDKEEIRRIWRQANNGDDATAGLTHIMIWHSNLNDTTYQRTRAL
VRTGMDPRMCSLMQGSTLPRRSGAAGAAVKGVGTMVLELIRMIKRGINDRNFWRGENGRKTRIAYERMCNILKGKFQ
TAAQKAMMDQVRESRNPGNAEIEDLTFLARSALILRGSVAHKSCLPACVYGPAVASGYDFEKEGYSLVGVDPFKLLQ
TSQVYSLIRPNENPAHKSQLVWMACNSAAFEDLRVSSFIRGTRVLPRGKLSTRGVQIASNENMDAIVSSTLELRSRY
WAIRTRSGGNTNQQRASAGQISTQPTFSVQRNLPFDKTTIMAAFTGNTEGRTSDMRAEIIKMMESARPEEVSFQGRG
VFELSDERATNPIVPSFDMSNEGSYFFGDNAEEYDN

SEQ ID NO: 57 (M1, A/New Caledonia/20/1999)

MSLLTEVETYVLSIVPSGPLKAEIAQRLENVFAGKNTDLEALMEWLKTRPILSPLTKGILGFVFTLTVPSERGLQRR
RFVQNALNGNGDPNNMDRAVKLYRKLKREITFHGAKEIALSYSAGALASCMGLIYNRMGAVTTESAFGLICATCEQI
ADSQHKSHRQMVTTTNPLIRHENRMVLASTTAKAMEQMAGSSEQAAEAMEVASQARQMVQAMRAIGTHPSSSTGLKN
DLLENLQAYQKRMGVQMQRFK

SEQ ID NO: 58 (NA, A/New Caledonia/20/1999)

MNPNQKIITIGSISIAIGIISLMLQIGNIISIWASHSIQTGSQNHTGVCNQRIITYENSTWVNHTYVNINNTNVVAG
KDKTSVTLAGNSSLCSISGWAIYTKDNSIRIGSKGDVFVIREPFISCSHLECRTFFLTQGALLNDKHSNGTVKDRSP
YRALMSCPLGEAPSPYNSKFESVAWSASACHDGMGWLTIGISGPDNGAVAVLKYNGIITETIKSWKKRILRTQESEC
VCVNGSCFTIMTDGPSNGAASYKIFKIEKGKVTKSIELNAPNFHYEECSCYPDTGTVMCVCRDNWHGSNRPWVSFNQ
NLDYQIGYICSGVFGDNPRPKDGEGSCNPVTVDGADGVKGFSYKYGNGVWIGRTKSNRLRKGFEMIWDPNGWTDTDS
DFSVKQDVVAITDWSGYSGSFVQHPELTGLDCIRPCFWVELVRGLPRENTTIWTSGSSISFCGVNSDTANWSWPDGA
ELPFTIDK

SEQ ID NO: 59 (PA, A/Wisconsin/67/2005)

MEDFVRQCFNPMIVELAEKAMKEYGEDLKIETNKFAAICTHLEVCFMYSDFHFINEQGESIVVELDDPNALLKHRFE
IIEGRDRTMAWTVVNSICNTTGAGKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKANKIKSENTHIHIFSFTGEE
MATKADYTLDEESRARIKTRLFTIRQEMANRGLWDSFRQSERGEETIEEKFEITGTMRRLADQSLPPNFSCLENFRA
YVDGFEPNGCIEGKLSQMSKEVNAQIEPFLKTTPRPIKLPNGPPCYQRSKFLLMDALKLSIEDPSHEGEGIPLYDAI
KCMKTFFGWKEPYIVKPHEKGINSNYLLSWKQVLSELQDIENEEKIPRTKNMKKTSQLKWALGENMAPEKVDFENCR
DISDLKQYDSDEPELRSLSSWIQNEFNKACELTDSVWIELDEIGEDVAPIEHIASMRRNYFTAEVSHCRATEYIMKG
VYINTALLNASCAAMDDFQLIPMISKCRTKEGRRKTNLYGFIIKGRSHLRNDTDVVNFVSMEFSLTDPRLEPHKWEK
YCVLEIGDMLLRSAIGQISRPMFLYVRTNGTSKVKMKWGMEMRRCLLQSLQQIESMIEAESSVKEKDMTKEFFENKS
EAWPIGESPKGVEEGSIGKVCRTLLAKSVFNSLYASPQLEGFSAESRKLLLVVQALRDNLEPGTFDLGGLYEAIEEC
LINDPWVLLNASWFNSFLTHALK

SEQ ID NO: 60 (PB1, A/Wisconsin/67/2005)

MDVNPTLLFLKVPAQNAISTTFPYTGDPPYSHGTGTGYTMDTVNRTHQYSEKGKWTTNTETGAPQLNPIDGPLPEDN
EPSGYAQTDCVLEAMAFLEESHPGIFENSCLETMEAVQQTRVDRLTQGRQTYDWTLNRNQPAATALANTIEVFRSNG
LTANESGRLIDFLKDVMESMDKEEMEITTHFQRKRRVRDNMTKKMVTQRTIGKKKQRVNKRGYLIRALTLNTMTKDA
ERGKLKRRAIATPGMQIRGFVYFVETLARSICEKLEQSGLPVGGNEKKAKLANVVRKMMTNSQDTELSFTITGDNTK
WNENQNPRMFLAMITYITKNQPEWFRNILSIAPIMFSNKMARLGKGYMFESKRMKLRTQIPAEMLASIDLKYFNEST
RKKIEKIRPLLIDGTASLSPGMMMGMFNMLSTVLGVSILNLGQKKYTKTTYWWDGLQSSDDFALIVNAPNHEGIQAG
VNRFYRTCKLVGINMSKKKSYINKTGTFEFTSFFYRYGFVANFSMELPSFGVSGINESADMSIGVTVIKNNMINNDL
GPATAQMALQLFIKDYRYTYRCHRGDTQIQTRRSFELKKLWDQTQSRAGLLVSDGGPNLYNIRNLHIPEVCLKWELM
DENYRGRLCNPLNPFVSHKEIESVNNAVVMPAHGPAKSMEYDAVATTHSWIPKRNRSILNTSQRGILEDEQMYQKCC
NLFEKFFPSSSYRRPIGISSMVEAMVSRARIDARIDFESGRIKKEEFSEIMKICSTIEELRRQR

SEQ ID NO: 61 (PB2, A/Wisconsin/67/2005)

MERIKELRNLMSQSRTREILTKTTVDHMAIIKKYTSGRQEKNPSLRMKWMMAMKYPITADKRITEMVPERNEQGQTL
WSKMSDAGSDRVMVSPLAVTWWNRNGPVTSTVHYPKVYKTYFDKVERLKHGTFGPVHFRNQVKIRRRVDINPGHADL
SAKEAQDVIMEVVFPNEVGARILTSESQLTITKEKKEELRDCKISPLMVAYMLERELVRKTRFLPVAGGTSSIYIEV
LHLTQGTCWEQMYTPGGEVRNDDVDQSLIIAARNIVRRAAVSADPLASLLEMCHSTQIGGTRMVDILRQNPTEEQAV
DICKAAMGLRISSSFSFGGFTFKRTSGSSVKKEEEVLTGNLQTLKIRVHEGYEEFTMVGKRATAILRKATRRLVQLI
VSGRDEQSIAEAIIVAMVFSQEDCMIKAVRGDLNFVNRANQRLNPMHQLLRHFQKDAKVLFQNWGIEHIDSVMGMVG
VLPDMTPSTEMSMRGIRVSKMGVDEYSSTERVVVSIDRFLRVRDQRGNVLLSPEEVSETQGTERLTITYSSSMMWEI
NGPESVLVNTYQWIIRNWEAVKIQWSQNPAMLYNKMEFEPFQSLVPKAIRSQYSGFVRTLFQQMRDVLGTFDTTQII
KLLPFAAAPPKQSRMQFSSLTVNVRGSGMRILVRGNSPVFNYNKTTKRLTILGKDAGTLIEDPDESTSGVESAVLRG
FLIIGKEDRRYGPALSINELSNLAKGEKANVLIGQGDVVLVMKRKRDSSILTDSQTATKRIRMAIN

SEQ ID NO: 62 (NP, A/Wisconsin/67/2005)

MASQGTKRSYEQMETDGDRQNATEIRASVGKMIDGIGRFYIQMCTELKLSDYEGRLIQNSLTIEKMVLSAFDERRNK
YLEEHPSAGKDPKKTGGPIYRRVDGKWMRELVLYDKEEIRRIWRQANNGEDATAGLTHIMIWHSNLNDATYQRTRAL
VRTGMDPRMCSLMQGSTLPRRSGAAGAAVKGIGTMVMELIRMVKRGINDRNFWRGENGRKTRSAYERMCNILKGKFQ
TAAQRAMVDQVRESRNPGNAEIEDLIFLARSALILRGSVAHKSCLPACVYGPAVSSGYNFEKEGYSLVGIDPFKLLQ
NSQVYSLIRPNENPAHKSQLVWMACHSAAFEDLRLLSFIRGTKVSPRGKLSTRGVQIASNENMDNMGSGTLELRSGY
WAIRTRSGGNTNQQRASAGQTSVQPTFSVQRNLPFEKSTIMAAFTGNTEGRTSDMRAEIIRMMEGAKPEEVSFRGRG
VFELSDEKATNPIVPSFDMSNEGSYFFGDNAEEYDN

SEQ ID NO: 63 (M1, A/Wisconsin/67/2005)

MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEALMEWLKTRPILSPLTKGILGFVFTLTVPSERGLQRR
RFVQNALNGNGDPNNMDKAVKLYRKLKREITFHGAKEIALSYSAGALASCMGLIYNRMGAVTTEVAFGLVCATCEQI
ADSQHRSHRQMVATTNPLIRHENRMVLASTTAKAMEQMAGSSEQAAEAMEIASQARQMVQAMRAIGTHPSSSTGLRD
DLLENLQTYQKRMGVQMQRFK

SEQ ID NO: 64 (M2, A/Wisconsin/67/2005)

MSLLTEVETPIRNEWGCRCNDSSDPLVVAANIIGILHLILWILDRLFFKCVYRLFKHGLKRGPSTEGVPESMREEYR
KEQQNAVDADDSHFVSIELE

SEQ ID NO: 65 (NS, A/Wisconsin/67/2005)

AATGGATTCCAACACTGTGTCAAGTTTCCAGGTAGATTGCTTTCTTTGGCATATCCGGAAACAAGTTGTAGACCAAG
AACTGAGTGATGCCCCATTCCTTGATCGGCTTCGCCGAGATCAGAGGTCCCTAAGGGGAAGAGGCAATACTCTCGGT
CTAGACATCAAAGCAGCCACCCATGTTGGAAAGCAAATTGTAGAAAAGATTCTGAAAGAAGAATCTGATGAGGCACT
TAAAATGACCATGGTCTCCACACCTGCTTCGCGATACATAACTGACATGACTATTGAGGAATTGTCAAGAAACTGGT
TCATGCTAATGCCCAAGCAGAAAGTGGAAGGACCTCTTTGCATCAGAATGGACCAGGCAATCATGGAGAAAAACATC
ATGTTGAAAGCGAATTTCAGTGTGATTTCTGACCGACTAGAGACCATAGTATTACTAAGGGCTTTCACCGAAGAGGG
AGCAATTGTTGGCGAAATCTCACCATTGCCTTCTTTTCCAGGACATACTATTGAGGATGTCAAAAATGCAATTGGGG
TCCTCATCGGAGGACTTGAATGGAATGATAACACAGTTCGAGTCTCTAAAAATCTACAGAGATTCGCTTGGAGAAGC
AGTAATGAGAATGGGGGACCTCCACTTACTCCAAAACAGAAACGGAAAATGGCGAGAACAGCTAGGTCAAAAGTTTG
AAGAGATAAGATGGCTGATTGAAGAAGTGAGACACAGACTAAAAACAACTGAAAATAGCTTTGAACAAATAACATTC
ATGCAAGCATTACAACTGCTGTTTGAAGTGGAACAGGAGATAAGAACTTTCTCATTTCAGCTTATTTAATGATAAA

SEQ ID NO: 66 (HA, A/Wisconsin/67/2005)

MKTIIALSYILCLVFAQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVQSSSTGGICDSPHQILDG
ENCTLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASSGTLEFNDESFNWTGVTQNGTSSS
CKRRSNNSFFSRLNWLTHLKFKYPALNVTMPNNEKFDKLYIWGVHHPVTDNDQIFLYAQASGRITVSTKRSQQTVIP
NIGSRPRIRNIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCNSECITPNGSIPNDK
PFQNVNRITYGACPRYVKQNTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSEGIGQAADLKS
TQAAINQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNK
LFERTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISFAIS
CFLLCVALLGFIMWACQKGNIRCNICI

SEQ ID NO: 67 (NA, A/Wisconsin/67/2005)

MNPNQKIITIGSVSLTISTICFFMQIAILITTVTLHFKQYEFNSPPNNQVMLCEPTIIERNITEIVYLTNTTIEKEI
CPKLAEYRNWSKPQCNITGFAPFSKDNSIRLSAGGDIWVTREPYVSCDPDKCYQFALGQGTTLNNVHSNDTVHDRTP
YRTLLMNELGVPFHLGTKQVCIAWSSSSCHDGKAWLHVCVTGDDKNATASFIYNGRLVDSIVSWSKEILRTQESECV
CINGTCTVVMTDGSASGKADTKILFIEEGKIVHTSTLSGSAQHVEECSCYPRYLGVRCVCRDNWKGSNRPIVDINIK
DYSIVSSYVCSGLVGDTPRKNDSSSSSHCLDPNNEEGGHGVKGWAFDDGNDVWMGRTISEKLRSGYETFKVIEGWSN
PNSKLQINRQVIVDRGNRSGYSGIFSVEGKSCINRCFYVELIRGRKEETEVLWTSNSIVVFCGTSGTYGTGSWPDGA
DINLMPI

SEQ ID NO: 68 (PA, 105p30)

MEDFVRQCFNPMIVELAEKAMKEYGEDPKIETNKFAAICTHLEVCFMYSDFHFIDERGESIIVESGDPNALLKHRFE
IIEGRDRIMAWTVINSICNTTGVEKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKANKIKSEKTHIHIFSFTGEE
MATKADYTLDEESRARIKTRLFTIRQEMASKSLWDSFRQSERGEETIEEKFEITGTMRKLADQSLPPNFPSLENFRA
YVDGFEPNGCIEGKLSQMSKEVNAKIEPFLRTTPRPLRLPDGPLCHQRSKFLLMDALKLSIEDPSHEGEGIPLYDAI
KCMKTFFGWKEPNIVKPHEKGINPNYLMAWKQVLAELQDIENEEKIPRTKNMKRTSQLKWALGENMAPEKVDFDDCK
DVGDLKQYDSDEPEPRSLASWVQNEFNKACELTDSSWIELDEIGEDVAPIEHIASMRRNYFTAEVSHCRATEYIMKG

VYINTALLNASCAAMDDFQLIPMISKCRTKEGRRKTNLYGFIIKGRSHLRNDTDVVNFVSMEFSLTDPRLEPHKWEK
YCVLEIGDMLLRTAIGQVSRPMFLYVRTNGTSKIKMKWGMEMRRCLLQSLQQIESMIEAESSVKEKDMTKEFFENKS
ETWPIGESPRGVEEGSIGKVCRTLLAKSVFNSLYASPQLEGFSAESRKLLLIVQALRDNLEPGTFDLGGLYEAIEEC
LINDPWVLLNASWFNSFLTHALK

SEQ ID NO: 69 (M1, 105p30)

MSLLTEVETYVLSIVPSGPLKAEIAQRLENVFAGKNTDLEALMEWLKTRPILSPLTKGILGFVFTLTVPSERGLQRR
RFVQNALNGNGDPNNMDKAVKLYRKLKREITFHGAKEIALSYSAGALASCMGLIYNRMGAVTTESAFGLICATCEQI
ADSQHKSHRQMVTTTNPLIRHENRMVLASTTAKAMEQMAGSSEQAAEAMEVASQARQMVQAMRAIGTHPSSSTGLKN
DLLENLQAYQKRMGVQMQRFK

SEQ ID NO: 70 (HA, A/California/04/09)

MKAILVVLLYTFATANADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNGKLCKLRGVAPLHLGKCNIAGW
ILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAACP
HAGAKSFYKNLIWLVKKGNSYPKLSKSYINDKGKEVLVLWGIHHPSTSADQQSLYQNADTYVFVGSSRYSKKFKPEI
AIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFAMERNAGSGIIISDTPVHDCNTTCQTPKGAINTSLP
FQNIHPITIGKCPKYVKSTKLRLATGLRNIPSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSGYAADLKST
QNAIDEITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLLENERTLDYHDSNVKNL
YEKVRSQLKNNAKEIGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREEIDGVKLESTRIYQILAIYSTVAS
SLVLVVSLGAISFWMCSNGSLQCRICI

SEQ ID NO: 71 (PA, B/Brisbane/60/08)

