(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 October 2011 (20.10.2011)

PCT

(10) International Publication Number
WO 2011/130652 A2

(51) International Patent Classification:
A61K 39/145 (2006.01)

(21) International Application Number:
PCT/US2011/032719

(22) International Filing Date:
15 April 2011 (15.04.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/324,703 15 April 2010 (15.04.2010) US

(72) Inventor; and
(71) Applicant : **BAER, George** [US/US]; Whiskey Hill Studio, Whiskey Hill Road, Walkill, NY 12589 (US).

(74) Agents: **WILLIAMS, Kathleen** et al.; Edwards Angell Palmer & Dodge LLP, P.O. Box 55874, Boston, MA 02205 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

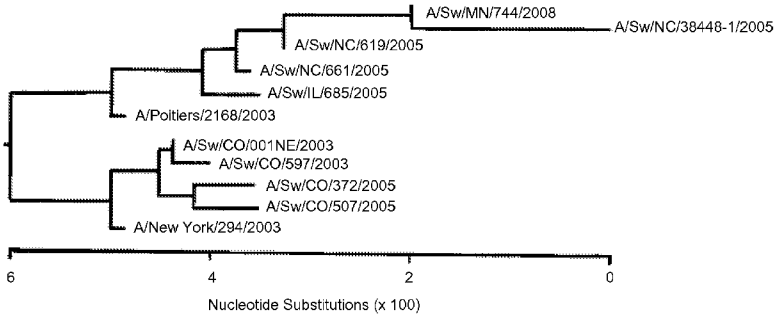
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) **Title**: COMPOSITIONS AND METHODS FOR VACCINATING HUMANS AND ANIMALS AGAINST ENVELOPED VIRUSES

Figure 1



(57) **Abstract**: Immunogenic compositions and methods for vaccinating humans and animals against enveloped viruses, and more particularly against influenza viruses. Immunogenic compositions are composed of internal moieties of an enveloped virus. Such internal moieties are more highly conserved between different viral strains than are their external moiety counterparts. Consequently, these immunogenic compositions have an increased likelihood of generating immuno-protective responses in humans and animals against enveloped viruses that are strain independent. Methods are also disclosed that increase the ability of the immunogenic compositions to generate immuno-protective responses against their target viruses.

WO 2011/130652 A2

COMPOSITIONS AND METHODS FOR VACCINATING HUMANS AND ANIMALS AGAINST ENVELOPED VIRUSES

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 61/324,703, filed April 15, 2010, the entire contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Eukaryotic viruses represent a large and diverse group of infectious agents known for the wide variety of diseases they cause. Based on their viral particle structure, viruses may be broadly classified as being either "non-enveloped" or "enveloped." Non-enveloped viruses contain a viral genome that is surrounded by a proteinaceous capsid, which is formed by products encoded by the viral genome and synthesized by the machinery of the host cell. Enveloped viruses also have a viral genome surrounded by a proteinaceous capsid, however, the capsid is further encapsulated within an "envelope" comprised of a protein containing phospholipid bilayer. This envelope is acquired as the viral capsid buds through the cell membrane, and is usually derived from the outer membrane of the host cell, but may also be derived from the nuclear membrane, Golgi apparatus, or endoplasmic reticulum.

Enveloped viruses represent a diverse family of viruses that include, but are not limited to, arenaviridae, arteriviridae, asfarviridae, baculoviridae, bornaviridae, bunyaviridae, coronaviridae, filoviridae, flaviviridae, hepadnaviridae, herpesviridae, orthomyxoviridae, paramyxoviridae, poxviridae, retroviridae, rhabdoviridae, and togaviridae. These virus families are responsible for a wide variety of human and animal diseases including, but not limited to, encephalitis, gastro-intestinal disease, hemorrhagic disease, hepatitis, immunosuppressive diseases, ocular disease, pox (*e.g.* chickenpox, cowpox, smallpox, monkeypox, felinepox, swinepox, and pseudo-cowpox), respiratory disease, sexually transmitted disease, and cancer, and result in billions of infections, and millions of deaths, world wide every year. For example, infections with influenza viruses in humans are a common and significant cause of respiratory disease and result in an average of approximately 20,000 deaths and 114,000 hospitalizations

per year in the U.S. alone. Severe outbreaks of influenza periodically affect millions of people. In one instance, the 1918-1919 Spanish flu pandemic was responsible for killing an estimated 20 million people. In another instance, 80% of the U.S. Army's casualties during World War I were attributed to influenza.

Vaccinating humans and animals against influenza viruses is difficult because the virally encoded proteins present in the envelope, which are the primary targets of the immune system, undergo significant antigenic variation as a result of the processes of antigenic drift and antigenic shift. Antigenic "drift" involves minor antigenic changes in the envelope proteins hemagglutinin (HA) and neuraminidase (NA), while antigenic "shift" involves major antigenic changes in these molecules. Changes in the conformation of these two antigens are accompanied by changes in antigenicity, which facilitates the ability of the viral particle to evade detection by the immune system. The combination of antigenic drift and shift allows the genetic constitution of influenza particles to change very rapidly.

In the United States vaccines are prepared each year for the annual vaccination program, with the quantity of immunoreactive HA in each dose standardized to contain the amount recommended by the Bureau of Biologics, U.S. Food and Drug Administration (FDA). The resistance to infection after vaccine administration correlates with the level of serum antibody produced to the specific strain active that year. In other words, antigenic changes within the HA and NA subtypes described above require concomitant changes in the vaccine, according to the type(s) of antigen present.

The challenge of vaccinating human and animal populations is further complicated by the fact that there is a growing complexity of influenza viruses operating at the human-animal interface. For example, the isolation of viruses with a seemingly high affinity for reassortment indicates that the U.S. swine population is an increasingly important reservoir of viruses that may have human pandemic potential. Recently, multiple lineages of antigenically and genetically diverse swine influenza viruses (SIV) have been identified in U.S. swine populations. As another example, reassortment between human and avian influenza viruses produced the strains that caused both the 1957 and 1968 human influenza pandemics. Based on evidence from the three pandemics that occurred during the 20th century, scientists have determined that pandemic flu strains

tend to infect between 25% and 35% of the population. In September 2005, the World Health Organization (WHO) estimated that a new influenza pandemic would likely result in the death of between 2 million and 7 million people. New disease challenges such as novel reassorted viruses and the use of viruses as bioterrorism agents can be devastating for both animal and human populations. Therefore there is an urgent need for technologies that can enhance the immune system, prevent or greatly reduce the shed of virus, and improve clinical outcome of the disease in animal and human populations in order to help reduce the pandemic potential of enveloped viruses, and influenza viruses in particular.

SUMMARY OF THE INVENTION

The present disclosure relates to compositions and methods for vaccinating humans and animals against enveloped viruses, and more particularly against influenza viruses.

In one aspect, the invention provides an immunogenic composition that includes an effective amount of at least one internal moiety of an enveloped virus, or antigenic fragment thereof, and a pharmaceutically acceptable carrier or excipient.

In one embodiment, the enveloped virus is from the arenaviridae family, the arteriviridae family, the asfarviridae family, the baculoviridae family, the bornaviridae family, the bunyaviridae family, the coronaviridae family, the filoviridae family, the flaviviridae family, the hepadnaviridae family, the herpesviridae family, the orthomyxoviridae family, the paramyxoviridae family, the poxviridae family, the retroviridae family, the rhabdoviridae family, and the togaviridae family. In a preferred embodiment, the enveloped virus is influenza.

In one embodiment, the internal moiety is any one or more viral polypeptides or polynucleotides selected from the group consisting of the RNA genome (RNAg), nucleoprotein (NP), RNA polymerase (PA), RNA polymerase 1 (PB1), RNA polymerase 2 (PB2), non-structural protein 1(NS1), non-structural protein 2 (NS2), and matrix protein 1 (M1).