AGCAGAAGCGGTGCGTTTGATTTGTCATAATGGATACTTTTATTACAAGAAACTTCCAGACTACAATAATACAAAAG
GCCAAAAACACAATGGCAGAATTTAGTGAAGATCCTGAATTGCAACCAGCAATGCTATTCAATATCTGCGTCCATCT
AGAGGTTTGCTATGTAATAAGTGACATGAATTTTCTTGACGAAGAAGGAAAAGCATATACAGCATTAGAAGGACAAG
GGAAAGAACAAAACTTGAGACCACAATATGAAGTAATTGAGGGAATGCCAAGAACCATAGCATGGATGGTCCAGAGA
TCCTTAGCTCAAGAGCATGGAATAGAGACTCCCAAGTATCTGGCTGATTTGTTTGATTATAAAACCAAAAGATTTAT
AGAAGTTGGAATAACAAAGGGATTGGCTGATGATTACTTTTGGAAAAAGAAAGAAAAGTTGGGAAATAGCATGGAAC
TGATGATATTCAGCTACAATCAAGACTACTCGTTAAGTAATGAATCCTCATTGGATGAGGAAGGGAAAGGGAGAGTG
CTAAGCAGACTCACAGAACTTCAGGCTGAATTAAGTCTGAAAAATTTATGGCAAGTTCTCATAGGAGAAGAAGATGT
TGAAAAGGGAATTGATTTTAAACTTGGACAAACAATATCTAGACTAAGGGATATATCTGTTCCAGCTGGTTTCTCCA
ATTTTGAAGGAATGAGGAGCTACATAGACAATATAGACCCAAAAGGAGCAATAGAGAGAAATCTAGCAAGGATGTCT
CCCTTAGTATCAGTCACACCTAAAAAGTTAACATGGGAGGACCTAAGACCAATAGGGCCTCACATTTACGACCATGA
GCTACCAGAAGTTCCATATAATGCCTTTCTTCTAATGTCTGATGAACTGGGATTGGCCAATATGACTGAGGGAAAGT
CCAAAAAACCGAAGACATTAGCCAAAGAATGTCTAGAAAAGTACTCAACACTACGGGATCAAACTGACCCAATATTA
ATAATGAAAAGCGAAAAAGCTAACGAAAATTTCCTATGGAAGCTTTGGAGAGACTGTGTAAATACAATAAGTAATGA
GGAAACGAGTAACGAGTTACAGAAAACCAATTATGCCAAATGGGCCACAGGGGATGGATTAACATACCAGAAAATAA
TGAAAGAAGTAGCAATAGATGACGAAACAATGTGCCAAGAAGAGCCTAAAATCCCTAACAAATGTAGAGTGGCTGCT
TGGGTTCAAACAGAGATGAATCTATTGAGCACTCTGACAAGTAAAAGAGCTCTGGACCTACCAGAAATAGGGCCAGA
CATAGCACCCGTGGAGCATGTAGGAAGTGAAAGAAGGAAATACTTTGTTAATGAAATCAACTACTGTAAGGCCTCTA
CAGTTATGATGAAGTATGTGCTTTTTCACACTTCATTGTTGAATGAAAGCAATGCCAGCATGGGAAAATACAAAGTA
ATACCAATAACCAATAGAGTAGTAAATGAAAAAGGAGAAAGTTTCGACATGCTTTACGGTCTGGCGGTTAAAGGACA
ATCTCATCTGAGGGGAGATACTGATGTTGTAACAGTTGTAACTTTCGAATTTAGTAGTACAGATCCAAGAGTGGACT
CAGGAAAGTGGCCAAAATATACTGTGTTTAGGATTGGCTCCCTATTTGTGAGTGGGAGGGAAAAATCTGTGTACTTG
TATTGCCGAGTGAATGGCACAAATAAGATCCAAATGAAATGGGGAATGGAAGCTAGAAGATGTTTGCTTCAATCAAT
GCAACAAATGGAGGCAATTGTTGAACAGGAATCATCAATACAAGGATATGACATGACCAAAGCCTGTTTCAAGGGAG
ACAGAGTAAATAGCCCCAAAACTTTCAGTATTGGAACTCAAGAAGGAAAACTAGTAAAAGGATCCTTTGGAAAAGCA
CTAAGAGTAATATTTACTAAATGCTTGATGCACTATGTATTTGGAAATGCCCAATTGGAGGGGTTTAGTGCCGAGTC
TAGGAGACTTCTACTGTTGATTCAAGCATTAAAGGACAGAAAGGGCCCTTGGGTGTTCGACTTAGAGGGAATGTATT
CTGGAATAGAAGAATGTATTAGCAACAACCCTTGGGTAATACAGAGTGTATACTGGTTCAATGAATGGTTGGGCTTT
GAAAAGGAGGGGAATAAAGTGTTGGAATCAGTGGATGAAATAATGGATGAATAAAAGGAAATGGTACTCAATTTGGT
ACTATTTTGTTCATTATGTATCTAAACATCCAATAAAAAGAACCAAGAATCAAAAATGCACGTGTTTCTACT

SEQ ID NO: 72 (PB1, B/Brisbane/60/08)

AGCAGAAGCGGAGCCTTTAAGATGAATATAAATCCTTATTTTCTCTTCATAGATGTGCCCGTACAGGCAGCAATTTC
AACAACATTCCCATACACTGGTGTTCCCCCTTATTCCCATGGAACAGGAACAGGCTACACAATAGACACCGTGATCA
GAACGCATGAGTACTCAAACAAGGGGAAACAGTACATTTCTGATGTTACAGGATGCACAATGGTAGATCCAACAAAT
GGACCATTACCCGAAGATAATGAGCCGAGTGCCTATGCGCAATTAGATTGCGTTTTAGAGGCTTTGGATAGAATGGA
TGAAGAACACCCAGGTCTTTTTCAAGCAGCCTCACAGAATGCTATGGAGGCCCTAATGGTCACAACTGTAGACAAAT
TAACCCAGGGGAGACAGACTTTTGATTGGACAGTATGCAGAAACCAACCTGCTGCAACGGCACTGAACACAACAATA

ACCTCTTTTAGGTTGAATGATTTAAATGGAGCCGACAAAGGTGGATTAATACCTTTTTGCCAGGATATCATTGATTC
ATTAGACCGACCTGAAATGACTTTCTTCTCAGTAAAGAATATAAAGAAAAAATTGCCTGCCAAAAACAGAAAGGGTT
TCCTCATAAAGAGGATACCAATGAAGGTAAAAGACAAAATAACCAAAGTGGAATACATCAAAAGAGCATTATCATTA
AACACAATGACAAAAGACGCTGAAAGAGGCAAACTGAAAAGAAGAGCGATTGCCACTGCTGGAATACAAATCAGAGG
GTTTGTATTAGTAGTTGAAAACTTGGCTAAAAATATATGTGAAAATCTAGAACAAAGTGGTTTACCAGTAGGTGGAA
ACGAGAAGAAAGCCAAACTGTCAAACGCAGTGGCCAAAATGCTCAGTAACTGCCCACCAGGAGGGATTAGCATGACA
GTAACAGGAGACAATACAAAATGGAATGAATGTTTAAACCCAAGAATCTTTTTGGCTATGACTGAAAGAATAACCAG
AGACAGCCCAGTTTGGTTCAGGGATTTTTGTAGTATAGCACCGGTCCTGTTCTCCAATAAGATAGCAAGATTGGGGA
AAGGGTTTATGATAACAAGCAAAACAAAAAGACTGAAGGCTCAAATACCTTGTCCTGATCTGTTTAGTATACCGTTA
GAAAGATATAATGAAGAAACAAGGGCAAAATTGAAAAAGCTAAAACCATTCTTCAATGAAGAAGGAACTGCATCTTT
GTCGCCTGGGATGATGATGGGAATGTTTAATATGCTATCTACCGTGTTGGGAGTAGCTGCACTAGGTATCAAGAACA
TTGGAAACAAAGAATACTTATGGGATGGACTGCAATCTTCTGATGATTTTGCTCTGTTTGTTAATGCAAAGGATGAA
GAAACATGTATGGAAGGAATAAACGACTTTTACCGAACATGTAAATTATTGGGAGTAAACATGAGCAAAAAGAAAAG
TTACTGTAATGAGACTGGAATGTTTGAATTTACAAGCATGTTCTACAGAGATGGATTTGTATCTAATTTTGCAATGG
AACTCCCTTCGTTTGGGGTTGCTGGAGTAAATGAATCAGCAGATATGGCAATAGGAATGACAATAATAAAGAACAAC
ATGATCAACAATGGAATGGGTCCGGCAACAGCACAAACAGCCATACAGTTATTCATAGCTGATTATAGATACACCTA
CAAATGCCACAGGGGAGATTCCAAAGTAGAAGGAAAGAGAATGAAAATCATAAAGGAGTTATGGGAAAACACTAAAG
GAAGAGATGGTCTATTAGTAGCAGATGGTGGGCCCAACATTTACAATTTGAGAAACCTGCATATCCCAGAAATAGTA
TTAAAGTATAATCTAATGGACCCTGAATACAAAGGGCGGTTACTTCATCCTCAAAATCCCTTTGTGGGACATTTGTC
TATTGAGGGCATCAAAGAGGCAGACATAACCCCAGCACATGGTCCAGTAAAGAAAATGGACTACGATGCGGTGTCTG
GAACTCATAGTTGGAGAACCAAAAGAAACAGATCTATACTAAACACTGATCAGAGGAACATGATTCTTGAGGAACAA
TGCTACGCTAAATGTTGCAACCTATTTGAGGCCTGTTTTAACAGTGCATCATACAGGAAGCCAGTGGGTCAACATAG
CATGCTTGAGGCTATGGCCCACAGATTAAGAATGGATGCACGATTAGATTATGAATCAGGGAGAATGTCAAAGGATG
ATTTTGAGAAAGCAATGGCTCACCTTGGTGAGATTGGGTACATATAAGCTTCGAAGATGTTTATGGGGTTATTGGTC
ATCATTGAATACATGCGATACACAAATGATTAAAATGAAAAAAGGCTCGTGTTTCTACT

SEQ ID NO: 73 (PB2, B/Brisbane/60/08)

AGCAGAAGCGGAGCGTTTTCAAGATGACATTGGCCAAAATTGAATTGTTAAAACAACTGCTAAGGGACAATGAAGCC
AAAACAGTTTTGAAGCAAACAACGGTAGACCAATATAACATAATAAGAAAATTCAATACATCAAGGATTGAAAAGAA
TCCTTCACTAAGGATGAAGTGGGCCATGTGTTCTAATTTTCCCTTGGCTCTAACCAAGGGCGATATGGCAAACAGAA
TCCCCTTGGAATACAAAGGGATACAACTTAAAACAAATGCTGAAGACATAGGAACCAAAGGCCAAATGTGCTCAATA
GCAGCAGTTACTTGGTGGAATACATATGGACCAATAGGAGATACTGAAGGTTTCGAAAGGGTCTACGAAAGCTTTTT
TCTCAGAAAAATGAGACTTGACAACGCCACTTGGGGCCGAATAACTTTTGGCCCAGTTGAAAGAGTGAGAAAAAGGG
TACTGCTAAACCCTCTCACCAAGGAAATGCCTCCGGATGAGGCGAGCAATGTGATAATGGAAATATTGTTCCCTAAA
GAAGCAGGAATACCAAGAGAATCCACTTGGATACATAGGGAACTGATAAAAGAAAAAAGAGAAAAATTGAAAGGAAC
AATGATAACTCCAATCGTACTGGCATACATGCTTGAAAGAGAACTGGTTGCTCGAAGAAGATTCTTGCCAGTGGCAG
GAGCAACATCAGCTGAGTTCATAGAAATGCTACACTGCTTACAAGGTGAAAATTGGAGACAAATATATCACCCAGGA
GGGAATAAATTAACTGAGTCCAGGTCTCAATCAATGATAGTAGCTTGTAGAAAAATAATCAGAAGATCAATAGTCGC
TTCAAACCCACTGGAGCTAGCTGTAGAAATTGCAAACAAGACTGTGATAGATACTGAACCTTTAAAGTCATGTCTGG
CAGCCATAGACGGAGGTGATGTAGCTTGTGACATAATAAGAGCTGCATTAGGACTAAAGATCAGACAAAGACAAAGA
TTTGGACGGCTTGAGCTAAAAAGAATATCAGGAAGAGGATTCAAAAATGATGAAGAAATATTAATAGGGAACGGAAC
AATACAGAAGATTGGAATATGGGACGGGGAAGAGGAGTTCCATGTAAGATGTGGTGAATGCAGGGGAATATTAAAAA
AGAGTAAAATGAAACTGGAAAAACTACTGATAAATTCAGCCAAAAAGGAGGATATGAGAGATTTAATAATCTTATGC
ATGGTATTTTCTCAAGACACTAGGATGTTCCAAGGAGTGAGAGGAGAAATAAATTTTCTTAATCGAGCAGGCCAACT
TTTATCTCCAATGTACCAACTCCAACGATATTTTTTGAATAGAAGCAACGACCTTTTTGATCAATGGGGGTATGAGG
AATCACCCAAAGCAAGTGAACTACATGGGATAAATGAATCAATGAATGCATCTGACTATACATTGAAAGGGATTGTA
GTGACAAGAAATGTAATTGACGACTTTAGCTCTATTGAAACAGAAAAAGTATCCATAACAAAAAATCTTAGTTTAAT
AAAAAGGACTGGGGAAGTCATAATGGGAGCTAATGACGTGAGTGAATTAGAATCACAAGCACAGCTGATGATAACAT
ATGATACACCTAAAATGTGGGAAATGGGAACAACCAAAGAACTGGTGCAAAACACTTATCAATGGGTGCTAAAAAAC
TTGGTGACACTGAAGGCTCAGTTTCTTCTAGGAAAAGAGGACATGTTCCAATGGGATGCATTTGAAGCATTTGAGAG
CATAATTCCTCAGAAGATGGCTGGTCAGTACAGTGGATTTGCAAGAGCAGTGCTCAAACAAATGAGAGACCAGGAGG
TTATGAAAACTGACCAGTTCATAAAGTTGTTGCCTTTTTGTTTCTCACCACCAAAATTAAGGAGCAATGGGGAGCCT
TATCAATTCTTAAAACTTGTGTTGAAAGGAGGAGGGGAAAATTTCATCGAAGTAAGGAAAGGGTCCCCTCTATTTTC
CTATAATCCACAAACAGAAGTCCTAACTATATGCGGCAGAATGATGTCATTAAAAGGGAAAATTGAAGATGAAGAAA
GGAATAGATCAATGGGTAATGCAGTATTAGCAGGCTTTCTCGTTAGTGGCAAGTATGACCCAGATCTTGGAGATTTC
AAAACTATTGAAGAACTTGAAAAGCTGAAACCGGGGGGAAAAGGCAAACATCTTACTTTATCAAGGAAAACCAGTTAA
AGTAGTTAAAAGGAAAAGGTATAGTGCTTTGTCCAATGACATTTCACAAGGAATTAAGAGACAAAGAATGACAGTTG
AGTCTATGGGGTGGGCCTTGAGCTAATATAAATTTATCCATTAATTCAATGAACGCAATTGAGTGAAAAATGCTCGT
GTTTCTACT

SEQ ID NO: 74 (NP, B/Brisbane/60/08)

AGCAGAAGCACAGCATTTTCTTGTGAACTTCAAGCACCAGTAAAAGAACTGAAAATCAAAATGTCCAACATGGATAT
TGACGGTATAAACACTGGGACAATTGACAAAACACCGGAAGAAATAACTTCTGGAACCAGTGGGACAACCAGACCAA
TCATTAGACCAGCAACCCTTGCCCCACCAAGCAACAAACGAACCCGTAACCCATCCCCGGAAAGAGCAACCACAAGC
AGTGAAGATGATGTCGGAAGGAAAACCCAAAAGAAACAGACCCCGACAGAGATAAAGAAGAGCGTCTACAACATGGT
GGTGAAACTGGGCGAATTCTATAACCAGATGATGGTCAAAGCTGGACTCAATGATGACATGGAGAGAAATCTAATCC
AAAATGCGCATGCCGTGGAAAGAATTCTATTGGCTGCCACTGATGACAAGAAAACCGAGTTCCAGAAGAAAAAGAAT
GCCAGAGATGTCAAAGAAGGGAAAGAAGAAATAGATCACAACAAAACAGGAGGCACCTTTTACAAGATGGTAAGAGA
TGATAAAACCATCTACTTCAGCCCTATAAGAATTACCTTTTTAAAAGAAGAGGTGAAAACAATGTACAAAACCACCA
TGGGGAGTGATGGCTTCAGTGGACTAAATCACATAATGATTGGGCATTCACAGATGAATGATGTCTGTTTCCAAAGA
TCAAAGGCACTAAAAAGAGTTGGACTTGATCCTTCATTAATCAGTACCTTTGCGGGAAGCACAGTCCCCAGAAGATC
AGGTGCGACTGGTGTTGCAATCAAAGGAGGTGGAACCTTAGTGGCTGAAGCCATTCGATTTATAGGAAGAGCAATGG
CAGACAGAGGGCTATTGAGAGACATCAAAGCCAAGACTGCCTATGAAAAGATTCTTCTGAATCTAAAAAACAAATGC
TCTGCGCCCCAACAAAAGGCTCTAGTTGATCAAGTGATCGGAAGCAGAAATCCGGGGATTGCAGACATTGAAGATCT
AACCCTGCTTGCTCGTAGTATGGTCGTTGTTAGGCCCTCTGTGGCAAGCAAAGTGGTGCTTCCCATAAGCATTTACG
CCAAAATACCTCAACTAGGGTTCAATGTTGAAGAGTACTCTATGGTTGGGTACGAAGCCATGGCTCTTTACAATATG
GCAACACCTGTGTCCATATTAAGAATGGGAGATGATGCAAAAGATAAATCGCAATTATTCTTCATGTCTTGCTTCGG
AGCTGCCTATGAAGACCTGAGAGTTTTGTCTGCATTAACAGGCACAGAATTCAAGCCTAGATCAGCATTAAAATGCA
AGGGTTTCCATGTTCCAGCAAAGGAACAGGTAGAAGGAATGGGAGCAGCTCTGATGTCCATCAAGCTCCAGTTTTGG
GCTCCGATGACCAGATCTGGGGGGAACGAAGTAGGTGGAGACGGAGGGTCTGGCCAAATAAGCTGCAGCCCAGTGTT
TGCAGTGGAAAGACCTATTGCTCTAAGCAAGCAAGCTGTAAGAAGAATGCTGTCAATGAATATTGAGGGACGTGATG
CAGATGTCAAAGGAAATCTACTCAAGATGATGAATGACTCAATGGCTAAGAAAACCAGTGGAAATGCTTTCATTGGG
AAGAAAATGTTTCAAATATCAGACAAAAACAAAACCAATCCCATTGAAATTCCAATTAAGCAGACCATCCCCAATTT
CTTCTTTGGGAGGGACACAGCAGAGGATTATGATGACCTCGATTATTAAGGCAACAAAATAGACACTATGACTGTGA
TTGTTTCAATACGTTTGGAATGTGGGTGTTTATTCTTATTAAAATAAATATAAAAAATGCTGTTGTTTCTACT

SEQ ID NO: 75 (M, B/Brisbane/60/08)

AGCAGAAGCACGCACTTTCTTAAAATGTCGCTGTTTGGAGACACAATTGCCTACCTGCTTTCATTGACAGAAGATGG
AGAAGGCAAAGCAGAACTAGCAGAAAAATTACACTGTTGGTTTGGTGGGAAAGAATTTGACCTAGACTCTGCCTTGG
AATGGATAAAAAACAAAAGATGCTTAACTGATATACAAAAAGCACTAATTGGTGCCTCTATATGCTTTTTAAAACCC
AAAGACCAGGAAAGAAAAAGAAGATTCATCACAGAGCCCTTATCAGGAATGGGAACAACAGCAACAAAAAAGAAAGG
CCTGATTCTGGCTGAGAGAAAAATGAGAAGATGTGTGAGCTTTCATGAAGCATTTGAAATAGCAGAAGGCCATGAAA
GCTCAGCGCTACTATACTGTCTCATGGTCATGTACCTGAATCCTGGAAATTATTCAATGCAAGTAAAACTAGGAACG
CTCTGTGCTTTATGCGAGAAACAAGCATCACATTCACACAGGGCTCATAGCAGAGCAGCGAGATCTTCAGTGCCTGG
AGTGAGACGAGAAATGCAGATGGTCTCAGCTATGAACACAGCAAAAACAATGAATGGAATGGGAAAAGGAGAAGACG
TCCAAAAGCTGGCAGAAGAGTTGCAAAGCAACATTGGAGTGCTGAGATCTCTTGGGGCAAGCCAAAAGAATGGGGAA
GGGATTGCAAAGGATGTAATGGAAGTGCTAAAGCAGAGCTCCATGGGAAATTCAGCTCTTGTGAAGAAATATCTATA
ATGCTCGAACCATTTCAGATTCTTACAATTTGTTCTTTTATCTTATCAGCTCTCCATTTCATGGCTTGGACAATAGG
GCATTTGAATCAAATAAAAAGAGGAATAAACATGAAAATACGAATAAAAGGTCCAAACAAAGAGACAATAAACAGAG
AGGTATCAATTTTGAGACACAGTTACCAAAAAGAAATCCAGGCCAAAGAAACAATGAAGGAAGTACTCTCTGACAAC
ATGGAGGTATTGAATGACCACATAATAATTGAGGGGCTTTCTGCCGAAGAGATAATAAAAATGGGTGAAACAGTTTT
GGAGATAGAAGAATTGCATTAAATTCAATTTTACTGTATTTCTTACTATGCATTTAAGCAAATTGTAATCAATGTCA
GCAAATAAACTGGAAAAAGTGCGTTGTTTCTACT

SEQ ID NO: 76 (NS, B/Brisbane/60/08)

AGCAGAAGCAGAGGATTTGTTTAGTCACTGGCAAACAGGGAAAAATGGCGAACAACAACATGACCACAACACAAATT
GAGGTGGGTCCGGGAGCAACCAATGCCACCATAAACTTTGAAGCAGGAATTCTAGAGTGCTATGAAAGGCTTTCATG
GCAAAGAGCCCTTGACTACCCTGGTCAAGACCGCCTAAACAGACTAAAGAGAAAATTAGAGTCAAGAATAAAGACTC
ACAACAAAAGTGAGCCTGAAAGTAAAAGGATGTCCCTTGAAGAGAGAAAAGCAATTGGAGTAAAAATGATGAAAGTA
CTCCTATTTATGAATCCGTCTGCTGGAATTGAAGGGTTTGAGCCATACTGTATGAAAAGTTCCTCAAATAGCAACTG
TACGAAATACAATTGGACTGATTACCCTTCAACACCAGAGAGGTGCCTTGATGACATAGAGGAAGAACCAGAGGATG
TTGATGGCCCAACTGAAATAGTATTAAGGGACATGAACAACAAAGATGCAAGGCAAAAGATAAAGGAGGAAGTAAAC
ACTCAGAAAGAAGGGAAGTTCCGTTTGACAATAAAAAGGGATATGCGTAATGTATTGTCCTTGAGAGTGTTGGTAAA
CGGAACATTCCTCAAACACCCCAATGGACACAAGTCCTTATCAACTCTGCATAGATTGAATGCATATGACCAGAGTG
GAAGGCTTGTTGCTAAACTTGTTGCCACTGATGATCTTACAGTGGAGGATGAAGAAGATGGCCATCGGATCCTCAAC
TCACTCTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAATTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCA
ATTTGGTCAAGAGCACCGATTATCACCAGAAGAGGGAGACAATTAGACTGGTCACGGAAGAACTTTATCTTTTAAGT
AAAAGAATTGATGATAACATACTATTCCACAAAACAGTAATAGCTAACAGCTCCATAATAGCTGACATGGTTGTATC

ATTATCATTATTAGAAACATTGTATGAAATGAAGGATGTGGTTGAAGTGTACAGCAGGCAGTGCTTGTGAATTTAAA
ATAAAAATCCTCTTGTTACTACT

SEQ ID NO: 77 (PA, B/Panama/45/90)

AGCAGAAGCGGTGCGTTTGATTTGCCATAATGGATACTTTTATTACAAGAAACTTCCAGACTACAATAATACAAAAG
GCCAAAAACACAATGGCAGAATTTAGTGAAGATCCTGAATTACAACCAGCAATGCTATTCAACATCTGCGTCCATCT
AGAGGTTTGCTATGTAATAAGTGACATGAATTTTCTTGACGAAGAAGGAAAATCATATACAGCATTAGAAGGACAAG
GAAAAGAACAAAACTTGAGACCACAATATGAAGTAATTGAGGGAATGCCAAGAACCATAGCATGGATGGTCCAAAGA
TCCTTAGCTCAAGAGCATGGAATAGAGACTCCAAAGTATCTGGCTGATTTGTTTGATTATAAAACCAAGAGATTTAT
AGAAGTTGGAATAACAAAAGGATTGGCTGATGATTACTTTTGGAAAAAGAAAGAAAAGCTGGGAAATAGCATGGAAC
TGATGATATTCAGCTACAATCAAGACTATTCGTTAAGTAATGAATCCTCATTGGATGAGGAAGGGAAAGGGAGAGTG
CTAAGCAGACTCACAGAACTTCAGGCTGAATTAAGTCTGAAAAACCTATGGCAAGTTCTCATAGGAGAAGAAGATGT
TGAAAAGGGAATTGACTTTAAACTTGGACAAACAATATCTAGACTAAGGGATATATCTGTTCCAGCTGGTTTCTCCA
ATTTTGAAGGAATGAGGAGCTACATAGACAATATAGATCCTAAAGGAGCAATAGAAAGAAATCTAGCAAGGATGTCT
CCCTTAGTATCAGCCACACCTAAAAAGTTGAAATGGGAGGACCTAAGACCAATAGGGCCTCACATTTACAACCATGA
GTTACCAGAAGTTCCATATAATGCCTTTCTTCTAATGTCTGATGAATTGGGGCTGGCCAATATGACTGAGGGAAAGT
CCAAAAAACCGAAGACATTAGCCAAAGAATGTCTAGAAAAGTACTCAACACTACGGGATCAAACTGACCCAATATTA
ATAATGAAAAGCGAAAAAGCTAACGAAAATTTCCTATGGAAGCTGTGGAGGGACTGTGTAAATACAATAAGTAATGA
GGAAATGAGTAACGAGTTACAGAAAACCAATTATGCCAAGTGGGCCACAGGAGATGGATTAACATACCAGAAAATAA
TGAAAGAAGTAGCAATAGATGACGAAACAATGTGCCAAGAAGAGCCTAAAATCCCTAACAAATGTAGAGTGGCTGCT
TGGGTTCAAACAGAGATGAATTTATTGAGCACTCTGACAAGTAAAAGAGCTCTGGACCTACCAGAAATAGGGCCAGA
CGTAGCACCCGTGGAGCATGTAGGGAGTGAAAGAAGGAAATACTTTGTTAATGAAATCAACTGCTGTAAGGCCTCTA
CAGTTATGATGAAGTATGTGCTTTTTCACACTTCATTATTGAATGAAAGCAATGCCAGCATGGGAAAATATAAAGTA
ATACCAATAACCAATAGAGTAGTAAATGAAAAAGGAGAAAGTTTCGACATGCTTTATGGTCTGGCGGTTAAAGGACA
ATCTCATCTGAGGGGAGATACTGATGTTGTAACAGTTGTGACTTTCGAATTTAGTGGTACAGATCCCAGAGTGGACT
CAGGAAAGTGGCCAAAATATACTGTGTTTAGGATTGGCTCCCTATTTGTGAGTGGGAGGGAAAAATCTGTGTACCTA
TATTGCCGAGTGAATGGCACAAATAAGATCCAAATGAAATGGGGAATGGAAGCTAGAAGATGTCTGCTTCAATCAAT
GCAACAAATGGAAGCAATTGTTGAACAAGAATCATCGATACAAGGATATGACATGACCAAAGCTTGTTTCAAGGGAG
ACAGAGTAAATAGCCCCAAAACTTTTAGTATTGGGACTCAAGAAGGAAAACTAGTAAAAGGATCCTTTGGGAAAGCA
CTAAGAGTAATATTTACCAAATGTTTGATGCACTATGTATTTGGAAATGCCCAATTGGAGGGGTTTAGTGCCGAGTC
TAGGAGACTTCTACTGTTAATTCAAGCACTAAAGGACAGAAAGGGCCCTTGGGTGTTCGACTTAGAGGGAATGTATT
CTGGAATAGAAGAATGTATTAGTAACAACCCTTGGGTAATACAGAGTGCATACTGGTTCAATGAATGGTTGGGCTTT
GAAAAGGAGGGGAGTAAAGTATTAGAATCAGTAGATGAAATAATGAATGAATGAAAAAACATAGTACTCAATTTGGT
ACTATTTTGTTCATTATGTATCTAAACATCCAATAAAAAGAATCGAGAATCAAAAATGCACGTGTTTCTACT

SEQ ID NO: 78 (PB1, B/Panama/45/90)

AGCAGAAGCGGAGCCTTTAAGATGAATATAAATCCTTATTTTCTCTTCATAGATGTACCCATACAGGCAGCAATTTC
AACAACATTCCCATACACCGGTGTTCCCCCTTACTCCCATGGAACGGGAACAGGCCACACAATAGACACCGTGATCA
GAACACATGAGTACTCGAACAAGGGAAAACAGTATGTTTCTGACATCACAGGATGTACAATGGTAGATCCAACAAAT
GGGCCATTACCCGAAGACAATGAGCCGAGTGCCTATGCACAATTAGATTGCGTTCTGGAGGCTTTGGATAGAATGGA
TGAAGAACATCCAGGTTTGTTTCAAGCAGCCTCACAGAATGCCATGGAGGCACTAATGGTCACAACTGTAGACAAAT
TAACCCAGGGGAGACAGACTTTTGATTGGACAGTATGCAGAAACCAGCCTGCTGCAACGGCACTAAACACAACAATA
ACCTCCTTTAGGTTGAATGATTTGAATGGAGCTGACAAGGGTGGATTGGTACCCTTTTGCCAAGATATCATTGATTC
ATTGGACAAACCTGAAATGACTTTCTTCTCAGTAAAGAATATAAAGAAAAAATTGCCTGCTAAAAACAGAAAGGGTT
TCCTCATAAAGAGAATACCAATGAAAGTAAAAGACAGGATAACCAGAGTGGAATACATCAAAAGAGCATTATCATTA
AACACAATGACAAAAGATGCTGAAAGGGGCAAACTAAAAAGAAGAGCGATTGCAACCGCTGGAATACAAATCAGAGG
GTTTGTATTAGTAGTTGAAAACTTGGCTAAAAATATCTGTGAAAATCTAGAACAAAGTGGTTTGCCCGTAGGTGGAA
ATGAAAAGAAGGCCAAACTGTCAAATGCAGTGGCCAAAATGCTCAGTAACTGCCCACCAGGAGGGATCAGCATGACA
GTAACAGGAGACAATACTAAATGGAATGAATGCTTAAATCCAAGAATCTTTTTGGCTATGACTGAAAGGATAACAAG
AGACAGCCCAATTTGGTTCCGGGATTTTTGTAGTATAGCACCGGTCTTGTTCTCCAATAAAATAGCCAGATTGGGAA
AAGGATTTATGATAACAAGCAAAACAAAAAGACTGAAGGCTCAAATACCTTGTCCAGATCTGTTTAGCATACCATTA
GAAAGATATAATGAAGAAACAAGGGCAAAATTAAAAAAGCTGAAACCATTCTTCAATGAAGAAGGAACGGCATCTTT
GTCGCCTGGGATGATGATGGGAATGTTTAATATGCTATCTACCGTGTTGGGAGTAGCCGCACTAGGTATCAAAAACA
TTGGAAACAAAGAATATTTATGGGATGGACTGCAATCTTCTGATGATTTTGCTCTGTTTGTTAATGCAAAAGATGAA
GAGACATGTATGGAAGGAATAAACGACTTTTACCGAACATGTAAATTATTGGGAATAAACATGAGCAAAAAGAAAAG
TTACTGTAATGAAACTGGAATGTTTGAATTTACAAGCATGTTCTATAGAGATGGATTTGTATCTAATTTTGCAATGG
AAATTCCTTCATTTGGAGTTGCTGGAGTAAATGAATCAGCAGATATGGCAATAGGAATGACAATAATAAAGAACAAT
ATGATCAACAATGGGATGGGTCCAGCAACAGCACAAACAGCCATACAATTATTCATAGCTGATTATAGGTACACCTA
CAAATGCCACAGGGGAGATTCCAAAGTGGAAGGAAAAAGAATGAAAATTATAAAGGAGCTATGGGAAAACACTAAAG
GAAGAGATGGTCTGTTAGTGGCAGATGGTGGGCCCAACATTTACAATTTGAGAAACTTACATATCCCAGAAATAGTA

TTGAAGTACAACCTAATGGACCCTGAATACAAAGGGCGGTTACTTCATCCTCAAAATCCATTTGTAGGACATTTATC
TATTGAGGGCATCAAAGAAGCAGATATAACCCCAGCACATGGTCCCGTAAAGAAAATGGATTATGATGCAGTATCTG
GAACTCATAGTTGGAGAACCAAAAGGAACAGATCTATACTAAATACTGACCAGAGGAACATGATTCTTGAGGAACAA
TGCTACGCTAAGTGTTGCAACCTTTTTGAGGCCTGTTTTAATAGTGCATCATACAGGAAACCAGTAGGTCAGCACAG
CATGCTTGAGGCTATGGCCCACAGATTAAGAGTGGATGCACGACTAGATTATGAATCAGGAAGAATGTCAAAGGATG
ATTTTGAGAAAGCAATGGCTCACCTTGGTGAGATTGGGTACATATAAGCTCCGAAGATGTCTATGGGGTTATTGGTC
ATCATTGAATACATGTGATAAACAAATGATTAAAATGAAAAAAGGCTCGTGTTTCTACT

SEQ ID NO: 79 (PB2, B/Panama/45/90)

AGCAGAAGCGGAGCGTTTTCAAGATGACATTGGCTAAAATTGAATTGTTAAAACAACTGTTAAGGGACAATGAAGCC
AAAACAGTATTGAAACAAACAACGGTAGACCAATATAACATAATAAGAAAATTCAATACATCAAGAATTGAAAAGAA
CCCTTCATTGAGGATGAAGTGGGCAATGTGTTCTAATTTTCCCTTGGCTCTGACCAAGGGTGATATGGCAAACAGAA
TCCCCTTGGAATACAAGGGAATACAACTTAAAACAAATGCTGAAGACATAGGAACTAAAGGCCAAATGTGCTCAATA
GCAGCAGTTACCTGGTGGAATACATATGGACCAATAGGAGATACTGAAGGTTTCGAAAAGGTCTACGAAAGCTTTTT
TCTCAGAAAGATGAGACTTGACAATGCCACTTGGGGCCGAATAACTTTTGGCCCAGTTGAAAGAGTAAGAAAAAGGG
TACTGCTAAACCCTCTCACCAAGGAAATGCCTCCAGATGAAGCAAGTAATGTGATAATGGAAATATTGTTCCCTAAG
GAAGCAGGAATACCAAGAGAATCTACTTGGATACATAGGGAACTGATAAAAGAAAAAAGAGAAAAATTGAAAGGAAC
AATGATAACTCCCATTGTACTGGCATACATGCTTGAGAGAGAATTGGTTGCCAGAAGAAGGTTCCTGCCGGTGGCAG
GAGCAACATCAGCTGAGTTCATAGAAATGCTACACTGCTTACAAGGTGAAAATTGGAGACAAATATATCACCCAGGA
GGAAATAAACTAACTGAATCTAGGTCTCAATCGATGATTGTAGCTTGTAGAAAGATAATCAGAAGATCAATAGTCGC
ATCAAACCCATTAGAGCTAGCTGTAGAAATTGCAAACAAGACTGTGATAGATACTGAACCTTTAAAATCATGTCTGA
CAGCCATAGACGGAGGTGATGTAGCCTGTGACATAATAAGAGCTGCATTAGGACTAAAGATCAGACAAAGACAAAGA
TTTGGACGACTTGAACTAAAGAGAATATCAGGAAGAGGATTCAAAAATGATGAAGAAATATTAATCGGGAACGGAAC
AATACAGAAGATTGGAATATGGGACGGAGAAGAGGAGTTCCATGTAAGATGTGGTGAATGCAGGGGAATATTAAAAA
AGAGCAAAATGAGAATGGAAAAACTACTAATAAATTCAGCTAAAAAGGAAGACATGAAAGATTTAATAATCTTGTGC
ATGGTATTTTCTCAAGACACTAGGATGTTCCAAGGAGTGAGAGGAGAAATAAATTTTCTTAATAGAGCAGGCCAACT
TTTATCTCCAATGTACCAACTCCAAAGATATTTTTTGAATAGAAGCAACGATCTCTTTGATCAATGGGGGTATGAGG
AATCACCCAAAGCAAGTGAGCTACATGGAATAAATGAATTAATGAATGCATCTGACTACACTTTGAAAGGGGTTGTA
GTAACAAAAAATGTAATTGATGATTTTAGTTCTACTGAAACAGAAAAAGTATCTATAACAAAAAATCTTAGTTTAAT
AAAAAGGACTGGGGAAGTCATAATGGGGGCTAATGACGTAAGTGAATTAGAATCACAAGCTCAGCTAATGATAACAT
ATGATACACCTAAGATGTGGGAGATGGGAACAACCAAAGAACTGGTGCAAAACACCTACCAATGGGTGCTGAAAAAT
TTGGTAACACTGAAGGCTCAGTTTCTTCTAGGAAAAGAAGACATGTTCCAATGGGATGCATTTGAAGCATTTGAAAG
CATAATCCCCCAGAAGATGGCTGGCCAGTACAGTGGATTTGCAAGAGCAGTGCTCAAACAAATGAGAGACCAAGAGG
TTATGAAAACTGACCAGTTCATAAAGTTGTTGCCCTTTTGTTTCTCACCACCAAAATTAAGGAGAAATGGGGAGCCT
TATCAGTTCTTGAGGCTTGTATTGAAGGGAGGAGGAGAAAATTTCATCGAAGTAAGGAAAGGGTCCCCTCTATTCTC
TTACAATCCACAAACAGAAGTCCTAACTATATGCGGCAGAATGATGTCATTAAAAGGGAAAATTGAAGATGAAGAAA
GGAATAGATCAATGGGGAATGCAGTATTAGCGGGCTTTCTCGTTAGTGGCAAGTATGACCCAGATCTTGGAGATTTC
AAAACTATTGAAGAACTTGAAAAGCTGAAACCGGGGGGAGAAAGCAAACATCTTACTTTATCAAGGAAAGCCCGTTAA
AGTAGTTAAAAGGAAAAGATATAGTGCTTTATCCAATGACATTTCACAAGGAATTAAGAGACAAAGAATGACAGTTG
AGTCCATGGGGTGGGCCTTGAGCTAATATAAATTTATCCATTAATTCAATAAACACAATTGAGTGAAAAATGCTCGT
GTTTCTACT