In a preferred embodiment, the at least one internal moiety includes NP. In another preferred embodiment, the at least one internal moiety includes NP and RNAg. In yet another preferred embodiment, the at least one internal moiety is NP. In another preferred embodiment, the at least one internal moiety is NP and

RNAg.

In another aspect, the invention provides an immunogenic composition that includes an effective amount of a naked viral core particle of an enveloped virus and a pharmaceutically acceptable carrier or excipient.

In one embodiment, the enveloped virus is from the arenaviridae family, the arteriviridae family, the asfarviridae family, the baculoviridae family, the bornaviridae family, the bunyaviridae family, the coronaviridae family, the filoviridae family, the flaviviridae family, the hepadnaviridae family, the herpesviridae family, the orthomyxoviridae family, the paramyxoviridae family, the poxviridae family, the retroviridae family, the rhabdoviridae family, and the togaviridae family. In a preferred embodiment, the enveloped virus is influenza.

In one embodiment, the naked viral core particle includes any one or more viral polypeptides or polynucleotides from the following group: the RNA genome (RNAg), nucleoprotein (NP), RNA polymerase (PA), RNA polymerase 1 (PB1), RNA polymerase 2 (PB2), non-structural protein 1(NS1), and non-structural protein 2 (NS2).

In one embodiment, the naked viral core particle includes at least three viral polypeptides or polynucleotides from the following group: RNAg, NP, PA, PB1, PB2, NS1, and NS2. In a preferred embodiment, the naked viral core particle contains NP, NS1, and RNAg. In another preferred embodiment, the naked viral core particle contains NP, NS2, and RNAg. In yet another preferred embodiment, the naked viral core particle contains NP, NS1, NS2, and RNAg.

In another aspect, the invention provides an immunogenic composition comprising an effective amount of at least one virus like particle (VLP) of an enveloped virus and a pharmaceutically acceptable carrier or excipient.

In one embodiment, the enveloped virus is from the arenaviridae family, the arteriviridae family, the asfarviridae family, the baculoviridae family, the bornaviridae family, the bunyaviridae family, the coronaviridae family, the filoviridae family, the flaviviridae family, the hepadnaviridae family, the herpesviridae family, the orthomyxoviridae family, the paramyxoviridae family, the poxviridae family, the retroviridae family, the rhabdoviridae family, and the togaviridae family. In a preferred embodiment, the enveloped virus is influenza.

In one embodiment, the VLP includes any one or more viral polypeptides or polynucleotides, or antigenic fragments thereof, selected from the group consisting of RNA genome (RNAg), nucleoprotein (NP), RNA polymerase (PA), RNA polymerase

1 (PB1), RNA polymerase 2 (PB2), non-structural protein 1(NS1), and non-structural protein 2 (NS2). In another embodiment, the antigenic fragment is an N-terminal, a C-terminal, or an internal polypeptide fragment.

In another aspect, the invention provides an immunogenic composition comprising an effective amount of at least one recombinant fusion protein, or an antigenic fragment thereof, of an enveloped virus and a pharmaceutically acceptable carrier or excipient. In one embodiment, the at least one recombinant fusion protein includes a protein from the following group: nucleoprotein (NP), RNA polymerase (PA), RNA polymerase 1 (PB1), RNA polymerase 2 (PB2), non-structural protein 1(NS1), non-structural protein 2 (NS2), and/or matrix protein 1 (M1).

In one embodiment, the at least one recombinant fusion protein is joined with a viral polypeptide, or antigenic fragment thereof, selected from the group of viral proteins that includes: hemagglutinin (HA), neuraminidase (NA), and matrix protein 2 (M2). In another embodiment, the at least one recombinant fusion protein is NP, or an antigenic fragment thereof, joined to HA, or an antigenic fragment thereof.

In another aspect, the invention provides a method of synthesizing naked viral core particles from enveloped viruses that involves producing live virus from a host, purifying the live virus from the host, and removing the envelope from the purified live virus.

In another aspect, the invention provides a method of synthesizing naked viral core particles from enveloped viruses that involves producing live virus from a cell culture system, purifying the live virus from the cell culture system, and removing the envelope from the purified live virus.

In another aspect, the invention provides a method of synthesizing naked viral core particles that involves producing *in vitro* the genome and protein components of the virus of interest corresponding to the desired internal moieties.

In another aspect, the invention provides a method of inducing immunity against an enveloped virus in a mammal that involves administering the mammal with an immunologically effective amount of an immunogenic composition as described above. In one embodiment, the method of administration is selected from the group consisting of topical, oral, anal, vaginal, mucosal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal, nasopharyngeal, buccal, and intradermal administration. In a preferred embodiment, the method of administration is intradermal.

In another aspect, the invention provides a method of generating an immune response in a subject, the method comprising administering to said mammal an antigenic amount of any of the above described immunogenic compositions.

In another aspect, the invention provides an influenza vaccine that includes a therapeutically or prophylactically effective amount of a naked viral core particle, which may include any combination of one or more of the RNA genome (RNAg), nucleoprotein (NP), RNA polymerase (PA), RNA polymerase 1 (PB1), RNA polymerase 2 (PB2), non-structural protein 1(NS1), and non-structural protein 2 (NS2); a pharmaceutically acceptable carrier or excipient; and, a pharmaceutically acceptable adjuvant.

In another aspect, the invention provides a method of vaccinating a mammal against influenza that involves administering to the mammal intradermally a vaccine including: a therapeutically or prophylactically effective amount of a naked viral core particle, which may include any combination of one or more of the RNA genome (RNAg), nucleoprotein (NP), RNA polymerase (PA), RNA polymerase 1 (PB1), RNA polymerase 2 (PB2), non-structural protein 1(NS1), and non-structural protein 2 (NS2); a pharmaceutically acceptable carrier or excipient; and, a pharmaceutically acceptable adjuvant.

The present disclosure provides compositions comprised of naked viral core particles that recapitulate the internal moieties of an enveloped virus, including the viral genome and its genome associated proteins. Advantageously, such internal moieties generally display higher levels of amino acid conservation between strains than the external moieties associated with viral envelope proteins; therefore, the use of vaccine compositions comprising such internal moieties may provide immuno-protection against enveloped viruses that is independent of strain variation.

The present disclosure also provides compositions that are derived from naked viral core particles, including: virus like particles (viral genome in combination with one or more internal moieties), internal viral proteins (purified internal moieties, individually or in combination), derivative internal viral proteins (deletion derivatives of internal moieties, alone or in combination), and recombinant fusion proteins (internal moieties fused with extrinsic proteins or protein fragments).

The present disclosure further relates to a method of administering compositions containing naked viral core particles or naked viral core particle derivatives via an intradermal route.

DEFINITIONS

By "enveloped virus" is meant any virus in which the capsid is encapsulated within a phospholipid bilayer. The bilayer that may, or may not, contain host and/or virally encoded proteins. For example, enveloped viruses may belong to the family of viruses that includes, but is not limited to, arenaviridae, arteriviridae, asfarviridae, baculoviridae, bornaviridae, bunyaviridae, coronaviridae, filoviridae, flaviviridae, hepadnaviridae, herpesviridae, orthomyxoviridae, paramyxoviridae, poxviridae, retroviridae, rhabdoviridae, and togaviridae.