SEQ ID NO: 80 (NP, B/Panama/45/90)

AGCAGAAGCACAGCATTTTCTTATTAACTTCAAGTACCAACAAAAGAACTGAAAATCAAAATGTCCAACATGGATAT
TGACGGTATCAACACTGGGACAATTGACAAAACACCGGAAGAAATAACTTCTGGAACCAGTGGGACAACCAGACCAA
TCATCAGACCAGCAACCCTTGCCCCACCAAGCAACAAACGAACCCGGAACCCATCCCCGGAAAGAGCAACCACAAGC
AGTGAAGCTGATGTCGGAAGGAAAACCCAAAAGAAACAGACCCCGACAGAGATAAAGAAGAGCGTCTACAATATGGT
AGTGAAACTGGGTGAATTCTATAACCAGATGATGGTCAAAGCTGGACTCAACGATGACATGGAGAGAAACCTAATCC
AAAATGCGCATGCTGTGGAAAGAATTCTATTGGCTGCCACTGATGACAAGAAAACTGAATTCCAGAGGAAAAAGAAT
GCCAGAGATGTCAAAGAAGGAAAAGAAGAAATAGACCACAACAAAACAGGAGGCACCTTTTACAAGATGGTAAGAGA
TGATAAAACCATCTACTTCAGCCCTATAAGAATTACCTTTTTAAAAGAAGAGGTGAAAACAATGTACAAAACCACCA
TGGGGAGTGATGGCTTCAGTGGACTAAATCACATAATGATTGGGCATTCACAGATGAATGATGTCTGTTTCCAAAGA
TCAAAGGCCCTAAAAAGAGTTGGACTTGACCCTTCATTAATCAGTACCTTTGCAGGAAGCACACTCCCCAGAAGATC
AGGTGCAACTGGTGTTGCAATCAAAGGAGGTGGAACTTTAGTGGCTGAAGCCATTCGATTTATAGGAAGAGCAATGG
CAGACAGAGGGCTATTGAGAGACATCAAAGCCAAGACTGCCTATGAAAAGATTCTTCTGAATCTAAAAAACAAATGC
TCTGCGCCCCAACAAAAGGCTCTAGTTGATCAAGTGATCGGAAGTAGAAATCCAGGGATTGCAGACATTGAAGACCT
AACCCTGCTTGCTCGTAGTATGGTCGTTGTTAGGCCCTCTGTGGCGAGCAAAGTAGTGCTTCCCATAAGCATTTATG
CTAAAATACCTCAACTAGGGTTCAATGTTGAAGAATACTCTATGGTTGGGTATGAAGCCATGGCTCTCTACAATATG
GCAACACCTGTTTCCATATTAAGAATGGGAGATGATGCAAAAGATAAATCGCAATTATTCTTCATGTCTTGCTTCGG
AGCTGCCTATGAAGACCTGAGAGTTTTGTCTGCATTAACAGGCATAGAATTCAAGCCTAGATCAGCATTAAAATGCA

AGGGTTTCCATGTTCCAGCAAAGGAACAGGTGGAAGGAATGGGGGCAGCTCTGATGTCCATCAAGCTCCAGTTTTGG
GCTCCAATGACCAGATCTGGAGGGAACGAAGTAGGTGGAGACGGAGGGTCTGGCCAAATAAGTTGCAGCCCAGTGTT
TGCAGTAGAAAGACCTATTGCTCTAAGCAAGCAAGCTGTAAGAAGAATGCTTTCAATGAATATTGAGGGACGTGATG
CAGATGTCAAAGGAAATCTACTCAAGATGATGAATGACTCAATGGCTAAGAAAACCAATGGAAATGCTTTCATTGGG
AAGAAAATGTTTCAAATATCAGACAAAAACAAAACCAATCCCGTTGAAATTCCAATTAAGCAGACCATCCCCAATTT
CTTCTTTGGGAGGGACACAGCAGAGGATTATGATGACCTCGATTATTAAAGCAACAAAATAGACACTATGACTGTGA
TTGTTTCAATACGTTTGGAATGTGGGTGTTTACTCTTATTGAAATAAATATAAAAAATGCTGTTGTTTCTACT

SEQ ID NO: 81 (M, B/Panama/45/90)

AGCAGAAGCACGCACTTTCTTAAAATGTCGCTGTTTGGAGACACAATTGCCTACCTGCTTTCATTGACAGAAGATGG
AGAAGGCAAAGCAGAACTAGCAGAAAAATTACACTGTTGGTTCGGTGGGAAAGAATTTGACCTAGACTCTGCCTTGG
AATGGATAAAAAACAAAAGATGCTTAACTGATATACAGAAAGCACTAATTGGTGCCTCTATCTGCTTTTTAAAACCA
AAAGACCAAGAAAGAAAAAGAAGATTCATCACAGAGCCCCTATCAGGAATGGGAACAACAGCAACAAAAAAGAAGGG
CCTGATTCTAGCTGAGAGAAAAATGAGAAGATGTGTGAGTTTTCATGAAGCATTTGAAATAGCAGAAGGCCATGAAA
GCTCAGCGCTACTATATTGTCTCATGGTCATGTACCTGAACCCTGGAAATTATTCAATGCAAGTAAAACTAGGAACG
CTCTGTGCTTTGTGCGAGAAACAAGCATCACATTCACACAGGGCTCATAGCAGAGCAGCAAGATCTTCAGTGCCTGG
AGTGAGGCGAGAAATGCAGATGGTCTCAGCTATGAACACAGCAAAAACAATGAATGGAATGGGAAAGGGAGAAGACG
TCCAAAAACTGGCAGAAGAGCTGCAAAGCAACATTGGAGTATTGAGATCTCTTGGGGCAAGTCAAAAGAATGGGGAA
GGAATTGCAAAGGATGTGATGGAAGTGCTAAAGCAGAGCTCTATGGGAAATTCAGCTCTTGTGAAGAAATACCTATA
ATGCTCGAACCATTTCAGATTCTTTCAATTTGTTCTTTCATCTTATCAGCTCTCCATTTCATGGCTTGGACAATAGG
GCATTTGAATCAAATAAAAAGAGGAGTAAACATGAAAATACGAATAAAAAATCCAAATAAAGAGACAATAAACAGAG
AGGTATCAATTTTGAGACACAGTTACCAAAAAGAAATCCAGGCCAAAGAAACAATGAAGGAAGTACTCTCTGACAAC
ATGGAGGTATTGAGTGACCACATAGTAATTGAGGGGCTTTCTGCTGAAGAGATAATAAAAATGGGTGAAACAGTTTT
GGAGGTAGAAGAATTGCATTAAATTCAATTTTTACTGTATTTCTTGCTATGCATTTAAGCAAATTGTAATCAATGTC
AGCAAATAAACTGGAAAAAGTGCGTTGTTTCTACT

SEQ ID NO: 82 (NS, B/Panama/45/90)

AGCAGAAGCAGAGGATTTGTTTAGTCACTGGCAAACGAAAAAATGGCGGACAACATGACCACAACACAAATTGAGGT
GGGTCCGGGAGCAACCAATGCCACCATAAACTTTGAAGCAGGAATTTTGGAGTGCTATGAAAGGCTTTCATGGCAAA
GAGCCCTTGACTACCCTGGTCAAGACCGCCTAAACAAACTAAAGAGAAAATTGGAATCAAGAATAAAGACTCACAAC
AAAAGTGAGCCAGAAAGTAAAAGGATGTCTCTTGAAGAGAGAAAAGCTATTGGGGTAAAAATGATGAAAGTGCTCCT
ATTTATGAACCCATCTGCTGGAGTTGAAGGGTTTGAGCCATATTGTATGAAAAATCCCTCCAATAGCAACTGTCCAG
ACTGCAATTGGGCTGATTACCCTCCAACACCAGGAAAGTACCTTGATGGCATAGAAGAAGAACCGGAGAATGTTGGT
GACTCAACTGAAATAGTATTAAGGGACATGAACAACAAAGATGCAAGGCAAAAGATAAAAGAGGAAGTAAACACTCA
GAAAGAAGGGAAATTCCGTTTGACAATAAAAAGGGATATACGTAATGTGTTGTCCTTGAGAGTGTTGGTAAACGGAA
CATTCATCAAGCACCCTAATGGATACAAGTCCTTATCAACTCTGCATAGATTGAATGCATATGACCAGAGTGGAAGA
CTTGTTGCTAAACTTGTTGCTACTGATGATCTTACAGTGGAGGATGAAGAAGATGGCCATCGGATCCTCAACTCACT
CTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAATTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCAATTTG
GTCAAGAGCACCGATTATCACCAGAAGAGAGAGACAATTAGACTGGTTACGGAAGAACTTTATCTTTTAAGTAAAAG
AATTGATGATAACATATTGTTCCACAAAACAGTAATAGCCAACAGCTCCATAATAGCTGACATGATTGTATCATTAT
CATTATTGGAAACATTGTATGAAATGAAGGATGTGGTTGAAGTGTACAGCAGGCAGTGCTTGTGAATTTAAAATAAA
AATCCTCTTGTTACTACT

SEQ ID NO: 83 (PA, B/Brisbane/60/08)

MDTFITRNFQTTIIQKAKNTMAEFSEDPELQPAMLFNICVHLEVCYVISDMNFLDEEGKAYTALEGQGKEQNLRPQY
EVIEGMPRTIAWMVQRSLAQEHGIETPKYLADLFDYKTKRFIEVGITKGLADDYFWKKKEKLGNSMELMIFSYNQDY
SLSNESSLDEEGKGRVLSRLTELQAELSLKNLWQVLIGEEDVEKGIDFKLGQTISRLRDISVPAGFSNFEGMRSYID
NIDPKGAIERNLARMSPLVSVTPKKLTWEDLRPIGPHIYDHELPEVPYNAFLLMSDELGLANMTEGKSKKPKTLAKE
CLEKYSTLRDQTDPILIMKSEKANENFLWKLWRDCVNTISNEETSNELQKTNYAKWATGDGLTYQKIMKEVAIDDET
MCQEEPKIPNKCRVAAWVQTEMNLLSTLTSKRALDLPEIGPDIAPVEHVGSERRKYFVNEINYCKASTVMMKYVLFH
TSLLNESNASMGKYKVIPITNRVVNEKGESFDMLYGLAVKGQSHLRGDTDVVTVVTFEFSSTDPRVDSGKWPKYTVF
RIGSLFVSGREKSVYLYCRVNGTNKIQMKWGMEARRCLLQSMQQMEAIVEQESSIQGYDMTKACFKGDRVNSPKTFS
IGTQEGKLVKGSFGKALRVIFTKCLMHYVFGNAQLEGFSAESRRLLLLIQALKDRKGPWVFDLEGMYSGIEECISNN
PWVIQSVYWFNEWLGFEKEGNKVLESVDEIMDE

SEQ ID NO: 84 (PB1, B/Brisbane/60/08)

MNINPYFLFIDVPVQAAISTTFPYTGVPPYSHGTGTGYTIDTVIRTHEYSNKGKQYISDVTGCTMVDPTNGPLPEDN
EPSAYAQLDCVLEALDRMDEEHPGLFQAASQNAMEALMVTTVDKLTQGRQTFDWTVCRNQPAATALNTTITSFRLND
LNGADKGGLIPFCQDIIDSLDRPEMTFFSVKNIKKKLPAKNRKGFLIKRIPMKVKDKITKVEYIKRALSLNTMTKDA

ERGKLKRRAIATAGIQIRGFVLVVENLAKNICENLEQSGLPVGGNEKKAKLSNAVAKMLSNCPPGGISMTVTGDNTK
WNECLNPRIFLAMTERITRDSPVWFRDFCSIAPVLFSNKIARLGKGFMITSKTKRLKAQIPCPDLFSIPLERYNEET
RAKLKKLKPFFNEEGTASLSPGMMMGMFNMLSTVLGVAALGIKNIGNKEYLWDGLQSSDDFALFVNAKDEETCMEGI
NDFYRTCKLLGVNMSKKKSYCNETGMFEFTSMFYRDGFVSNFAMELPSFGVAGVNESADMAIGMTIIKNNMINNGMG
PATAQTAIQLFIADYRYTYKCHRGDSKVEGKRMKIIKELWENTKGRDGLLVADGGPNIYNLRNLHIPEIVLKYNLMD
PEYKGRLLHPQNPFVGHLSIEGIKEADITPAHGPVKKMDYDAVSGTHSWRTKRNRSILNTDQRNMILEEQCYAKCCN
LFEACFNSASYRKPVGQHSMLEAMAHRLRMDARLDYESGRMSKDDFEKAMAHLGEIGYI

SEQ ID NO: 85 (PB2, B/Brisbane/60/08)

MTLAKIELLKQLLRDNEAKTVLKQTTVDQYNIIRKFNTSRIEKNPSLRMKWAMCSNFPLALTKGDMANRIPLEYKGI
QLKTNAEDIGTKGQMCSIAAVTWWNTYGPIGDTEGFERVYESFFLRKMRLDNATWGRITFGPVERVRKRVLLNPLTK
EMPPDEASNVIMEILFPKEAGIPRESTWIHRELIKEKREKLKGTMITPIVLAYMLERELVARRRFLPVAGATSAEFI
EMLHCLQGENWRQIYHPGGNKLTESRSQSMIVACRKIIRRSIVASNPLELAVEIANKTVIDTEPLKSCLAAIDGGDV
ACDIIRAALGLKIRQRQRFGRLELKRISGRGFKNDEEILIGNGTIQKIGIWDGEEEFHVRCGECRGILKKSKMKLEK
LLINSAKKEDMRDLIILCMVFSQDTRMFQGVRGEINFLNRAGQLLSPMYQLQRYFLNRSNDLFDQWGYEESPKASEL
HGINESMNASDYTLKGIVVTRNVIDDFSSIETEKVSITKNLSLIKRTGEVIMGANDVSELESQAQLMITYDTPKMWE
MGTTKELVQNTYQWVLKNLVTLKAQFLLGKEDMFQWDAFEAFESIIPQKMAGQYSGFARAVLKQMRDQEVMKTDQFI
KLLPFCFSPPKLRSNGEPYQFLKLVLKGGGENFIEVRKGSPLFSYNPQTEVLTICGRMMSLKGKIEDEERNRSMGNA
VLAGFLVSGKYDPDLGDFKTIEELEKLKPGEKANILLYQGKPVKVVKRKRYSALSNDISQGIKRQRMTVESMGWALS

SEQ ID NO: 86 (NP, B/Brisbane/60/08)

MSNMDIDGINTGTIDKTPEEITSGTSGTTRPIIRPATLAPPSNKRTRNPSPERATTSSEDDVGRKTQKKQTPTEIKK
SVYNMVVKLGEFYNQMMVKAGLNDDMERNLIQNAHAVERILLAATDDKKTEFQKKKNARDVKEGKEEIDHNKTGGTF
YKMVRDDKTIYFSPIRITFLKEEVKTMYKTTMGSDGFSGLNHIMIGHSQMNDVCFQRSKALKRVGLDPSLISTFAGS
TVPRRSGATGVAIKGGGTLVAEAIRFIGRAMADRGLLRDIKAKTAYEKILLNLKNKCSAPQQKALVDQVIGSRNPGI
ADIEDLTLLARSMVVVRPSVASKVVLPISIYAKIPQLGFNVEEYSMVGYEAMALYNMATPVSILRMGDDAKDKSQLF
FMSCFGAAYEDLRVLSALTGTEFKPRSALKCKGFHVPAKEQVEGMGAALMSIKLQFWAPMTRSGGNEVGGDGGSGQI
SCSPVFAVERPIALSKQAVRRMLSMNIEGRDADVKGNLLKMMNDSMAKKTSGNAFIGKKMFQISDKNKTNPIEIPIK
QTIPNFFFGRDTAEDYDDLDY

SEQ ID NO: 87 (M_1, B/Brisbane/60/08)

MSLFGDTIAYLLSLTEDGEGKAELAEKLHCWFGGKEFDLDSALEWIKNKRCLTDIQKALIGASICFLKPKDQERKRR
FITEPLSGMGTTATKKKGLILAERKMRRCVSFHEAFEIAEGHESSALLYCLMVMYLNPGNYSMQVKLGTLCALCEKQ
ASHSHRAHSRAARSSVPGVRREMQMVSAMNTAKTMNGMGKGEDVQKLAEELQSNIGVLRSLGASQKNGEGIAKDVME
VLKQSSMGNSALVKKYL

SEQ ID NO: 88 (M_2, B/Brisbane/60/08)

MLEPFQILTICSFILSALHFMAWTIGHLNQIKRGINMKIRIKGPNKETINREVSILRHSYQKEIQAKETMKEVLSDN
MEVLNDHIIIEGLSAEEIIKMGETVLEIEELH

SEQ ID NO: 89 (NS_1, B/Brisbane/60/08)

MANNNMTTTQIEVGPGATNATINFEAGILECYERLSWQRALDYPGQDRLNRLKRKLESRIKTHNKSEPESKRMSLEE
RKAIGVKMMKVLLFMNPSAGIEGFEPYCMKSSSNSNCTKYNWTDYPSTPERCLDDIEEEPEDVDGPTEIVLRDMNNK
DARQKIKEEVNTQKEGKFRLTIKRDMRNVLSLRVLVNGTFLKHPNGHKSLSTLHRLNAYDQSGRLVAKLVATDDLTV
EDEEDGHRILNSLFERLNEGHSKPIRAAETAVGVLSQFGQEHRLSPEEGDN

SEQ ID NO: 90 (NS2, B/Brisbane/60/08)

MANNNMTTTQIEWRMKKMAIGSSTHSSSVLMKDIQSQFEQLKLRWESYPNLVKSTDYHQKRETIRLVTEELYLLSKR
IDDNILFHKTVIANSSIIADMVVSLSLLETLYEMKDVVEVYSRQCL

SEQ ID NO: 91 (PA, B/Panama/45/90)