By "influenza virus" is meant any virus belonging to the orthomyxoviridae genera. Exemplary influenza viruses include, but are not limited to, influenza A, influenza B, and influenza C. Of these, the most virulent human pathogen is influenza A, which is a negative stranded, segmented, enveloped RNA virus comprised of a helical ribonucleocapsid known as the viral ribonucleoprotein (vRNP). The vRNP contains a genome comprised of eight negative sense RNA segments that encode eleven viral proteins, including: hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), M 1 , M2, NS1, NS2 (aka NEP), PA, PB1, PB1-F2, and PB2. The genome segments are packed in a helical form with NP, and the resulting RNP structures are associated with the three individual subunits (PB1, PB2, and PA) of the viral polymerase, which is known as the 3P complex in its assembled form. The vRNP may also be associated with NS2. Viral particles are packaged when RNPs accumulate at the cell surface near regions of the host cell membrane that have accumulated the viral transmembrane envelope proteins, HA, NA, and M2. The virus particle acquires its envelope as it buds from the host cell. HA trimers and NA tetramers display prominent glycoprotein spikes on the exterior surface of the envelope, with HA being the predominant envelope protein. The M2 protein encodes an ion channel, and is present in the envelope at a very low concentration estimated to be 16-20 molecules per virion. The viral protein M1 is located on the interior surface of the viral envelope, and is believed to play a role in linking the internal domains of HA and NA with the RNP. The morphology of influenza viral particles is highly variable, ranging from discrete spherical particles to filamentous particles that can be up to 2000 nm long. The most striking feature of influenza virions is the dense

layer of HA and NA spikes projecting radially outward over the surface of the viral envelope, and the epitopes encoded by these spikes represent important targets for the immune system of the host (*e.g.* animal or human).

Influenza subtypes include, but are not limited to, fifteen HA subtypes (H1-H15) and nine NA subtypes (N1-N9) that have been identified to date. Characterized influenza A subtypes include, but are not limited to, H1N1 (1918 Spanish flu pandemic), H2N2 (1957 Asian flu pandemic), H3N2 (1968 Hong Kong flu pandemic), H5N1 (current pandemic threat), H7N7 (current zoonotic threat), H1N2 (endemic in humans and swine), H9N2, H7N2, H7N3, and H10N7.

By "internal moiety" is meant any protein or polynucleotide component of an enveloped virus internal to the viral envelope (*i.e.* not present in the envelope) or antigenic fragment thereof. For example, internal moieties of influenza include the viral genome and its genome associated proteins, and antigenic fragments. Exemplary internal moieties include, but are not limited to the RNAg, NP, PB1, PB2, PA, NS1, NS2, and/or M1.

By "naked viral core particle (NVCP)" is meant an enveloped virus that has been stripped of its envelope and isolated. An influenza NVCP may be comprised of the viral genome (RNAg) in combination with one or more associated proteins, but does not include the viral envelope. For example, an influenza NVCP may include RNAg, NP, PB1, PB2, PA, and/or NS2.

By "virus-like particles (VLP)" is meant a structure that in at least one attribute resembles a virus, but which has not been demonstrated to be infectious. In general, virus-like particles lack a complete viral genome and, therefore, are noninfectious. In addition, virus-like particles can often be produced in large quantities by heterologous expression and can be easily purified.

For example, an influenza VLP composition may be comprised of RNAg and NP, RNAg and PB1, RNAg and PB2, RNAg and PA, or RNAg and NS2. As another example, an influenza VLP composition may also include, but is not limited to, RNAg and NP plus PB1, or RNAg and NP plus PB2, or RNAg and NP plus PA, or RNAg and NP plus NS2.

By "recombinant fusion protein (RFP)" is meant any fusion of two polypeptides, or fragments thereof that are not naturally contiguous. Exemplary fusion proteins include N-terminal or C-terminal fusions of NP, PB1, PB2, PA, and NS2 with proteins or protein fragments that may increase the

immunogenicity of the resulting recombinant protein. The term also encompasses the fusion of an internal fragment of NP, PB1, PB2, PA, and NS2 with proteins or protein fragments that may increase the immunogenicity of the resulting recombinant protein. For example, candidate proteins or protein fragments for use with influenza specific compositions may include, or be derived from, hemagglutinin (HA), neuraminidase (NA), and matrix protein 2 (M2).

By "nucleoprotein (NP) nucleic acid molecule" is meant a polynucleotide encoding a NP polypeptide. An exemplary NP nucleic acid molecule is provided at NCBI Accession No. NC_002019.1. Additional exemplary NP nucleic acid molecules include, but are not limited to, the following strain specific variant transcripts: variant 2, NCBI Accession No. NC_007360.1 and variant 3, NCBI Accession No. NC_007360.1.

By "nucleoprotein (NP) polypeptide" is meant a polypeptide, or fragment thereof, having at least about 85% amino acid identity to NP_040982.1, and having nucleic acid binding activity. Additional exemplary NP polypeptide molecules include, but are not limited to, the following strain specific variants: polypeptide variant 2, NCBI Accession No. YP_308667.1 and polypeptide variant 3, NCBI Accession No. YP_308843.1.

By "PB1 nucleic acid molecule" is meant a polynucleotide encoding a PB1 polypeptide. An exemplary PB1 nucleic acid molecule is provided at NCBI Accession No. NC_002021.1. Additional exemplary PB1 nucleic acid molecules include, but are not limited to, the following strain specific variant transcripts: variant 2, NCBI Accession No. NC_007358.1 and variant 3, NCBI Accession No. NC_007375.1.

By "PB1 polypeptide" is meant a polypeptide, or fragment thereof, having at least about 85% amino acid identity to NP_040985.1, and having RNA polymerase activity. Additional exemplary PB1 polypeptide molecules include, but are not limited to, the following strain specific variants: polypeptide variant 2, NCBI Accession No. YP_308665.1 and polypeptide variant 3, NCBI Accession No. YP_308851.1.

By "PB2 nucleic acid molecule" is meant a polynucleotide encoding a PB2 polypeptide. An exemplary PB2 nucleic acid molecule is provided at NCBI Accession No. NC_002023.1. Additional exemplary PB2 nucleic acid molecules include, but are not limited to, the following strain specific variant transcripts: variant

2, NCBI Accession No. NC_007357.1 and variant 3, NCBI Accession No. NC_007373.1.

By "PB2 polypeptide" is meant a polypeptide, or fragment thereof, having at least about 85% amino acid identity to NP_040987.1, and having RNA polymerase activity. Additional exemplary PB2 polypeptide molecules include, but are not limited to, the following strain specific variants: polypeptide variant 2, NCBI Accession No. YP_308664.1 and polypeptide variant 3, NCBI Accession No. YP_308849.1.

By "PA nucleic acid molecule" is meant a polynucleotide encoding a PA polypeptide. An exemplary PA nucleic acid molecule is provided at NCBI Accession No. NC_002022.1. Additional exemplary PA nucleic acid molecules include, but are not limited to, the following strain specific variant transcripts: variant 2, NCBI Accession No. NC_007359.1 and variant 3, NCBI Accession No. NC_007376.1.

By "PA polypeptide" is meant a polypeptide, or fragment thereof, having at least about 85% amino acid identity to NP_040986.1, and having RNA polymerase activity. Additional exemplary PA polypeptide molecules include, but are not limited to, the following strain specific variants: polypeptide variant 2, NCBI Accession No. YP_308666.1 and polypeptide variant 3, NCBI Accession No. YP_308852.1.

By "non structural protein 2 (NS2) nucleic acid molecule" is meant a polynucleotide encoding a NS2 polypeptide. An exemplary NS2 nucleic acid molecule is provided at NCBI Accession No. NC_002020.1. Additional exemplary NS2 nucleic acid molecules include, but are not limited to, the following strain specific variant transcripts: variant 2, NCBI Accession No. NC_004906.1 and variant 3, NCBI Accession No. NC_007370.1.

By "non structural protein 2 (NS2) polypeptide" is meant a polypeptide, or fragment thereof, having at least about 85% amino acid identity to NP_040983.1, and having antigenic activity. Additional exemplary NS2 polypeptide molecules include, but are not limited to, the following strain specific variants: polypeptide variant 2, NCBI Accession No. YP_581750.1 and polypeptide variant 3, NCBI Accession No. YP_308844.1.

By "matrix protein 1 (M1) nucleic acid molecule" is meant a polynucleotide encoding a M1 polypeptide. An exemplary M1 nucleic acid molecule is provided at NCBI Accession No. NC_002016.1. Additional exemplary M1 nucleic acid molecules include, but are not limited to, the following strain specific variant

transcripts: variant 2, NCBI Accession No. NC_007377.1 and variant 3, NCBI Accession No. NC_007363.1.

By "matrix protein 1 (M1) polypeptide" is meant a polypeptide, or fragment thereof, having at least about 85% amino acid identity to NP_040978.1, and having nucleic acid binding activity. Additional exemplary M1 polypeptide molecules include, but are not limited to, the following strain specific variants: polypeptide variant 2, NCBI Accession No. YP_308854.1 and polypeptide variant 3, NCBI Accession No. YP_308671.1.

By "non structural protein 1 (NS1) nucleic acid molecule" is meant a polynucleotide encoding a NS2 polypeptide. An exemplary NS1 nucleic acid molecule is provided at NCBI Accession No. NC_002020.1. Additional exemplary NS1 nucleic acid molecules include, but are not limited to, the following strain specific variant transcripts: variant 2, NCBI Accession No. NC_007370.1 and variant 3, NCBI Accession No. NC_004906.1.

By "non structural protein 1 (NS1) polypeptide" is meant a polypeptide, or fragment thereof, having at least about 85% amino acid identity to NP_040984.1, and having nucleic acid binding activity. Additional exemplary NS1 polypeptide molecules include, but are not limited to, the following strain specific variants: polypeptide variant 2, NCBI Accession No. YP_308845.1 and polypeptide variant 3, NCBI Accession No. NP_859034.1.

By "RNAg" is meant the nucleic acid segments, or subsets thereof, that comprise the viral genome and encode the viral polypeptides. An exemplary RNAg, or subset thereof, has at least 85%, 90%, 95%, or 100% identity to CY080633.1, CY080632.1, CY080631.1, CY080626.1, CY080629.1, CY080628.1, CY080627.1, and CY080630.1

As used herein, the singular forms "a", "an", and "the" include plural forms unless the context clearly dictates otherwise.

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. The term "about" can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term "about."

As used herein, the terms "comprises," "comprising," "containing," "having" and

the like can have the meaning ascribed to them in U.S. Patent law and can mean "includes," "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including, but not limited to."

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive.

The term "adjuvant" as used herein refers to a compound or mixture that enhances the immune response and/or promotes the proper rate of absorption following inoculation, and, as used herein, encompasses any uptake-facilitating agent. Acceptable adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol, and others. The term refers to a compound or mixture that enhances the immune response and/or promotes the proper rate of absorption following inoculation, and, as used herein, encompasses any uptake-facilitating agent. Acceptable adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, alum, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol, monophosphoryl lipid A, and others.

By "alteration" is meant any change in a nucleic acid or amino acid sequence relative to a reference sequence.

By "ameliorate" is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

By "analog" is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a corresponding naturally-occurring polypeptide, while having certain biochemical modifications that enhance the analog's function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog's protease resistance, membrane permeability, or half-life, without altering, for example, ligand

binding. An analog may include an unnatural amino acid.

The term "capsid" is meant to refer to the protein shell of the virus. In embodiments, the capsid refers to the protein shell of the orthomyxovirus. A viral capsid may consist of multimers of oligomeric protein subunits.

By "disease" is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ.

The term "epithelial surface" is meant to refer to a continuous sheet of one or more cellular layers that lines a vertebrate body compartment. An epithelial surface can be the skin. Epithelial surfaces according to certain embodiments of the invention can be cervicovaginal, oral, nasal, penile, anal, epidermal and respiratory surfaces.

The term "expression vector" is meant to refer to a vector, such as a plasmid or viral particle, which is capable of promoting expression of a foreign or heterologous nucleic acid incorporated therein. In embodiments, the nucleic acid to be expressed is "operably linked" to a promoter and/or enhancer, and is subject to transcription regulatory control by the promoter and/or enhancer.

The term "fragment" is meant to refer to a portion of a protein or nucleic acid that is substantially identical to a reference protein or nucleic acid. In embodiments, the fragment is a fragment of a gene. In embodiments, the fragment is a fragment of a viral gene. In embodiments, the fragment is a fragment of a viral protein. In embodiments, the portion can retain at least 50%, 75%, or 80%, or more preferably 90%, 95%, or even 99% of the biological activity of the reference protein or nucleic acid described herein. In other embodiments, the fragment comprises at least 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids of a reference protein or is a nucleic acid molecule encoding such a fragment.

The term "host" as used herein refers to an animal, preferably a mammal, and most preferably a human. In embodiments, the term host cell refers to a cell that contains a heterologous nucleic acid, such as a vector, and supports the replication or expression of the nucleic acid. In certain examples, host cells can be prokaryotic cells such as E. coli, or eukaryotic cells such as yeast, insect, amphibian, avian or mammalian cells, including human and porcine cells. Exemplary host cells include, but are not limited to, 293TT, 293ORF6, PERC.6, CHO, HEp-2, HeLa, BSC40, Vero, BHK-21, 293, C12 immortalized cell lines and primary mouse or human dendritic cells.

By "immunogenic composition" is meant an agent that induces an immune response in a subject.

By "immune response" is meant any cellular or humoral response against an

antigen.

The term "in combination" in the context of the administration of immunogenic compositions is meant to refer to the use of more than one immunogenic agent. In embodiments, an immunogenic composition and an agent or treatment to disrupt an epithelial surface are administered. The use of the term "in combination" does not restrict the order in which agents or therapies are administered to a subject with an infection. A first agent or therapy can be administered before (*e.g.*, 1 minute, 45 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks), concurrently, or after (*e.g.*, 1 minute, 45 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks) the administration of a second agent or therapy to a subject. Any additional agent or therapy can be administered in any order with the other additional treatments. Non-limiting examples of therapies that can be administered in combination with the immunogenic compositions of the invention include analgesic agents, anesthetic agents, antibiotics, or immunomodulatory agents or any other agent listed in the U.S. Pharmacopoeia and/or Physician's Desk Reference.

By "isolated nucleic acid molecule" is meant a nucleic acid (*e.g.*, a DNA) that is free of the genes that, in the naturally occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences.

"Live attenuated virus" or "attenuated recombinant virus" refer to a virus that has been genetically altered by modern molecular biological methods, *e.g.*, restriction endonuclease and ligase treatment, and rendered less virulent than wild type virus. In embodiments, attenuation results from deletion of specific genes, by serial passage in a non-natural host cell line, or by serial passage at cold temperatures. In embodiments, the live attenuated virus is a live attenuated influenza virus.

The term "nucleic acid" or "nucleic acid segment" is meant to refer to an oligomer or polymer of ribonucleic acid or deoxyribonucleic acid, or analog thereof. This term includes oligomers consisting of naturally occurring bases, sugars, and intersugar (backbone) linkages as well as oligomers having non-naturally occurring portions which

function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of properties such as, for example, enhanced stability in the presence of nucleases. A nucleic acid segment can include a gene.

The term "pharmaceutically acceptable" as used herein means being approved by a regulatory agency of the Federal or a state government, or listed in the U.S. Pharmacopia, European Pharmacopia or other generally recognized pharmacopia for use in animals, *e.g.*, humans.

"Pharmaceutically acceptable excipient, carrier or adjuvant" refers to an excipient, carrier or adjuvant that can be administered to a subject, together with an agent, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the agent.