MDTFITRNFQTTIIQKAKNTMAEFSEDPELQPAMLFNICVHLEVCYVISDMNFLDEEGKSYTALEGQGKEQNLRPQY
EVIEGMPRTIAWMVQRSLAQEHGIETPKYLADLFDYKTKRFIEVGITKGLADDYFWKKKEKLGNSMELMIFSYNQDY
SLSNESSLDEEGKGRVLSRLTELQAELSLKNLWQVLIGEEDVEKGIDFKLGQTISRLRDISVPAGFSNFEGMRSYID
NIDPKGAIERNLARMSPLVSATPKKLKWEDLRPIGPHIYNHELPEVPYNAFLLMSDELGLANMTEGKSKKPKTLAKE
CLEKYSTLRDQTDPILIMKSEKANENFLWKLWRDCVNTISNEEMSNELQKTNYAKWATGDGLTYQKIMKEVAIDDET
MCQEEPKIPNKCRVAAWVQTEMNLLSTLTSKRALDLPEIGPDVAPVEHVGSERRKYFVNEINCCKASTVMMKYVLFH
TSLLNESNASMGKYKVIPITNRVVNEKGESFDMLYGLAVKGQSHLRGDTDVVTVVTFEFSGTDPRVDSGKWPKYTVF

RIGSLFVSGREKSVYLYCRVNGTNKIQMKWGMEARRCLLQSMQQMEAIVEQESSIQGYDMTKACFKGDRVNSPKTFS
IGTQEGKLVKGSFGKALRVIFTKCLMHYVFGNAQLEGFSAESRRLLLLIQALKDRKGPWVFDLEGMYSGIEECISNN
PWVIQSAYWFNEWLGFEKEGSKVLESVDEIMNE

SEQ ID NO: 92 (PB1, B/Panama/45/90)

MNINPYFLFIDVPIQAAISTTFPYTGVPPYSHGTGTGHTIDTVIRTHEYSNKGKQYVSDITGCTMVDPTNGPLPEDN
EPSAYAQLDCVLEALDRMDEEHPGLFQAASQNAMEALMVTTVDKLTQGRQTFDWTVCRNQPAATALNTTITSFRLND
LNGADKGGLVPFCQDIIDSLDKPEMTFFSVKNIKKKLPAKNRKGFLIKRIPMKVKDRITRVEYIKRALSLNTMTKDA
ERGKLKRRAIATAGIQIRGFVLVVENLAKNICENLEQSGLPVGGNEKKAKLSNAVAKMLSNCPPGGISMTVTGDNTK
WNECLNPRIFLAMTERITRDSPIWFRDFCSIAPVLFSNKIARLGKGFMITSKTKRLKAQIPCPDLFSIPLERYNEET
RAKLKKLKPFFNEEGTASLSPGMMMGMFNMLSTVLGVAALGIKNIGNKEYLWDGLQSSDDFALFVNAKDEETCMEGI
NDFYRTCKLLGINMSKKKSYCNETGMFEFTSMFYRDGFVSNFAMEIPSFGVAGVNESADMAIGMTIIKNNMINNGMG
PATAQTAIQLFIADYRYTYKCHRGDSKVEGKRMKIIKELWENTKGRDGLLVADGGPNIYNLRNLHIPEIVLKYNLMD
PEYKGRLLHPQNPFVGHLSIEGIKEADITPAHGPVKKMDYDAVSGTHSWRTKRNRSILNTDQRNMILEEQCYAKCCN
LFEACFNSASYRKPVGQHSMLEAMAHRLRVDARLDYESGRMSKDDFEKAMAHLGEIGYI

SEQ ID NO: 93 (PB2, B/Panama/45/90)

MTLAKIELLKQLLRDNEAKTVLKQTTVDQYNIIRKFNTSRIEKNPSLRMKWAMCSNFPLALTKGDMANRIPLEYKGI
QLKTNAEDIGTKGQMCSIAAVTWWNTYGPIGDTEGFEKVYESFFLRKMRLDNATWGRITFGPVERVRKRVLLNPLTK
EMPPDEASNVIMEILFPKEAGIPRESTWIHRELIKEKREKLKGTMITPIVLAYMLERELVARRRFLPVAGATSAEFI
EMLHCLQGENWRQIYHPGGNKLTESRSQSMIVACRKIIRRSIVASNPLELAVEIANKTVIDTEPLKSCLTAIDGGDV
ACDIIRAALGLKIRQRQRFGRLELKRISGRGFKNDEEILIGNGTIQKIGIWDGEEEFHVRCGECRGILKKSKMRMEK
LLINSAKKEDMKDLIILCMVFSQDTRMFQGVRGEINFLNRAGQLLSPMYQLQRYFLNRSNDLFDQWGYEESPKASEL
HGINELMNASDYTLKGVVVTKNVIDDFSSTETEKVSITKNLSLIKRTGEVIMGANDVSELESQAQLMITYDTPKMWE
MGTTKELVQNTYQWVLKNLVTLKAQFLLGKEDMFQWDAFEAFESIIPQKMAGQYSGFARAVLKQMRDQEVMKTDQFI
KLLPFCFSPPKLRRNGEPYQFLRLVLKGGGENFIEVRKGSPLFSYNPQTEVLTICGRMMSLKGKIEDEERNRSMGNA
VLAGFLVSGKYDPDLGDFKTIEELEKLKPGEKANILLYQGKPVKVVKRKRYSALSNDISQGIKRQRMTVESMGWALS

SEQ ID NO: 94 (NP, B/Panama/45/90)

MSNMDIDGINTGTIDKTPEEITSGTSGTTRPIIRPATLAPPSNKRTRNPSPERATTSSEADVGRKTQKKQTPTEIKK
SVYNMVVKLGEFYNQMMVKAGLNDDMERNLIQNAHAVERILLAATDDKKTEFQRKKNARDVKEGKEEIDHNKTGGTF
YKMVRDDKTIYFSPIRITFLKEEVKTMYKTTMGSDGFSGLNHIMIGHSQMNDVCFQRSKALKRVGLDPSLISTFAGS
TLPRRSGATGVAIKGGGTLVAEAIRFIGRAMADRGLLRDIKAKTAYEKILLNLKNKCSAPQQKALVDQVIGSRNPGI
ADIEDLTLLARSMVVVRPSVASKVVLPISIYAKIPQLGFNVEEYSMVGYEAMALYNMATPVSILRMGDDAKDKSQLF
FMSCFGAAYEDLRVLSALTGIEFKPRSALKCKGFHVPAKEQVEGMGAALMSIKLQFWAPMTRSGGNEVGGDGGSGQI
SCSPVFAVERPIALSKQAVRRMLSMNIEGRDADVKGNLLKMMNDSMAKKTNGNAFIGKKMFQISDKNKTNPVEIPIK
QTIPNFFFGRDTAEDYDDLDY

SEQ ID NO: 95 (M_1, B/Panama/45/90)

MSLFGDTIAYLLSLTEDGEGKAELAEKLHCWFGGKEFDLDSALEWIKNKRCLTDIQKALIGASICFLKPKDQERKRR
FITEPLSGMGTTATKKKGLILAERKMRRCVSFHEAFEIAEGHESSALLYCLMVMYLNPGNYSMQVKLGTLCALCEKQ
ASHSHRAHSRAARSSVPGVRREMQMVSAMNTAKTMNGMGKGEDVQKLAEELQSNIGVLRSLGASQKNGEGIAKDVME
VLKQSSMGNSALVKKYL

SEQ ID NO: 96 (M_2, B/Panama/45/90)

MLEPFQILSICSFILSALHFMAWTIGHLNQIKRGVNMKIRIKNPNKETINREVSILRHSYQKEIQAKETMKEVLSDN
MEVLSDHIVIEGLSAEEIIKMGETVLEVEELH

SEQ ID NO: 97 (NS_1, B/Panama/45/90)

MADNMTTTQIEVGPGATNATINFEAGILECYERLSWQRALDYPGQDRLNKLKRKLESRIKTHNKSEPESKRMSLEER
KAIGVKMMKVLLFMNPSAGVEGFEPYCMKNPSNSNCPDCNWADYPPTPGKYLDGIEEEPENVGDSTEIVLRDMNNKD
ARQKIKEEVNTQKEGKFRLTIKRDIRNVLSLRVLVNGTFIKHPNGYKSLSTLHRLNAYDQSGRLVAKLVATDDLTVE
DEEDGHRILNSLFERLNEGHSKPIRAAETAVGVLSQFGQEHRLSPEERDN

SEQ ID NO: 98 (NS_2, B/Panama/45/90)

MADNMTTTQIEWRMKKMAIGSSTHSSSVLMKDIQSQFEQLKLRWESYPNLVKSTDYHQKRETIRLVTEELYLLSKRI
DDNILFHKTVIANSSIIADMIVSLSLLETLYEMKDVVEVYSRQCL

SEQ ID NO: 99 (NA, A/California/04/09)

MNPNQKIITIGSVCMTIGMANLILQIGNIISIWISHSIQLGNQNQIETCNQSVITYENNTWVNQTYVNISNTNFAAG
QSVVSVKLAGNSSLCPVSGWAIYSKDNSVRIGSKGDVFVIREPFISCSPLECRTFFLTQGALLNDKHSNGTIKDRSP
YRTLMSCPIGEVPSPYNSRFESVAWSASACHDGINWLTIGISGPDNGAVAVLKYNGIITDTIKSWRNNILRTQESEC
ACVNGSCFTVMTDGPSNGQASYKIFRIEKGKIVKSVEMNAPNYHYEECSCYPDSSEITCVCRDNWHGSNRPWVSFNQ
NLEYQIGYICSGIFGDNPRPNDKTGSCGPVSSNGANGVKGFSFKYGNGVWIGRTKSISSRNGFEMIWDPNGWTGTDN
NFSIKQDIVGINEWSGYSGSFVQHPELTGLDCIRPCFWVELIRGRPKENTIWTSGSSISFCGVNSDTVGWSWPDGAE
LPFTIDK

SEQ ID NO: 100 (NP, B/Lee/40)

AGCATTTTCTTGTGAGCTTCGAGCACTAATAAAACTGAAAATCAAAATGTCCAACATGGATATTGACAGTATAAATA
CCGGAACAATCGATAAAAAACCAGAAGAACTGACTCCCGGAACCAGTGGGGCAACCAGACCAATCATCAAGCCAGCA
ACCCTTGCTCCGCCAAGCAACAAACGAACCCGAAATCCATCCCCAGAAAGGACAACCACAAGCAGTGAAACCGATAT
CGGAAGGAAAATCCAAAAGAAACAAACCCCAACAGAGATAAAGAAGAGCGTCTACAACATGGTGGTAAAGCTGGGTG
AATTCTACAACCAGATGATGGTCAAAGCTGGACTTAATGATGACATGGAAAGGAATCTAATCCAAAATGCACAAGCT
GTGGAGAGAATCCTATTGGCTGCAACTGATGACAAGAAAACTGAATACCAAAAGAAAAGGAATGCCAGAGATGTCAA
AGAAGGGAAGGAAGAAATAGACCACAACAAGACAGGAGGCACCTTTTATAAGATGGTAAGAGATGATAAAACCATCT
ACTTCAGCCCTATAAAAATTACCTTTTTAAAAGAAGAGGTGAAAACAATGTACAAGACCACCATGGGGAGTGATGGT
TTCAGTGGACTAAATCACATTATGATTGGACATTCACAGATGAACGATGTCTGTTTCCAAAGATCAAAGGCACTGAA
AAGGGTTGGACTTGACCCTTCATTAATCAGTACTTTTGCCGGAAGCACACTACCCAGAAGATCAGGTACAACTGGTG
TTGCAATCAAAGGAGGTGGAACTTTAGTGGCAGAAGCCATTCGATTTATAGGAAGAGCAATGGCAGACAGAGGGCTA
CTGAGAGACATCAAGGCCAAGACAGCCTATGAAAAGATTCTTCTGAATCTGAAAAACAAGTGCTCTGCGCCCCAACA
AAAGGCTCTAGTTGATCAAGTGATCGGAAGTAGGAACCCAGGGATTGCAGACATAGAAGACCTAACTCTGCTTGCCA
GAAGCATGATAGTTGTCAGACCCTCTGTAGCGAGCAAAGTGGTGCTTCCCATAAGCATTTATGCTAAAATACCTCAA
CTAGGATTCAATATCGAAGAATACTCTATGGTTGGGTATGAAGCCATGGCTCTTTATAATATGGCAACACCTGTTTC
CATATTAAGAATGGGAGATGACGCAAAAGATAAATCTCAACTATTCTTCATGTCGTGCTTCGGAGCTGCCTATGAAG
ATCTAAGAGTGTTATCTGCACTAACGGGCACCGAATTTAAGCCTAGATCAGCACTAAAATGCAAGGGTTTCCATGTC
CCGGCTAAGGAGCAAGTAGAAGGAATGGGGGCAGCTCTGATGTCCATCAAGCTTCAGTTCTGGGCCCCAATGACCAG
ATCTGGAGGGAATGAAGTAAGTGGAGAAGGAGGGTCTGGTCAAATAAGTTGCAGCCCTGTGTTTGCAGTAGAAAGAC
CTATTGCTCTAAGCAAGCAAGCTGTAAGAAGAATGCTGTCAATGAACGTTGAAGGACGTGATGCAGATGTCAAAGGA
AATCTACTCAAAATGATGAATGATTCGATGGCAAAGAAAACCAGTGGAAATGCTTTCATTGGGAAGAAAATGTTTCA
AATATCAGACAAAAACAAAGTCAATCCCATTGAGATTCCAATTAAGCAGACCATCCCCAGTTTCTTCTTTGGGAGGG
ACACAGCAGAGGATTATGATGACCTCGATTATTAAAGCAATAAAATAGACACTATGGCTGTGACTGTTTCAGTACGT
TTGGGATGTGGGTGTTTACTCTTATTGAAATAAATGTAAAA

SEQ ID NO: 101 (NP, B/Ann Arbor/1/66)

MSNMDIDGINTGTIDKTPEEITSGTSGATRPIIKPATLAPPSNKRTRNPSPERATTSSEAIVGRRTQKKQTPTEIKK
SVYNMVVKLGEFYNQMMVKAGLNDDMERNLIQNAHAVERILLAATDDKKTEYQKKKNARDVKEGKEEIDHNKTGGTF
YKMVRDDKTIYFSPIRITFLKEEVKTMYKTTMGSDGFSGLNHIMIGHSQMNDVCFQRSKALKRVGLDPSLISTFAGS
TLPRRSGATGVAIKGGGTLVAEAIRFIGRAMADRGLLRDIRAKTAYEKILLNLKNKCSAPQQKALVDQVIGSRNPGI
ADIEDLTLLARSMVVVRPSVASKVVLPISINAKIPQLGFNVEEYSMVGYEAMALYNMATPVSILRMGDDAKDKSQLF
FMSCFGAAYEDQRVLSALTGTEFKPRSALKCKGFHVPAKEQVEGMGAALMSIKLQFWAPMTRSGGNEVGGDGGSGQI
SCSPVFAVERPIALSKQAVRRMLSMNIEGRDADVKGNLLKMMNDSMAKKTNGNAFIGKKMFQISDKNKINPVDIPIK
QTIPNFFFGRDTAEDYDDLDY

SEQ ID NO: 102 (NP, B/Ann Arbor/1/66)

MSNMDIDGINTGTIDKTPEEITSGTSGATRPIIKPATLAPPSNKRTRNPSPERAATSSEADVGRRTQKKQTPTEIKK
SVYNMVVKLGEFYNQMMVKAGLNDDMERNLIQNAHAAERILLAATDDKKTEFQKKKNARDVKEGKEEIDHNKTGGTF
YKMVRDDKTIYFSPIRITFLKEEVKTMYKTTMGSDGFSGLNHIMIGHSQMNDVCFQRSKALKRVGLDPSLISTFAGS
TLPRRSGATGVAIKGGGTLVAEAIRFIGRAMADRGLLRDIRAKTAYEKILLNLKNKCSAPQQKALVDQVIGSRNPGI
ADIEDLTLLARSMVVVRPSVASKVVLPISINAKIPQLGFNVEEYSMVGYEAMALYNMATPVSILRMGDDAKDKSQLF
FMSCFGAAYEDQRVLSALTGTEFKHRSALKCKGFHVPAKEQVEGMGAALMSIKLQFWAPMTRSGGNEVGGDGGSGQI
SCSPVFAVERPIALSKQAVRRMLSMNIEGRDADVKGNLLKMMNDSMTKKTNGNAFIGKKMFQISDKNKTNPIEIPIK
QTIPNFFFGRDTAEDYDDLDY

SEQ ID NO: 103 (NP, B/Ann Arbor/1/66)

AGCAGAAGCACAGCATTTTCTTGTGAACTTCAAGTACCAACAAAAACTGAAAATCAAAATGTCCAACATGGATATTG
ACGGCATCAACACTGGAACAATTGACAAAACACCAGAAGAAATAACTTCCGGAACCAGTGGGGCAACCAGACCAATC
ATCAAGCCAGCAACCCTTGCCCCACCAAGCAATAAACGAACCCGAAACCCATCCCCAGAAAGGGCAACCACAAGCAG
CGAAGCGATTGTCGGAAGGAGAACCCAAAAGAAACAAACCCCGACAGAGATAAAGAAGAGCGTCTACAATATGGTAG

TGAAACTGGGTGAATTCTACAACCAGATGATGGTCAAAGCTGGACTCAACGATGACATGGAGAGAAACCTAATCCAA
AATGCACATGCTGTGGAAAGAATTCTATTGGCTGCTACTGATGACAAGAAAACTGAATACCAAAAGAAAAAGAATGC
CAGAGATGTCAAAGAAGGGAAAGAAGAAATAGACCACAACAAAACAGGAGGCACCTTTTATAAGATGGTAAGAGATG
ATAAAACCATCTACTTCAGCCCTATAAGAATTACCTTTTTAAAAGAAGAGGTGAAAACAATGTACAAGACCACCATG
GGGAGTGATGGTTTCAGTGGACTAAATCACATCATGATTGGGCATTCACAGATGAACGATGTCTGTTTCCAAAGATC
AAAGGCACTAAAAAGAGTTGGACTTGACCCTTCATTAATCAGTACTTTTGCAGGAAGCACACTCCCCAGAAGATCAG
GTGCAACTGGTGTTGCGATCAAAGGAGGTGGAACTTTAGTGGCAGAAGCCATTCGATTTATAGGAAGAGCAATGGCA
GACAGAGGGCTATTGAGAGACATCAGAGCCAAGACGGCCTATGAAAAGATTCTTCTGAATCTGAAAAACAAGTGCTC
TGCGCCCCAACAAAAGGCTCTAGTTGATCAAGTGATCGGAAGTAGAAACCCAGGGATTGCAGACATAGAAGACCTAA
CCCTGCTTGCCCGAAGCATGGTCGTTGTCAGGCCCTCTGTAGCGAGCAAAGTGGTGCTTCCCATAAGCATTAATGCT
AAAATACCTCAACTAGGGTTCAATGTTGAAGAATACTCTATGGTTGGGTATGAAGCCATGGCTCTTTATAATATGGC
AACACCTGTTTCCATATTAAGAATGGGAGACGATGCAAAAGATAAATCACAATTATTCTTCATGTCTTGCTTTGGAG
CTGCCTATGAAGACCAAAGAGTTTTGTCTGCACTAACCGGCACAGAATTCAAGCCTAGGTCAGCATTAAAGTGCAAG
GGTTTCCACGTTCCAGCAAAGGAGCAAGTGGAAGGAATGGGGGCAGCTCTGATGTCCATCAAGCTCCAGTTTTGGGC
CCCAATGACCAGATCTGGGGGGAACGAAGTAGGTGGAGACGGAGGGTCTGGTCAAATAAGTTGCAGCCCCGTGTTTG
CAGTAGAGAGACCTATTGCTCTAAGCAAGCAAGCTGTAAGAAGAATGCTGTCAATGAATATTGAGGGACGTGATGCA
GATGTCAAAGGAAATCTACTCAAGATGATGAATGATTCAATGGCTAAGAAAACCAATGGAAATGCTTTCATTGGGAA
GAAAATGTTTCAAATATCAGACAAAAACAAAATCAATCCCGTTGATATTCCAATTAAGCAGACCATCCCCAATTTCT
TCTTTGGGAGGGACACAGCAGAGGATTATGATGACCTCGATTATTAAAGCAACAAAATAGACACTATGGCTGTGACT
GTTTCAGTACGTTTGGAATGTGGGTGTTTACTCTTATTGAAATAAATGTAAAAAATGCTGTTGTTTCTACT