By "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification.

As used herein, the terms "prevent," "preventing," "prevention," "prophylactic treatment," and the like, refer to reducing the probability of developing a disease or condition in a subject, who does not have, but is at risk of or susceptible to developing a disease or condition, *e.g.*, viral infection.

"Potentiating" or "enhancing" an immune response means increasing the magnitude and/or the breadth of the immune response, *e.g.*, the number of cells induced by a particular epitope may be increased and/or the numbers of epitopes that are recognized may be increased. Preferably, the immune response is enhanced 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, or greater after administration of the immunogenic compositions described herein. Such enhancement may also be measured relative to a control subject where the immune response was not enhanced. In one embodiment, such an "enhanced immune response" refers to an increase in the immune response in a subject by at least 10% relative to a control subject where the immune response was not enhanced. Preferably, the immune response is increased by 25%, 50%, 75% or 100%.

The term "promoter" refers to a DNA sequence that is recognized by RNA polymerase and initiates transcription.

A "retrovirus" is a virus containing an RNA genome and an enzyme, reverse transcriptase, which is an RNA-dependent DNA polymerase that uses an RNA molecule as a template for the synthesis of a complementary DNA strand. The DNA form of a retrovirus commonly integrates into the host-cell chromosomes and remains part of the host

cell genome for the rest of the cell's life. Non-limiting examples of retroviruses include lentiviruses such as HIV and SIV.

By "subject" is meant a mammal, such as a human patient or an animal (*e.g.*, a rodent, bovine, equine, porcine, ovine, canine, feline, or other domestic mammal).

A "therapeutically effective amount" is an amount sufficient to effect a beneficial or desired clinical result. For example, a therapeutically effective amount is an amount sufficient to induce an immune response that prevents or treats an influenza infection.

By "treat" is meant to stabilize, reduce, or ameliorate the symptoms of any disease or disorder. It will be appreciated that, although not precluded, treating a disease or condition does not require that the disease, condition, or symptoms associated therewith be completely eliminated.

The term "vector" is meant to refer to the means by which a nucleic acid can be propagated and/or transferred between organisms, cells, or cellular components. Vectors include plasmids, viruses, bacteriophage, pro-viruses, phagemids, transposons, and artificial chromosomes, and the like, that replicate autonomously or can integrate into a chromosome of a host cell. A vector can also be a naked RNA polynucleotide, a naked DNA polynucleotide, a polynucleotide composed of both DNA and RNA within the same strand, a poly-lysine-conjugated DNA or RNA, a peptide-conjugated DNA or RNA, a liposome-conjugated DNA, or the like, that are not autonomously replicating.

The term "vector priming" is meant to refer to the delivery of a gene encoding a vaccine antigen by way of an expression vector. In embodiments, it means that the vector-based gene delivery will be a first exposure to the immunogenic composition, followed by one or more subsequent "booster" dose or doses of immunogenic composition.

"Viral load" is the amount of virus present in the blood of a patient. Viral load is also referred to as viral titer or viremia. Methods for evaluating viral load are well-known in the art.

Any compounds, compositions, or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

DESCRIPTION OF DRAWINGS

The abovementioned and other features and advantages of the present disclosure will be better understood when reading the following detailed description taken together with the following drawings in which:

Figure 1 depicts a phylogenetic tree representing 2003 and 2005 HA subtypes.

Human-Swine reassortant viruses and selected human influenza virus reference strains. Nucleotide sequences were aligned via Clustal W software and phylograms created by the Phylip program. The scale is number of nucleotide substitutions per 100.

Figure 2 depicts an experimental timeline for testing vaccine dose response curves.

Figure 3A and 3B depict an exemplary NP encoding nucleotide sequence and polypeptide, respectively.

Figure 4A and 4B depict an exemplary variant 2 NP encoding nucleotide sequence and polypeptide, respectively.

Figure 5A and 5B depict an exemplary variant 3 NP encoding nucleotide sequence and polypeptide, respectively.

Figure 6A and 6B depict an exemplary PB1 encoding nucleotide sequence and polypeptide, respectively.

Figure 7A and 7B depict an exemplary variant 2 PB1 encoding nucleotide sequence and polypeptide, respectively.

Figure 8A and 8B depict an exemplary variant 3 PB1 encoding nucleotide sequence and polypeptide, respectively.

Figure 9A and 9B depict an exemplary PB2 encoding nucleotide sequence and polypeptide, respectively.

Figure 10A and 10B depict an exemplary variant 2 PB2 encoding nucleotide sequence and polypeptide, respectively.

Figure 11A and 11B depict an exemplary variant 3 PB2 encoding nucleotide sequence and polypeptide, respectively.

Figure 12A and 12B depict an exemplary PA encoding nucleotide sequence and polypeptide, respectively.

Figure 13A and 13B depict an exemplary variant 2 PA encoding nucleotide sequence and polypeptide, respectively.

Figure 14A and 14B depict an exemplary variant 3 PA encoding nucleotide sequence and polypeptide, respectively.

Figure 15A and 15B depict an exemplary NS2 encoding nucleotide sequence and polypeptide, respectively.

Figure 16A and 16B depict an exemplary variant 2 NS2 encoding nucleotide sequence and polypeptide, respectively.

Figure 17A and 17B depict an exemplary variant 3 NS2 encoding nucleotide sequence and polypeptide, respectively.

Figure 18A and 18B depict an exemplary M1 encoding nucleotide sequence and polypeptide, respectively.

Figure 19A and 19B depict an exemplary variant 2 M1 encoding nucleotide sequence and polypeptide, respectively.

Figure 20A and 20B depict an exemplary variant 3 M1 encoding nucleotide sequence and polypeptide, respectively.

Figure 21A and 21B depict an exemplary NS1 encoding nucleotide sequence and polypeptide, respectively.

Figure 22A and 22B depict an exemplary variant 2 NS1 encoding nucleotide sequence and polypeptide, respectively.

Figure 23A and 23B depict an exemplary variant 3 NS1 encoding nucleotide sequence and polypeptide, respectively.

Figure 24 depicts an RNAg sequence corresponding to segment 1 of an exemplary influenza A genome.

Figure 25 depicts an RNAg sequence corresponding to segment 2 of an exemplary influenza A genome.

Figure 26 depicts an RNAg sequence corresponding to segment 3 of an exemplary influenza A genome.

Figure 27 depicts an RNAg sequence corresponding to segment 4 of an exemplary influenza A genome.

Figure 28 depicts an RNAg sequence corresponding to segment 5 of an exemplary influenza A genome.

Figure 29 depicts an RNAg sequence corresponding to segment 6 of an exemplary influenza A genome.

Figure 30 depicts an RNAg sequence corresponding to segment 7 of an exemplary influenza A genome.

Figure 31 depicts an RNAg sequence corresponding to segment 8 of an exemplary influenza A genome.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure relates to compositions and methods for vaccinating humans and animals against enveloped viruses. More particularly, the present

disclosure relates to compositions and methods for vaccinating humans and animals against influenza.

Eukaryotic viruses represent a large and diverse group of infectious agents known for the wide variety of diseases they cause. Viruses may be classified by a variety of criteria including genome structure (*e.g.* linear single strand or double strand DNA, circular double strand DNA, plus or minus strand RNA, or segmented RNA), capsid symmetry (*e.g.* helical, icosahedral, or complex), mode of replication (*e.g.* asymmetric, bidirectional/asymmetric, circularization/rolling circle, and concatemerization/asymmetric, reverse transcription, *etc.*), host specificity, and viral particle structure.