SEQ ID NO: 104 (NP, B/Ann Arbor/1/66)

AGCAGAAGCACAGCATTTTCTTGTGAACTTCAAGTACCAACAAAAACTGAAAATCAAAATGTCCAACATGGATATTG
ACGGCATCAACACTGGAACAATTGACAAAACACCAGAAGAAATAACTTCCGGAACCAGTGGGGCAACCAGACCAATC
ATCAAACCAGCAACCCTTGCCCCACCAAGCAACAAACGAACCCGAAACCCATCCCCGGAAAGGGCAGCCACAAGCAG
TGAAGCTGATGTCGGAAGGAGAACCCAAAAGAAACAAACCCCGACAGAGATAAAGAAGAGCGTCTACAATATGGTAG
TGAAACTGGGTGAATTCTACAACCAGATGATGGTCAAAGCTGGACTCAACGATGACATGGAGAGAAACCTAATCCAA
AATGCACATGCTGCGGAAAGAATTCTATTGGCTGCTACTGATGACAAGAAAACTGAATTCCAAAAGAAAAAGAATGC
CAGAGATGTCAAAGAAGGGAAAGAAGAAATAGACCACAACAAAACAGGAGGCACCTTTTACAAGATGGTAAGAGATG
ATAAAACCATCTACTTCAGCCCTATAAGAATTACCTTTTTAAAAGAAGAGGTGAAAACAATGTACAAAACCACCATG
GGGAGTGATGGTTTCAGTGGACTAAATCACATCATGATTGGGCATTCACAGATGAACGATGTCTGTTTCCAAAGATC
AAAGGCACTAAAAAGAGTTGGACTTGACCCTTCATTAATCAGTACTTTTGCAGGAAGCACACTCCCCAGAAGATCAG
GTGCAACTGGTGTTGCGATCAAAGGAGGTGGAACTTTAGTGGCAGAAGCCATTCGATTTATAGGAAGAGCAATGGCA
GACAGAGGGCTATTGAGAGACATCAGAGCCAAGACGGCCTATGAAAAGATTCTTCTGAATCTGAAAAACAAGTGCTC
TGCGCCCCAACAAAAGGCTCTAGTTGATCAAGTGATCGGAAGTAGAAATCCAGGGATTGCAGACATAGAAGACCTAA
CCCTGCTTGCCCGAAGCATGGTCGTTGTCAGGCCCTCTGTAGCGAGCAAAGTGGTGCTTCCCATAAGCATTAATGCC
AAAATACCTCAACTAGGGTTCAATGTTGAAGAATACTCTATGGTTGGGTATGAAGCCATGGCTCTTTATAATATGGC
AACACCTGTTTCCATATTAAGAATGGGAGACGATGCAAAAGATAAATCACAATTATTCTTCATGTCTTGCTTCGGAG
CTGCCTATGAAGACCAAAGAGTTTTGTCTGCACTAACAGGCACAGAATTCAAGCATAGGTCAGCATTAAAGTGCAAG
GGTTTCCACGTTCCAGCAAAGGAGCAAGTGGAAGGAATGGGGGCAGCTCTGATGTCCATCAAGCTCCAGTTTTGGGC
TCCAATGACCAGATCTGGGGGGAATGAAGTAGGTGGAGACGGAGGGTCTGGTCAAATAAGTTGCAGCCCCGTGTTTG
CAGTAGAAAGACCTATTGCTCTAAGCAAGCAAGCTGTAAGAAGAATGCTGTCAATGAATATTGAGGGACGTGATGCA
GATGTCAAAGGAAATCTACTCAAGATGATGAATGATTCAATGACTAAGAAAACCAATGGAAATGCTTTCATTGGGAA
GAAAATGTTTCAAATATCAGACAAAAACAAAACCAATCCCATTGAGATTCCAATTAAGCAGACCATCCCCAATTTCT
TCTTTGGGAGGGACACAGCAGAGGATTATGATGACCTCGATTATTAAAGCAACAAAATAGACACTATGGCTGTGACT
GTTTCAGTACGTTTGGAATGTGGGTGTTTACTTTTATTGAAATAAATGTAAAAAATGCTGTTGTTTCTACT

SEQ ID NO: 105 (5'- HA NCR, 105p30)

AGCAAAAGCAGGGGAAAATAAAAGCAACCAAA

SEQ ID NO: 106 (HA SP, 105p30)

ATGAAAGTAAAACTACTGGTTCTGTTATGTACATTTACAGCTACATATGCA

SEQ ID NO: 107 (HA TM domain, 105p30)

AGATTCTGGCGATCTACTCAACAGTCGCCAGTTCCCTGGTTCTTTTGGTCTCCCTGGGGGCAATCAGCTTCTGGATG

SEQ ID NO: 108 (HA CT domain, 105p30)

TGTTCCAATGGGTCTTTGCAGTGTAGAATATGCATCTAA

SEQ ID NO: 109 (HA 3'- NCR, 105p30)

GACCAGAATTTCAGAAATATAAGGAAAAACACCCTTGTTTCTACT

SEQ ID NO: 110 (NA 5'- NCR, 105p30)

AGCAAAAGCAGGAGTTTAAA

SEQ ID NO: 111 (NA CT, 105p30)

ATGAATCCAAATCAAAAA

SEQ ID NO: 112 (NA TM domain, 105p30)

ATAATAACCATTGGATCAATCAGTATAGCAATCGGAATAATTAGTCTAATGTTGCAAATAGGAAATATTATTTCAAT
ATGGGCTAGT

SEQ ID NO: 113 (NA 3'- NCR, 105p30)

CTCGTTGAAAAAAACTCCTTGTTTCTACT

SEQ ID NO: 114 (5'- HA NCR, PR8-X)

AGCAAAAGCAGGGGAAAATAAAAACAACCAAA

SEQ ID NO: 115 (HA SP, PR8-X)

ATGAAGGCAAACCTACTGGTCCTGTTATGTGCACTTGCAGCTGCAGATGCA

SEQ ID NO: 116 (HA TM domain, PR8-X)

CAGATTCTGGCGATCTACTCAACTGTCGCCAGTTCACTGGTGCTTTTGGTCTCCCTGGGGGCAATCAGTTTCTGGAT
G

SEQ ID NO: 117 (HA CT domain, PR8-X)

TGTTCTAATGGATCTTTGCAGTGCAGAATATGCATCTGA

SEQ ID NO: 118 (HA 3'- NCR, PR8-X)

GATTAGAATTTCAGAGATATGAGGAAAAACACCCTTGTTTCTACT

SEQ ID NO: 119 (NA 5'- NCR, PR8-X)

AGCAAAAGCAGGGGTTTAAA

SEQ ID NO: 120 (NA CT, PR8-X)

ATGAATCCAAATCAGAAA

SEQ ID NO: 121 (NA TM domain, PR8-X)

ATAATAACCATTGGATCAATCTGTCTGGTAGTCGGACTAATTAGCCTAATATTGCAAATAGGGAATATAATCTCAAT
ATGGATTAGC

SEQ ID NO: 122 (NA 3'- NCR, PR8-X)

TCTGTTCAAAAAACTCCTTGTTTCTACT

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CLAIMS

1. A chimeric influenza hemagglutinin segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a signal peptide, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are from a second influenza strain which is not A/Puerto Rico/8/34, A/WSN/33 or B/Lee/40, wherein the first and the second influenza strains are both influenza A or influenza B strains.

2. A chimeric influenza hemagglutinin segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a signal peptide, a transmembrane domain and a cytoplasmic domain, wherein the ectodomain is from a first influenza A strain which is not a H1 or H5 influenza strain, and one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are from a second influenza strain, wherein the first and the second influenza strains are both influenza A or influenza B strains.

3. A chimeric influenza hemagglutinin segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a signal peptide, a transmembrane domain and a cytoplasmic domain, wherein the ectodomain is from a first influenza B strain, and one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are from a second influenza strain which is an influenza B strain or an influenza A strain which is not a H1 strain or a H3 strain.

4. A chimeric influenza hemagglutinin segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a signal peptide, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are from a second influenza strain, wherein the segment comprises one or more of:

   a) guanine in the position corresponding to nucleotide 24 of SEQ ID NO: 15, and/or
   b) adenine in the position corresponding to nucleotide 38 of SEQ ID NO: 15; and/or
   c) thymine in the position corresponding to nucleotide 40 of SEQ ID NO: 15; and/or
   d) adenine in the position corresponding to nucleotide 44 of SEQ ID NO: 15; and/or
   e) thymine in the position corresponding to nucleotide 53 of SEQ ID NO: 15; and/or
   f) adenine in the position corresponding to nucleotide 63 of SEQ ID NO: 15; and/or
   g) thymine in the position corresponding to nucleotide 66 of SEQ ID NO: 15; and/or
   h) adenine in the position corresponding to nucleotide 69 of SEQ ID NO: 15; and/or

i) adenine in the position corresponding to nucleotide 75 of SEQ ID NO: 15; and/or

j) thymine in the position corresponding to nucleotide 78 of SEQ ID NO: 15; and/or

k) adenine in the position corresponding to nucleotide 1637 of SEQ ID NO: 15, and/or

l) cytosine in the position corresponding to nucleotide 1649 of SEQ ID NO: 15, and/or

m) thymine in the position corresponding to nucleotide 1655 of SEQ ID NO: 15, and/or

n) cytosine in the position corresponding to nucleotide 1682 of SEQ ID NO: 15, and/or

o) cytosine in the position corresponding to nucleotide 1697 of SEQ ID NO: 15, and/or

p) guanine in the position corresponding to nucleotide 1703 of SEQ ID NO: 15, and/or

q) thymine in the position corresponding to nucleotide 1715 of SEQ ID NO: 15, and/or

r) adenine in the position corresponding to nucleotide 1729 of SEQ ID NO: 15, and/or

s) cytosine in the position corresponding to nucleotide 1733 of SEQ ID NO: 15, and/or

t) cytosine in the position corresponding to nucleotide 1734 of SEQ ID NO: 15, and/or

u) adenine in the position corresponding to nucleotide 1746 of SEQ ID NO: 15, and/or

v) adenine in the position corresponding to nucleotide 1751 of SEQ ID NO: 15;

when aligned to SEQ ID NO: 15 using a pairwise alignment algorithm.

5. The chimeric hemagglutinin segment of claim 4, wherein the segment comprises all of the nucleotides of (a) to (v).

6. The chimeric hemagglutinin segment of any preceding claim, comprising one or more of the 5'- NCR domain of SEQ ID NO: 105; and/or the SP of SEQ ID NO: 106; and/or the TM domain of SEQ ID NO: 107; and/or the CT domain of SEQ ID NO: 108; and/or the 3'- NCR of SEQ ID NO: 109.

7. The chimeric hemagglutinin segment of any one of claims 4 to 6, wherein the first and the second influenza strains are both influenza A or influenza B viruses.

8. The chimeric hemagglutinin segment of any preceding claim wherein the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are all from the second influenza strain.

9. The chimeric hemagglutinin of any preceding claim, wherein the segment encodes a protein that does not have tyrosine in the position corresponding to amino acid 545, when aligned to SEQ ID NO: 7.

10. A chimeric influenza neuraminidase segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'-

non-coding region, the transmembrane domain and the cytoplasmic domain are from a second influenza strain which is not A/Puerto Rico/8/34 or A/WSN/33.

11. A chimeric influenza neuraminidase segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is from a first influenza strain and the 5'- non-coding region, the 3'- non-coding region, the transmembrane domain and the cytoplasmic domain are from a second influenza strain wherein the first and the second influenza strain are both influenza A strains or both influenza B strains.

12. A chimeric neuraminidase segment having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a transmembrane domain and a cytoplasmic domain, wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the transmembrane domain and the cytoplasmic domain are from a second influenza strain, wherein the segment comprises:

a) adenine in the position corresponding to nucleotide 13 of SEQ ID NO: 16; and/or
b) adenine in the position corresponding to nucleotide 35 of SEQ ID NO: 16; and/or
c) adenine in the position corresponding to nucleotide 60 of SEQ ID NO: 16; and/or
d) adenine in the position corresponding to nucleotide 63 of SEQ ID NO: 16; and/or
e) adenine in the position corresponding to nucleotide 65 of SEQ ID NO: 16; and/or
f) cytosine in the position corresponding to nucleotide 67 of SEQ ID NO: 16; and/or
g) adenine in the position corresponding to nucleotide 69 of SEQ ID NO: 16; and/or
h) adenine in the position corresponding to nucleotide 75 of SEQ ID NO: 16; and/or
i) thymine in the position corresponding to nucleotide 83 of SEQ ID NO: 16; and/or
j) guanine in the position corresponding to nucleotide 89 of SEQ ID NO: 16; and/or
k) adenine in the position corresponding to nucleotide 101 of SEQ ID NO: 16; and/or
l) thymine in the position corresponding to nucleotide 107 of SEQ ID NO: 16; and/or
m) thymine in the position corresponding to nucleotide 110 of SEQ ID NO: 16; and/or
n) guanine in the position corresponding to nucleotide 120 of SEQ ID NO: 16; and/or
o) cytosine in the position corresponding to nucleotide 121 of SEQ ID NO: 16; and/or
p) thymine in the position corresponding to nucleotide 125 of SEQ ID NO: 16; and/pr
q) thymine in the position corresponding to nucleotide 127 of SEQ ID NO: 16;

when aligned to SEQ ID NO: 16 using a pairwise alignment algorithm.

13. The chimeric neuraminidase segment of claim 12, wherein the segment comprises all of the nucleotides of (a) to (q).

14. The chimeric neuraminidase segment of any one of claims 10 to 13, comprising one or more of the 5'- NCR domain of SEQ ID NO: 110; and/or the CT domain of SEQ ID NO: 111; and/or the TM domain of SEQ ID NO: 112; and/or the 3'- NCR of SEQ ID NO: 113.

15. The chimeric neuraminidase segment of any one of claims 10 to 14 wherein the 5'- non-coding region, the 3'- non-coding region, the transmembrane domain and the cytoplasmic domain are all from the second influenza strain.

16. The chimeric hemagglutinin segment or the chimeric neuraminidase segment of any preceding claim wherein the second influenza strain is a H1 influenza strain.

17. A chimeric hemagglutinin protein encoded by the chimeric hemagglutinin segment of any one of claims 1 to 9, or a chimeric neuraminidase protein encoded by the chimeric neuraminidase segment of any of claims 10 to 16.

18. A reassortant influenza virus comprising the chimeric hemagglutinin segment and/or the chimeric neuraminidase segment of any one of claims 1 to 17.

19. The reassortant influenza virus of claim 18, wherein all of the backbone segments are from an influenza strain selected from the group consisting of 105p30 and PR8-X.

20. A reassortant influenza virus comprising:

a) a chimeric hemagglutinin protein having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a signal peptide, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is from a first influenza strain and one or more of the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain are from a second influenza strain; and/or a chimeric neuraminidase protein having an ectodomain, a 5'- non-coding region, a 3'- non-coding region, a transmembrane domain and a cytoplasmic domain wherein the ectodomain is from a first influenza strain and one or more of the non-coding regions, the cytoplasmic domain, and the transmembrane domain are from a second influenza strain; and

b) one or more of:

i. backbone segments from two or more different donor strains

ii. backbone segments from two or more donor strains, wherein the PB1 and the PB2 segments are from the same donor strain;

iii. backbone segments from two or three donor strains, wherein each donor strain provides more than one backbone segment;

iv. backbone segments from two or more donor strains, wherein the PB1 segment is not from the A/Texas/1/77 influenza strain;

v. backbone segments from two or more donor strains, wherein at least the PA, NP, or M segment are not from A/Puerto Rico/8/34;

vi. backbone segments from two or more donor strains, wherein the HA segment and the PB1 segment are from different influenza A strains with the same influenza virus HA subtype.

vii. backbone segments from two or more donor strains, wherein the HA segment and the PB1 segment are from different influenza A strains with the same influenza virus HA subtype.

21. The reassortant influenza virus of claim 20 wherein the 5'- non-coding region, the 3'- non-coding region, the signal peptide, the transmembrane domain and the cytoplasmic domain of the chimeric hemagglutinin are all from the second influenza strain, and/or wherein the 5'- non-coding region, the 3'- non-coding region, the transmembrane domain and the cytoplasmic domain of the chimeric neuraminidase protein are all from the second influenza strain.

22. The reassortant influenza virus of any one of claims 18 to 21, wherein the second influenza strain is selected from the group consisting of 105p30 and PR8-X.

23. The reassortant influenza virus of any one of claims 18 to 22, wherein the reassortant influenza virus comprises a M genome segment which has lysine in the position corresponding to amino acid 95 of SEQ ID NO: 33 when aligned to SEQ ID NO: 33 using a pairwise alignment algorithm.

24. The reassortant influenza virus of any one of claims 18, or 20 to 23, wherein the influenza virus comprises at least one backbone viral segment from a donor strain selected from the group consisting of 105p30 and PR8-X.

25. The reassortant influenza virus of claim 24 wherein the at least one backbone viral segment has a sequence having at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity or 100% identity with a sequence selected from the group consisting of SEQ ID NOs 9-14 or SEQ ID NOs 17-22

26. The reassortant influenza virus of claim 24 or claim 25 wherein the at least one backbone viral segment has the sequence of SEQ ID NO: 17 or SEQ ID NO: 20.