Based on their viral particle structure, viruses may be broadly classified as being either "non-enveloped" or "enveloped." Non-enveloped viruses contain a viral genome that is surrounded by a proteinaceous capsid, which is formed by products encoded by the viral genome and synthesized by the machinery of the host cell. Enveloped viruses also have a viral genome surrounded by a proteinaceous capsid, however, the capsid is further encapsulated within an "envelope" comprised of a protein containing phospholipid bilayer. This envelope is acquired as the viral capsid buds through the cell membrane, and is usually derived from the outer membrane of the host cell, but may also be derived from the nuclear membrane, Golgi apparatus, or endoplasmic reticulum.

Enveloped viruses contain a variety of proteins within the phospholipid bilayer of their envelope that include both host cell encoded proteins (*e.g.* integral and trans-membrane proteins) and viral encoded proteins (*e.g.* glycoproteins). The composition of host cell encoded proteins found in the envelope of a particular enveloped virus varies with the cell type of the particular host cell that has been infected. The envelope serves an important function during viral replication by facilitating the entry of a virus particle into a new host cell. In this capacity, the viral glycoproteins within the envelope may function to recognize and bind specific molecules on the cell surface membrane of the target cell. For example, these molecules may include, but are not limited to, receptors, proteins, and lipids. Viral glycoprotein binding may then mediate fusion of the viral envelope with the target cell membrane and entry of the virus into the cell (*e.g.* by receptor-mediated endocytosis or direct fusion).

Enveloped viruses represent a diverse family of viruses that include, but are not limited to, arenaviridae, arteriviridae, asfarviridae, baculoviridae,

bornaviridae, bunyaviridae, coronaviridae, filoviridae, flaviviridae, hepadnaviridae, herpesviridae, orthomyxoviridae, paramyxoviridae, poxviridae, retroviridae, rhabdoviridae, and togaviridae. These virus families are responsible for a wide variety of human and animal diseases including, but not limited to, encephalitis, gastro-intestinal disease, hemorrhagic disease, hepatitis, immunosuppressive diseases, ocular disease, pox (*e.g.* chickenpox, cowpox, smallpox, monkeypox, felinepox, swinepox, and pseudo-cowpox), respiratory disease, sexually transmitted disease, and cancer, and result in billions of infections, and millions of deaths, world wide every year. For example, infections with influenza viruses in humans are a common and significant cause of respiratory disease and result in an average of approximately 20,000 deaths and 114,000 hospitalizations per year in the U.S. alone. Severe outbreaks of influenza periodically affect millions of people. In one instance, the 1918-1919 Spanish flu pandemic was responsible for killing an estimated 20 million people. In another instance, 80% of the U.S. Army's casualties during World War I were attributed to influenza.

Influenza belongs to the orthomyxoviridae family of viruses, which encompasses several genera including influenza A, influenza B, and influenza C. Of these, the most virulent human pathogen is influenza A, which is a negative stranded, segmented, enveloped RNA virus comprised of a helical ribonucleocapsid known as the viral ribonucleoprotein (vRNP). The vRNP contains a genome comprised of eight negative sense RNA segments that encode eleven viral proteins, including: hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), M 1 , M2, NS1, NS2 (aka NEP), PA, PB1, PB1-F2, and PB2. The genome segments are packed in a helical form with NP, and the resulting RNP structures are associated with the three individual subunits (PB1, PB2, and PA) of the viral polymerase, which is known as the 3P complex in its assembled form. The vRNP may also be associated with NS2. Viral particles are packaged when RNPs accumulate at the cell surface near regions of the host cell membrane that have accumulated the viral transmembrane envelope proteins, HA, NA, and M2. The virus particle acquires its envelope as it buds from the host cell. HA trimers and NA tetramers display prominent glycoprotein spikes on the exterior surface of the envelope, with HA being the predominant envelope protein. The M2 protein encodes an ion channel, and is present in the envelope at a very low concentration estimated to be 16-20 molecules per virion. The viral protein M1 is

located on the interior surface of the viral envelope, and is believed to play a role in linking the internal domains of HA and NA with the RNP.

The morphology of influenza viral particles is highly variable, ranging from discrete spherical particles to filamentous particles that can be up to 2000 nm long. The most striking feature of influenza virions is the dense layer of HA and NA spikes projecting radially outward over the surface of the viral envelope, and the epitopes encoded by these spikes represent important targets for the immune system of the host (*e.g.* animal or human).

HA and NA play essential roles in the viral replication process. HA is believed to facilitate virus binding by interacting with sialic sugars present on all glycoproteins and glycolipids on the cell surface of target epithelial cells. Once bound, viral particles enter the cell by endocytosis. Enzymatic or proteolytic cleavage of the HA protein is essential for viral infection, as cleaved HA allows the endocytosed particle to fuse with the endosomal membrane. The cleavage of HA is modulated by the number of basic amino acid residues located at the cleavage site, and this composition is important in determining overall virulence. NA is important during the shedding process, as this protein cleaves sialic acid residues, thereby facilitating the release of the viral particle.

Influenza viruses periodically undergo significant antigenic variation as a result of the processes of antigenic drift and antigenic shift. Antigenic "drift" involves minor antigenic changes in the envelope proteins hemagglutinin (HA) and neuraminidase (NA), while antigenic "shift" involves major antigenic changes in these molecules. Changes in the conformation of these two antigens are accompanied by changes in antigenicity, which facilitates the ability of the viral particle to evade detection by the immune system. The genomic changes associated with antigenic drift are facilitated by the fact that the 3P polymerase complex has no proofreading activity; consequently, the polymerase introduces errors into the genome during viral replication at a rate of approximately one base change error per genome per replication cycle. This represents a substantial rate of mutation. Furthermore, antigenic shift is facilitated by the division of the RNA genome into multiple segments because the infection of a single cell by multiple strains of influenza virus may lead to reassortment of RNA segments from multiple strains into a single viral particle, effectively "shuffling" the viral genome. The combination of antigenic drift and shift allows the genetic constitution of influenza particles to change very rapidly.

These changes in genetic composition are tracked within influenza virus nomenclature, which categorizes strains by their origin and antigenic constitution of their HA and NA loci. For example, a swine isolate from Iowa may be categorized A/swine/Iowa (H1N1), where the H and N refer to the HA and NA subtypes, respectively. Extent influenza subtypes include, but are not limited to, fifteen HA subtypes (H1-H15) and nine NA subtypes (N1-N9) that have been identified to date. Characterized influenza A subtypes include, but are not limited to, H1N1 (1918 Spanish flu pandemic), H2N2 (1957 Asian flu pandemic), H3N2 (1968 Hong Kong flu pandemic), H5N1 (current pandemic threat), H7N7 (current zoonotic threat), H1N2 (endemic in humans and swine), H9N2, H7N2, H7N3, and H10N7.

In the United States vaccines are prepared each year for the annual vaccination program, with the quantity of immunoreactive HA in each dose standardized to contain the amount recommended by the Bureau of Biologics, U.S. Food and Drug Administration (FDA). The resistance to infection after vaccine administration correlates with the level of serum antibody produced to the specific strain active that year. In other words, antigenic changes within the HA and NA subtypes described above require concomitant changes in the vaccine, according to the type(s) of antigen present.

Influenza A viruses have been isolated from a number of animal hosts including humans, birds, and pigs. Pandemic influenza in humans can be a zoonotic disease caused by transfer of influenza A viral genome segments from animal reservoirs. In recent years the prevalence of swine influenza virus (SI) has increased in U.S. swine populations and several new subtypes have been identified. Pigs are postulated to serve as the "missing vessel" hosts in which reassortment between avian and human viruses can generate genetically novel viruses with pandemic potential. Additionally, the likelihood of a human pandemic has increased dramatically with the natural transmission of the highly pathogenic avian influenza virus (H5N1) directly to humans.