27. The reassortant influenza virus of claim 24 or claim 25, wherein the PB1 and PB2 viral segments have at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity or 100% identity with the sequences of SEQ ID NOs: 18 and 19.

28. The reassortant influenza virus of claim 27, wherein the virus further comprises a viral segment having at least 95% identity with a sequence selected from the group consisting of SEQ ID NOs 17-22.

29. The reassortant influenza virus of claim 25, wherein the virus comprises all the segments of SEQ ID NOs 9 to 14.

30. The reassortant influenza virus of claim 25, wherein the virus comprises the PA segment of SEQ ID NO: 9, the M segment of SEQ ID NO: 13, the NS segment of SEQ ID NO: 14, the PB1

segment of SEQ ID NO: 18, the PB2 segment of SEQ ID NO: 19, and the NP segment of SEQ ID NO: 20.

31. The reassortant influenza virus of claim 25, wherein the virus comprises the PA segment of SEQ ID NO: 9, the M segment of SEQ ID NO: 13, the NS segment of SEQ ID NO: 14, the PB1 segment of SEQ ID NO: 52, the PB2 segment of SEQ ID NO: 11, and the NP segment of SEQ ID NO: 12.

32. A method of preparing a reassortant influenza A virus comprising steps of

(i) introducing into a culture host one or more expression construct(s) which encode(s) the viral segments required to produce the influenza virus of any one of claims 18 to 28; and

(ii) culturing the culture host in order to produce reassortant virus.

33. A method of preparing a reassortant influenza virus comprising steps of (a) infecting a culture host with the reassortant influenza virus of claims 17-31 or the virus obtained by the method of claim 32; (b) culturing the host from step (a) to produce the virus; and optionally (c) purifying the virus obtained in step (b).

34. A method of preparing a vaccine, comprising steps of (a) preparing a virus by the method of claim 32 or claim 33; and (b) preparing a vaccine from the virus.

35. The method of any one of claims 32 to 34, wherein the culture host is an embryonated hen egg.

36. The method of any one of claims 32 to 34, wherein the culture host is a mammalian cell.

37. The method of claim 36, wherein the cell is an MDCK, Vero or PerC6 cell.

38. The method of claim 37, wherein the cell grows adherently.

39. The method of claim 37, wherein the cell grows in suspension.

40. The method of claim 39, wherein the MDCK cell is of the cell line MDCK 33016 (DSM ACC2219).

41. The method of any one of claims 34 to 40, wherein step (b) involves inactivating the virus.

42. The method of any one of claims 34 to 41, wherein the vaccine is a whole virion vaccine.

43. The method of any one of claims 34 to 41, wherein the vaccine is a split virion vaccine.

44. The method of any one of claims 34 to 41, wherein the vaccine is a surface antigen vaccine.

45. The method of any one of claims 34 to 41, wherein the vaccine is a virosomal vaccine.

46. The method of any one of claims 34 to 45, wherein the vaccine contains less than 10ng of residual host cell DNA per dose.

47. An expression system comprising one or more expression construct(s) encoding the segments of the reassortant influenza virus of any one of claims 17 to 31.

FIG. 1A

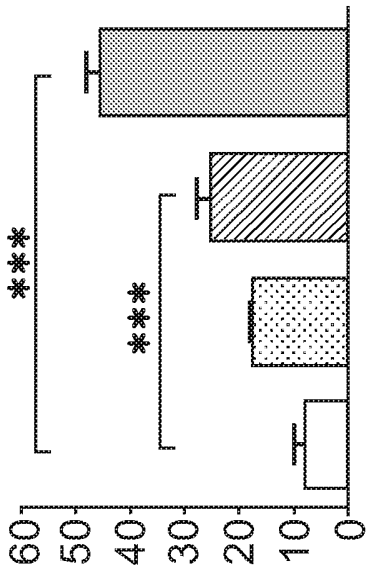


FIG. 1B

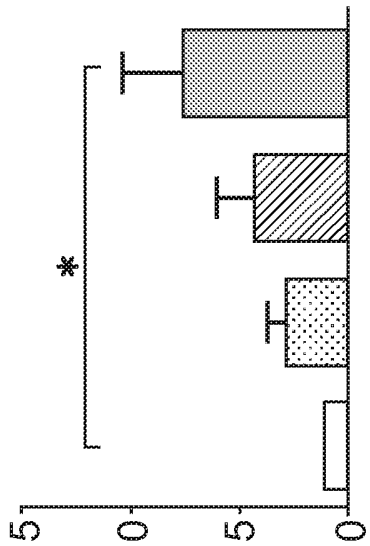


FIG. 1C

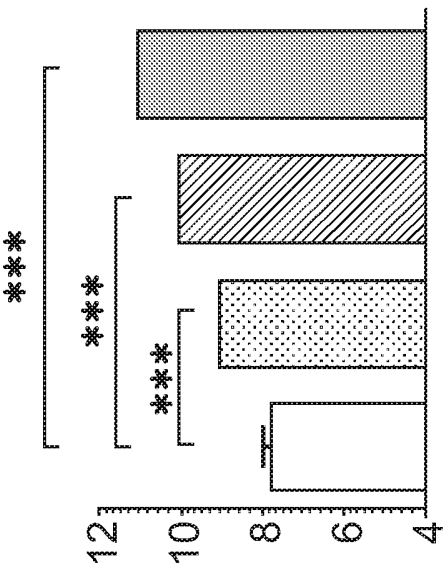


FIG. 1D

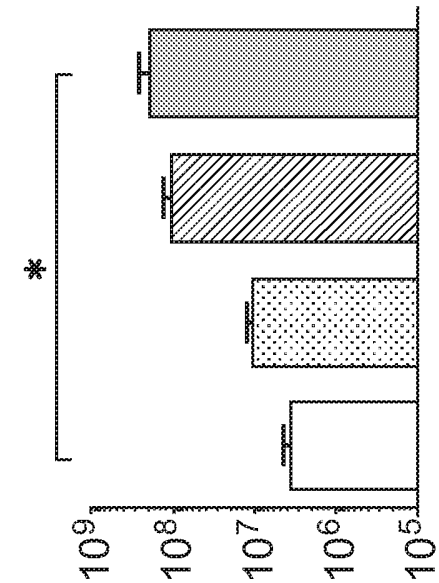


FIG. 2

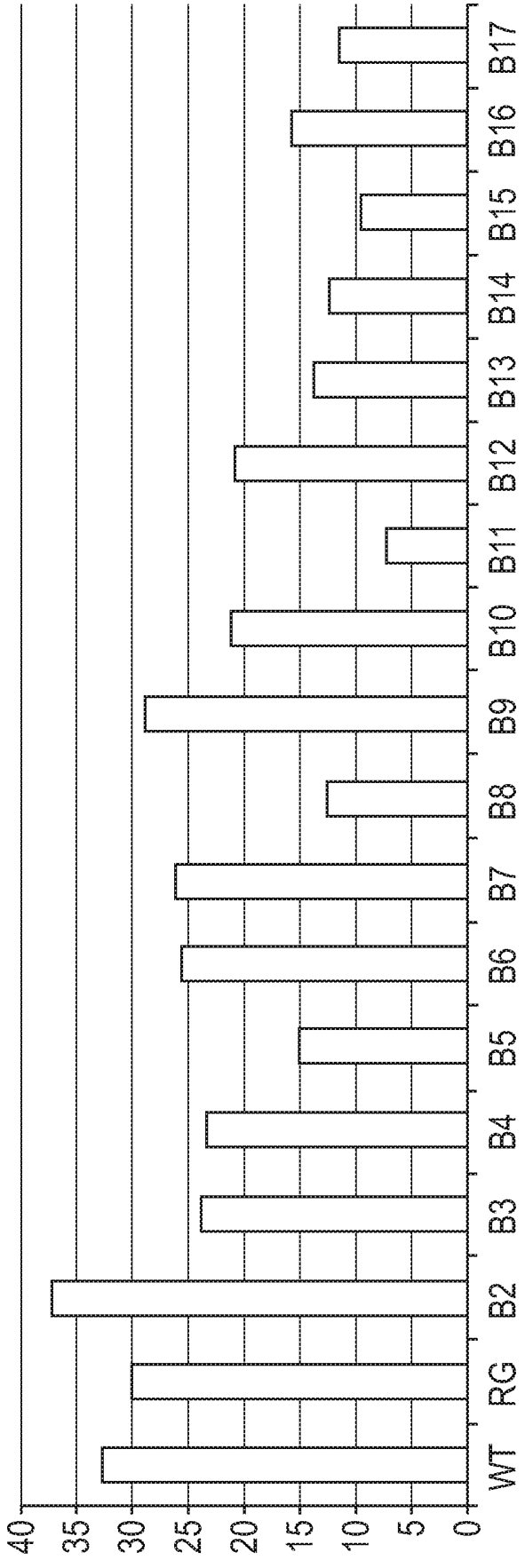

40
35
30
25
20
15
10
5
0
WT
RG
B2
B3
B4
B5
B6
B7
B8
B9
B10
B11
B12
B13
B14
B15
B16
B17

FIG. 3

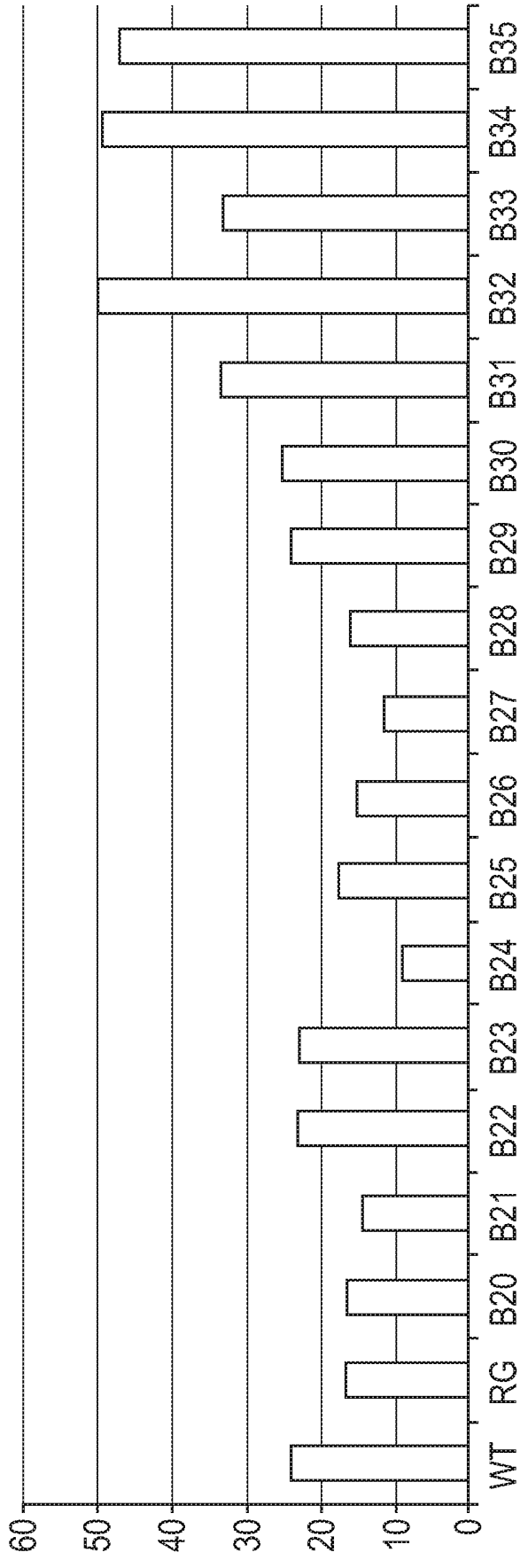

60
50
40
30
20
10
0
WT
RG
B20
B21
B22
B23
B24
B25
B26
B27
B28
B29
B30
B31
B32
B33
B34
B35

FIG. 4A

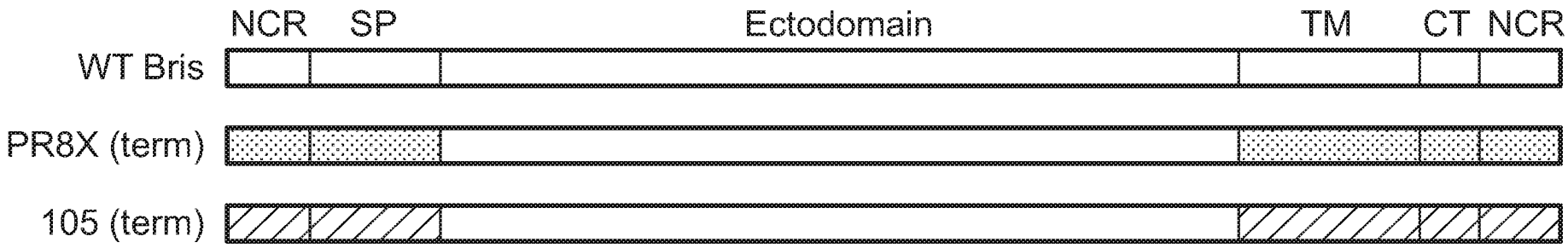

NCR
SP
Ectodomain
TM
CT
NCR
WT Bris
PR8X (term)
105 (term)

FIG. 4C

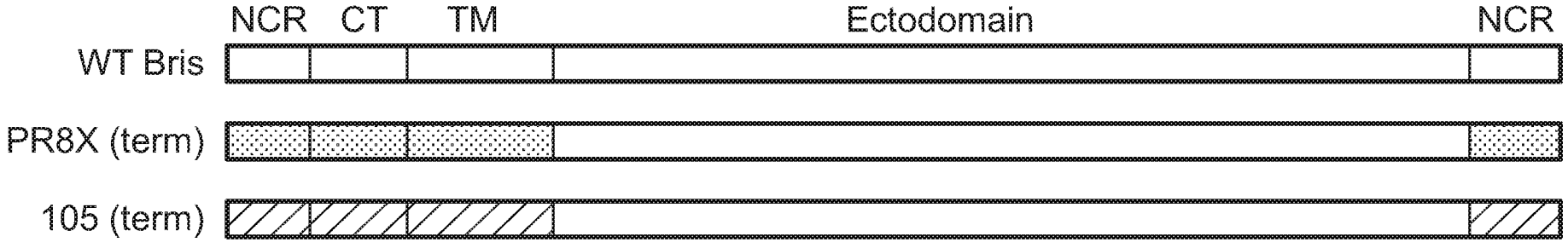

NCR
CT
TM
Ectodomain
NCR
WT Bris
PR8X (term)
105 (term)

FIG. 4B

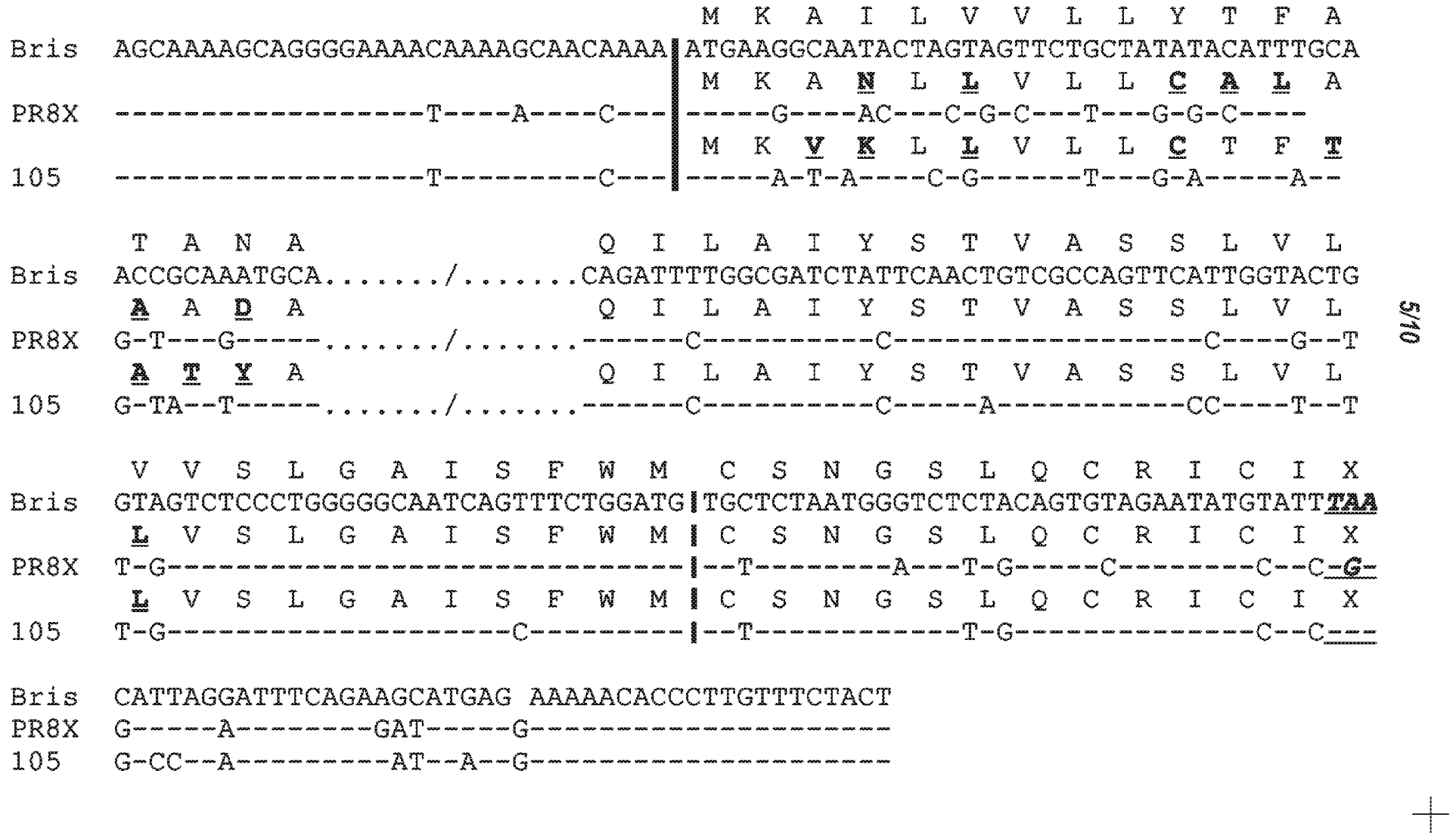

M K A I L V V L L Y T F A
Bris AGCAAAAGCAGGGGAAAACAAAAGCAACAAAA ATGAAGGCAATACTAGTAGTTCTGCTATATACATTTGCA
M K A N L L V L L C A L A
PR8X ----------T----A----C--- -----G----AC---C-G-C---T---G-G-C----
M K V K L L V L L C T F T
105 ----------T---------C--- -----A-T-A----C-G------T---G-A-----A--
T A N A Q I L A I Y S T V A S S L V L
Bris ACCGCAAATGCA...../.....CAGATTTTGGCGATCTATTCAACTGTCGCCAGTTCATTGGTACTG
A A D A Q I L A I Y S T V A S S L V L
PR8X G-T---G-----...../.....-----C----------C----------C----G--T
A T Y A Q I L A I Y S T V A S S L V L
105 G-TA--T-----...../.....-----C----------C-----A----------CC-----T--T
V V S L G A I S F W M C S N G S L Q C R I C I X
Bris GTAGTCTCCCTGGGGGCAATCAGTTTCTGGATG TGCTCTAATGGGTCTCTACAGTGTAGAATATGTATTTAA
L V S L G A I S F W M C S N G S L Q C R I C I X
PR8X T-G----------|--T---------A---T-G-----C---------C--C-G-
L V S L G A I S F W M C S N G S L Q C R I C I X
105 T-G----------C---------|--T----------T-G----------C--C---
Bris CATTAGGATTTCAGAAGCATGAG AAAAACACCCTTGTTTCTACT
PR8X G-----A---------GAT-----G----------
105 G-CC--A---------AT--A--G----------