SIV is an important etiological agent involved both in epizootic and enzootic forms of influenza. The swine disease is an important model for human influenza with similar clinical symptoms and pathogenesis that results in nearly 100% morbidity. Clinical signs of the epizootic form are a deep-dry, "barking" cough, fever above 42°C and anorexia. Sows infected during pregnancy may abort as a result of the high fever. Clinical signs of the enzootic form are coughing, fever, anorexia, and poor

performance. Human-like H1N1 and H1N2 reassortant SIVs have been isolated from pigs, and are associated with high morbidity.

When exposed to SIV under natural conditions, pigs develop antibodies to HA epitopes on the viral envelope via the humoral immune response. This humoral immunity appears to be subtype-and strain-specific, meaning H1N1-induced antibodies will not confer protection against an H3N2 infection and vice versa. In addition to the humoral response, natural exposure to SIV also generates anti-SIV antibodies via the cell-mediated immune (CMI) response. Interestingly, preliminary research in pigs suggests that the CMI response is directed primarily against non-envelope proteins, for example, NP and M1. In contrast, vaccination against SIV generates a markedly different response in pigs. As with natural exposure to the virus, vaccination induces a humoral immune response to the HA subtype of the vaccine strain; however, in contrast to the case with natural exposure, vaccination does not induce an appreciable CMI response. Furthermore, protection provided by vaccination is incomplete in the face of heterologous challenge, allowing infection and replication of virus, albeit at reduced levels. The observation that the CMI response appears to preferentially target non-envelope proteins is significant because viral nucleoproteins (including NP of influenza A) are often highly conserved between strains, unlike their protein counterparts within the viral envelope.

There are few examples in the art of the use of non-envelope protein compositions as the basis for viral vaccines and therapeutics. Dietzschold *et al.* (1987) attempted to use purified vRNP from the rabies virus (an enveloped virus belonging to the Rhabdoviridae family) as a vaccine to generate an immuno-protective response. Disadvantageously, this approach failed to protect mice against a viral challenge when injected intra-cerebrally, whereas a purified rabies envelope protein (G protein) provided effective immuno-protection against a live virus challenge when administered intra-cerebrally. Dietzschold *et al.* (1987) showed that vRNP from rabies could generate an immuno-protective response when administered intra-peritoneally. Unfortunately, intra-peritoneal administration of purified rabies vRNP was only capable of generating a homologous and/or heterologous protective response in approximately 80% of the mouse and raccoon populations tested, an unacceptably low efficacy rate. A further disadvantage of this work is that the vRNP purification protocol used by Dietzschold et al (1987) does not preclude the presence of contaminating envelope proteins. Even at undetectable levels, such proteins could exert immunogenic

responses in test subjects that would affect the interpretation of the observed results. These authors also attempted to use two different peptide fragments of the vRNP associated protein N as vaccines. Disadvantageously, one of these peptides failed to confer any immuno-protection, while the other conferred protection that was highly variable within the experimental population, ranging between 62-82%.

Ertl *et al.* (1989) tested a panel of 40 overlapping peptides derived from the N protein of rabies for efficacy as a vaccine, and found that some of these peptides were able to augment the production of rabies neutralizing antibodies. Unfortunately, none of the 40 peptides tested were able to immunize mice against a rabies viral challenge.

Sumner *et al.* (1991) demonstrated that the use of recombinant N protein (fusion with vaccinia TX protein) from rabies was able to immunize mice against a viral challenge with street rabies. A problem with this approach is that this immuno-protective response required inoculation with recombinant N protein at a much higher concentration (107 PFU vs. 103 PFU) then that required for inoculation with the rabies envelope protein G. A further disadvantage of these studies is that they rely on the use of a fusion protein.

Wraith *et al.* (1987) attempted to use purified NP protein to immunize mice against the influenza virus. Disadvantageously, these authors found that the use of purified NP protein protected only 75% of the test subjects from subsequent challenge by the influenza virus (Wraith *et al.* 1987). A further disadvantage of these studies arises from the fact that the authors acknowledge that their 'purified' NP protein samples may contain up to 3% contamination with the envelope protein HA.

According to an illustrative embodiment, compositions of the current disclosure comprise naked viral core particles (NVCPs) that may be used as vaccines to generate an immuno-protective response in humans and animals, or as therapeutics to reduce viral shedding and improve post-challenge clinical outcome of infected patients or animals. NVCPs may be comprised of the viral genome (RNAg), as well as various genome associated proteins, but lack the viral envelope and are avirulent as a result. For example, an influenza NVCP may be comprised of, but is not limited to, RNAg, NP, PB1, PB2, PA, and NS2.

NVCPs may be prepared by using an animal or cell culture system to produce virus that may be isolated and subjected to standard biochemical and molecular biological techniques to remove the envelope and envelope associated proteins. For example, influenza virus may be produced using cell culture systems

such as, but not limited to, the ones described in U.S. Patent No. 4,500,513 and U.S. Publication No. 2008/0274141, hereby incorporated by reference in their entirety. Once the influenza virus has been produced and purified, influenza NVCPs may be isolated using standard biochemical purification protocols (see *e.g.*, Schneider et al, 1973). One of ordinary skill in the art will recognize that the NVCP isolation protocols may vary substantially depending on the type of enveloped virus being purified.

It is also contemplated within the scope of the invention that NVCPs may be synthesized *de novo*. For example, the ssRNA genome segments may be synthesized using vectors that allow ssRNA production and purification, while the genes encoding the genome associated proteins may be subcloned into suitable expression vector systems. The resulting RNA segments and expressed proteins may then be combined to produce synthetic NVCPs.

Internal moieties associated with NVCPs generally display high levels of amino acid conservation across strains, relative to the highly variable moieties associated with envelope proteins. Advantageously, this high level of internal moiety conservation means that NVCPs may provide immuno-protection that is strain independent (*i.e.* both homologous and heterologous protection). A further advantage of NVCPs is that they may provide immuno-protection without compromising the efficacy of immuno-detection assays used to detect viral infection, which are generally based on serotypes generated against proteins within the viral envelope.

Without being bound by any particular theory, it is believed that NVCPs may induce immuno-protection by multiple, non-exclusive, mechanisms. One mechanism may be the direct production of antibodies against the nucleoprotein components via the humoral response. Another mechanism may be the production of antibodies against the nucleoprotein components by the CMI response. Yet another mechanism may be that NVCPs provide immuno-protection as a result of a direct induction of T-helper cells that augment the activity of virus neutralizing antibody producing B cells. Protection induced in this manner may be attributed to the priming of influenza nucleoprotein by specific cytolytic T cells, which represents a direct activation of cellular immunity by a purified, non-enveloped portion of the virus. Yet another possible immuno-protective mechanism may comprise the direct targeting of the viral genome by siRNA or RNAi mediated pathways by the ssRNA

components of the NVCP.

It is contemplated within the scope of the disclosure that compositions for vaccinating humans and animals against influenza may further comprise virus-like particles (VLPs) that consist of the viral genome in combination with any one of the genome associated proteins. For example, such VLP compositions may be comprised of RNAg and NP, RNAg and PB1, RNAg and PB2, RNAg and PA, or RNAg and NS2.

It is further contemplated within the scope of the disclosure that such VLP compositions may also be comprised of the viral genome in combination with any two genome associated proteins. For example, such VLP compositions may include, but are not limited to, RNAg and NP plus PB1, or RNAg and NP plus PB2, or RNAg and NP plus PA, or RNAg and NP plus NS2. It is still further contemplated that such VLP compositions may also be comprised of the viral genome in combination with any three, or any four, of the genome associated proteins.