FIG. 4D

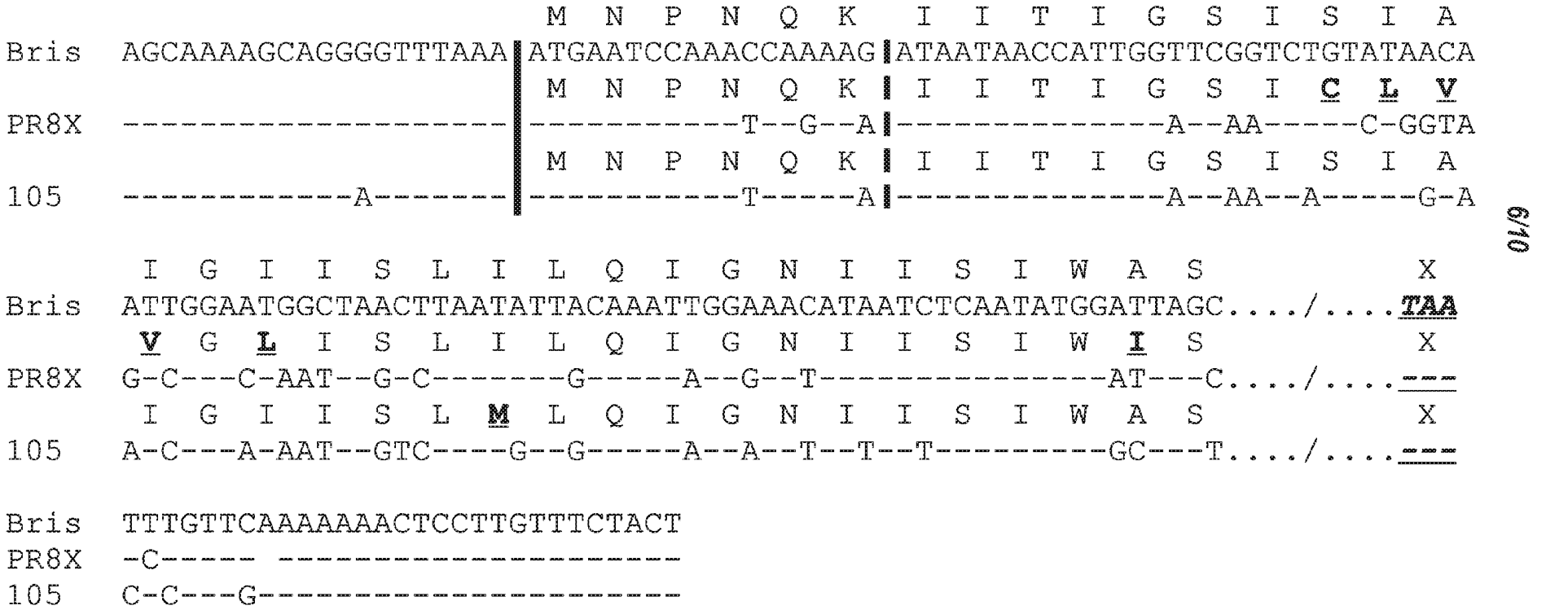

M N P N Q K I I T I G S I S I A
Bris AGCAAAAGCAGGGGTTTAAA ATGAATCCAAACCAAAAG ATAATAACCATTGGTTCGGTCTGTATAACA
M N P N Q K I I T I G S I C L V
PR8X ---------- ---------- T--G--A ---------- A--AA----- C-GGTA
M N P N Q K I I T I G S I S I A
105 ---------- A------- ---------- T----- A ---------- A--AA--A----- G-A
I G I I S L I L Q I G N I I S I W A S X
Bris ATTGGAATGGCTAACTTAATATTACAAATTGGAAACATAATCTCAATATGGATTAGC..../....TAA
V G L I S L I L Q I G N I I S I W I S X
PR8X G-C---C-AAT--G-C------- G----- A--G--T ---------- AT---C..../....---
I G I I S L M L Q I G N I I S I W A S X
105 A-C---A-AAT--GTC----G--G----- A--A--T--T--T---------- GC---T..../....---
Bris TTTGTTCAAAAAAACTCCTTGTTTCTACT
PR8X -C----- ----------
105 C-C---G----------

FIG.5A

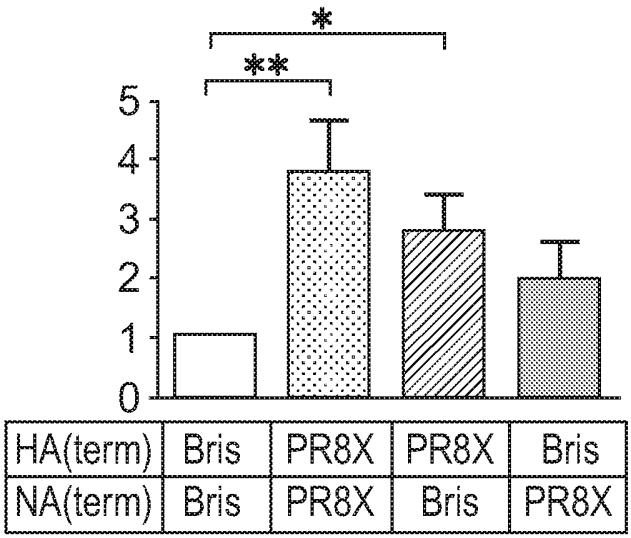


FIG. 5B

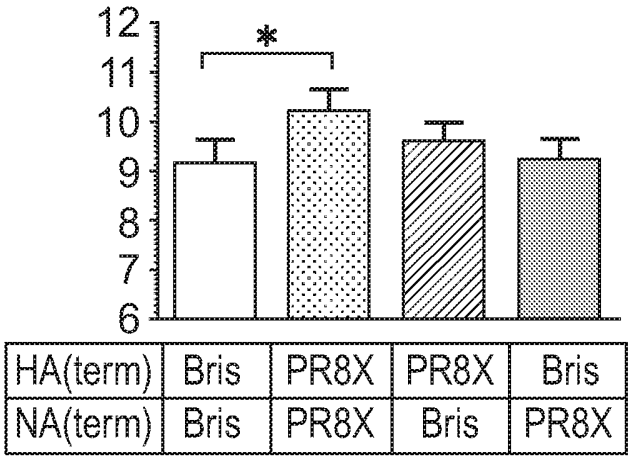


FIG. 5C

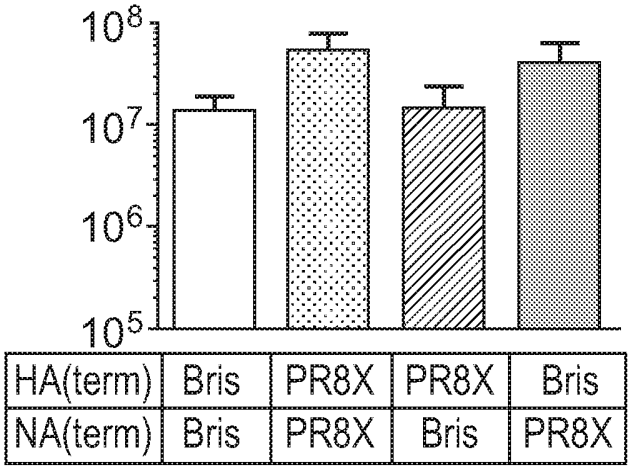


FIG. 6

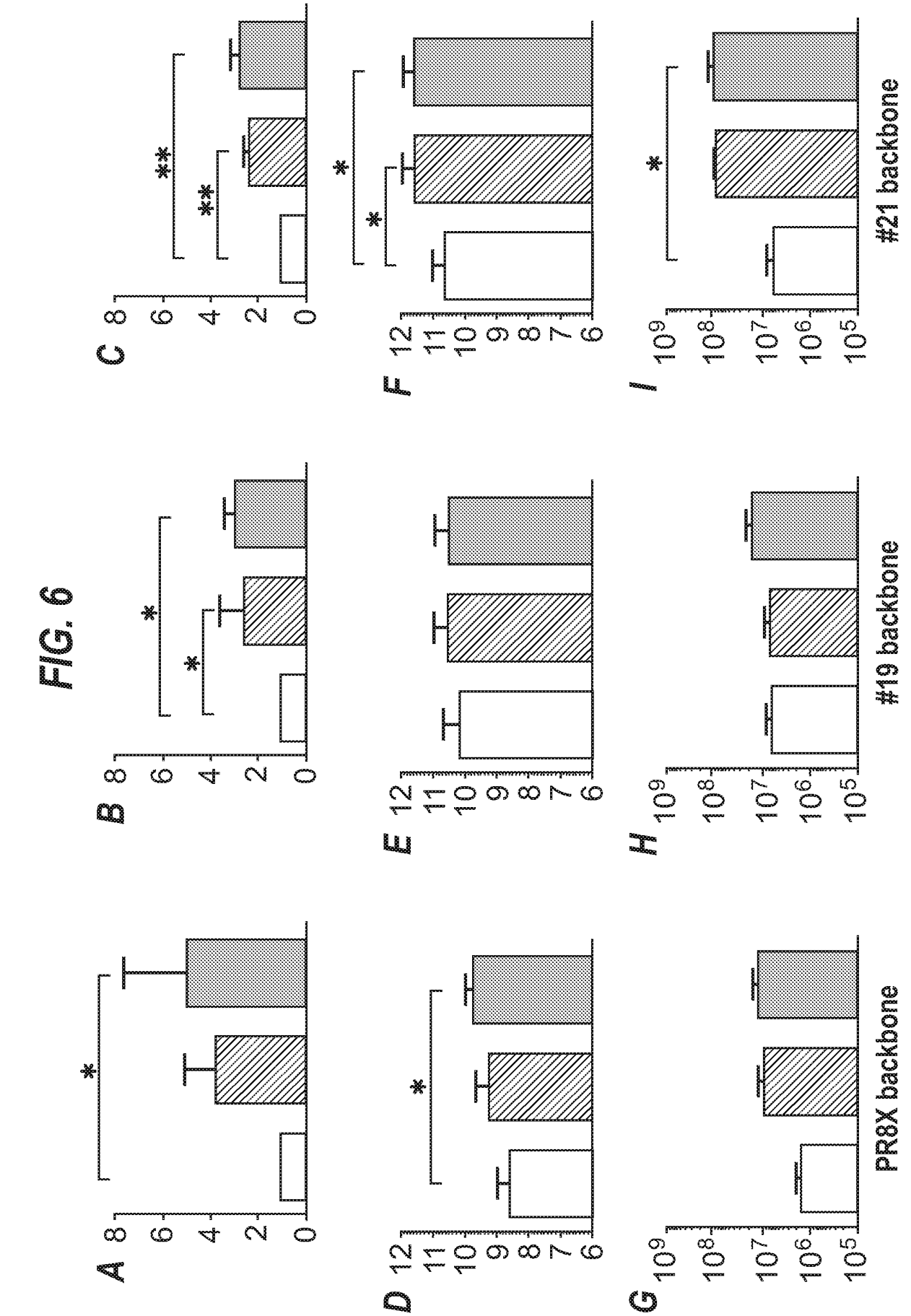


FIG. 7A

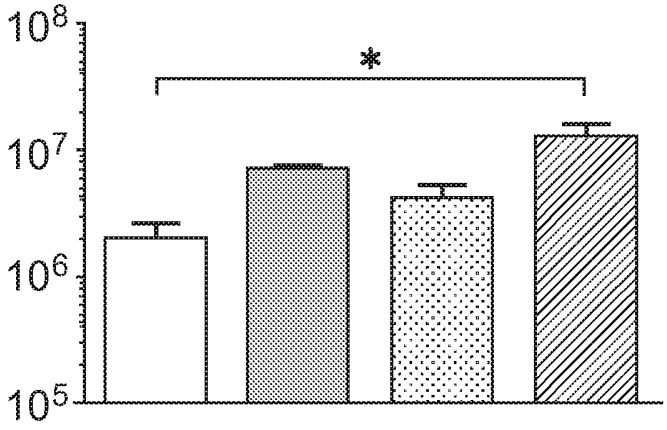


FIG. 7B

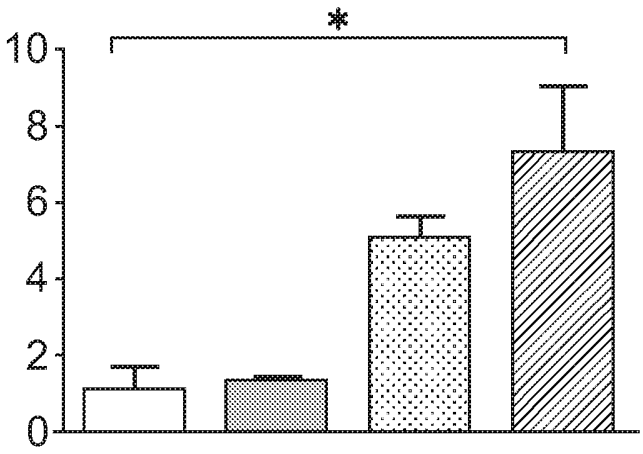


FIG. 8A

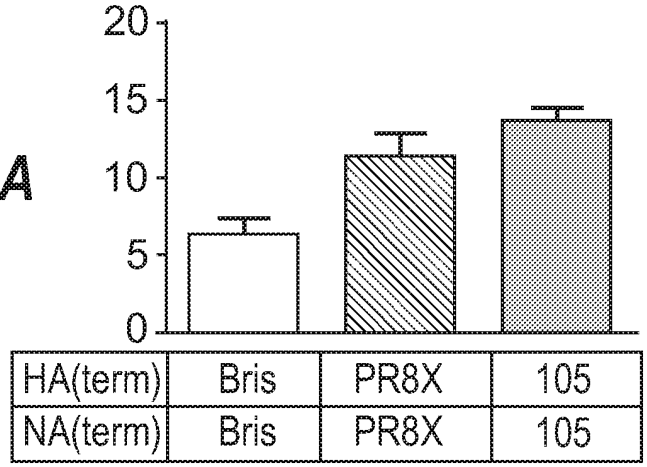


FIG. 8B

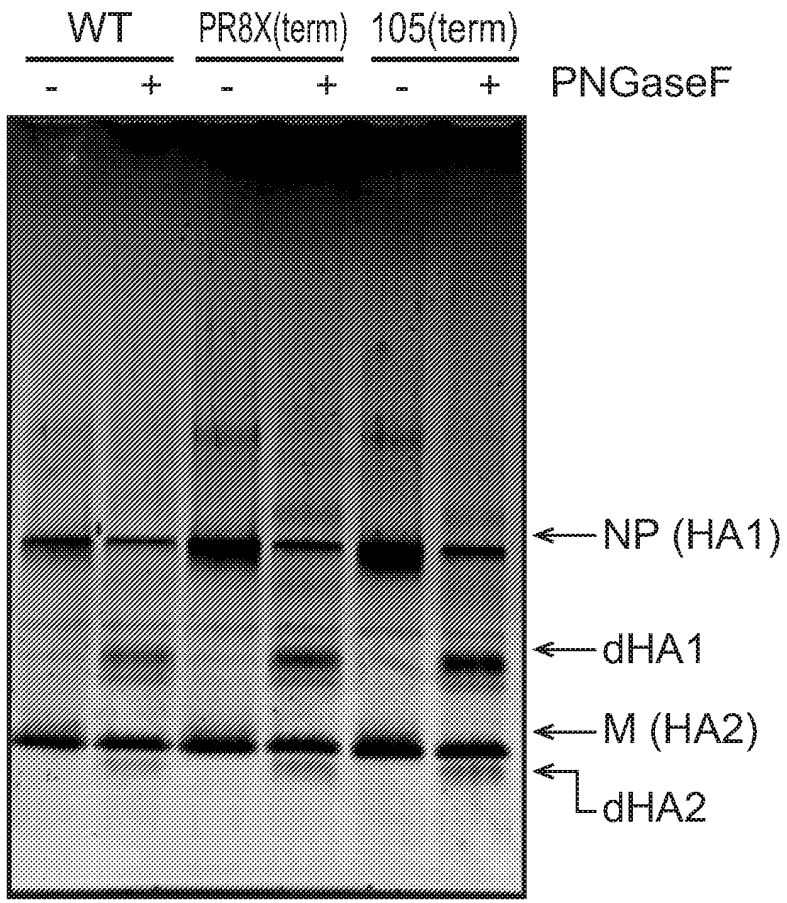
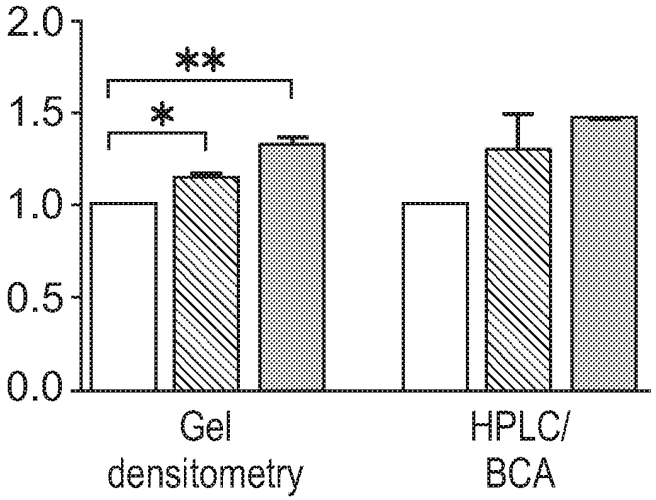


FIG. 8C



摘要

本發明提供重配流感病毒毒株。