The invention also provides constructs comprising a nucleic acid molecule and methods for producing a VLP comprising influenza polypeptides, or fragments thereof, in a nonpermissive cell type, as well as compositions and methods that increase the efficiency of VLP production in such cells. In various embodiments, the nucleic acid molecules are useful for *in vitro* or *in vivo* expression (*i.e.*, expression in a human or porcine subject having, or at risk of developing, an influenza infection). For example, the use of a cRNA promoter, or portions thereof, in an expression vector comprising a nucleic acid molecule of the invention can improve the efficiency of influenza protein production in a cell. In another example, a 3' UTR is included in the expression vector. A variety of expression systems exist for the production of the polypeptides of the invention. Expression vectors useful for producing such polypeptides include, without limitation, chromosomal, episomal, and virus-derived vectors, *e.g.*, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof.

Constructs and/or vectors provided herein comprise influenza

polynucleotides that encode structural polypeptides, or portions thereof as described herein. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. The constructs and/or vectors that comprise the nucleotides should be operatively linked to an appropriate promoter, such as the cRNA influenza promoter, CMV promoter, phage lambda PL promoter, the E. coli lac, phoA and tac promoters, the SV40 early and late promoters, and promoters of retroviral LTRs are non-limiting examples. In one embodiment, the promoter is an influenza cRNA promoter. Other suitable promoters will be known to the skilled artisan depending on the host cell and/or the rate of expression desired. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome-binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon appropriately positioned at the end of the polypeptide to be translated. If desired, the vector further comprises a 3' UTR, such as an influenza 3' UTR.

Expression vectors will typically include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Among vectors preferred are virus vectors, such as baculovirus, poxvirus (*e.g.*, vaccinia virus, avipox virus, canarypox virus, fowlpox virus, raccoonpox virus, swinepox virus, *etc.*), adenovirus (*e.g.*, canine adenovirus), herpesvirus, and retrovirus. Other vectors that can be used with the invention comprise vectors for use in bacteria, which comprise pQE70, pQE60 and pQE-9, pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5. Among preferred eukaryotic vectors are pFastBac1 pWINEO, pSV2CAT, pOG44, pXT1 and pSG, pSVK3, pBPV, pMSG, and pSVL. In particular embodiments, the vector is a bicistronic vector (*e.g.*, pIRES). Other suitable vectors will be readily apparent to the skilled artisan.

Recombinant constructs can be prepared and used to transfect, infect, or transform and can express viral proteins, including those described herein, into eukaryotic cells and/or prokaryotic cells. Thus, the invention provides for host cells which comprise a vector (or vectors) that contain nucleic acids which code for influenza proteins in a host cell under conditions which allow the formation of

VLPs.

In another embodiment, the vector and/or host cell comprise nucleotides that encode influenza proteins, or portions thereof as described herein. In another embodiment, the vector encodes a protein that consists essentially of influenza NP, PB1, PB2, PA, NS1, NS2, and M1, or portions thereof as described herein.

Once a recombinant polypeptide of the invention is expressed, it is isolated, *e.g.*, using affinity chromatography. In one example, an antibody (*e.g.*, produced as described herein) raised against a polypeptide of the invention may be attached to a column and used to isolate the recombinant polypeptide. Lysis and fractionation of polypeptide-harboring cells prior to affinity chromatography may be performed by standard methods (see, *e.g.*, Ausubel et al., supra).

Once isolated, the recombinant protein can, if desired, be further purified, *e.g.*, by high performance liquid chromatography (see, *e.g.*, Fisher, Laboratory Techniques In Biochemistry and Molecular Biology, eds., Work and Burdon, Elsevier, 1980). Polypeptides of the invention, particularly short peptide fragments, can also be produced by chemical synthesis (*e.g.*, by the methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984 The Pierce Chemical Co., Rockford, Ill.). These general techniques of polypeptide expression and purification can also be used to produce and isolate useful peptide fragments or analogs (described herein).

Methods to grow cells that produce VLPs of the invention include, but are not limited to, batch, batch-fed, continuous and perfusion cell culture techniques. In one embodiment, a cell comprising an influenza nucleic acid molecule is grown in a bioreactor or fermentation chamber where cells propagate and express protein (*e.g.* recombinant proteins) for purification and isolation. Typically, cell culture is performed under sterile, controlled temperature and atmospheric conditions. A bioreactor is a chamber used to culture cells in which environmental conditions such as temperature, atmosphere, agitation and/or pH can be monitored. In one embodiment, the bioreactor is a stainless steel chamber. In another embodiment, the bioreactor is a pre-sterilized plastic bag (*e.g.* Cellbag.RTM., Wave Biotech, Bridgewater, N.J.). In other embodiment, the pre-sterilized plastic bags are about 50 L to 1000 L bags.

The VLPs are isolated using methods that preserve the integrity thereof, such as by gradient centrifugation, *e.g.*, cesium chloride, sucrose and iodixanol, as

well as standard purification techniques including, *e.g.*, ion exchange and gel filtration chromatography. The following disclosure is an example of how VLPs of the invention can be made, isolated and purified. A person of skill in the art appreciates that there are additional methods that can be used to make and purify VLPs. Accordingly, the invention is not limited to the methods described herein.

The invention also provides for a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the polynucleotide and or VLP vaccine formulations of the invention. In a preferred embodiment, the kit comprises two or more containers, one containing VLPs, another containing a nucleic acid molecule and, optionally, another containing an adjuvant. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

The invention also provides that the nucleic acid molecules and/or VLP formulations be packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of composition. In one embodiment, the nucleic acid molecule and/or VLP composition is supplied as a liquid, in another embodiment, as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject.

The invention also features a kit comprising a nucleic acid molecule and/or VLP as described herein and instructions for use in an immunization method delineated herein.

In another embodiment, compositions of the disclosure may be comprised of the RNAg, in the absence of any of the internal viral proteins. It is further contemplated that RNAg-derivative compositions may comprise subsets of the viral genome. For example, an RNAg-derivative composition for influenza may comprise any combination of 1, 2, 3, 4, 5, 6, or 7 of the negative sense RNA segments of the RNAg.

In another embodiment, compositions of the disclosure may be comprised of any combination of 1, 2, 3, 4, or 5 of the internal viral proteins (IVPs)—NP, PB1, PB2, PA, and NS2—in the absence of the RNAg.

In yet another embodiment, compositions of the disclosure may be

comprised of derivative internal viral proteins (DIVPs), either individually or in combination. Such DIVPs may include C-terminal, N-terminal, or internal deletions of NP, PB1, PB2, PA, and/or NS2. It is also contemplated that such DIVPs may include peptides corresponding to discrete regions within NP, PB1, PB2, PA, and/or NS2.

In another embodiment, compositions of the disclosure may be comprised of recombinant fusion proteins (RFPs). Such RFPs may include either N-terminal or C-terminal fusions of NP, PB1, PB2, PA, and NS2 with proteins or protein fragments that may increase the immunogenicity of the resulting recombinant protein. For example, candidate proteins or protein fragments for use with influenza specific compositions may include, or be derived from, HA, NA, or M2.

It is contemplated within the scope of the invention that such IVPs, DIVPs, and/or RFPs may be synthesized by a variety of standard molecular biology methods. For example, the entire open reading frame of SIV H1NI (A/Swine/North Carolina/38448-1/2005) may be amplified and cloned into the GatewayTM (Invitrogen, Carlsbad CA) baculovirus cloning system per manufacturer's recommendations. The Gateway® Technology is based on the bacteriophage lambda site-specific recombination system which facilitates the integration of lambda into the E. coli chromosome and the switch between the lytic and lysogenic pathways (Ptashne, 1992). In the Gateway® Technology, the components of the lambda recombination system are modified to improve the specificity and efficiency of the system (Bushman *et al.*, 1985). In brief, an IVP gene may be cloned by lambda recombination into a baculodirect linear DNA vector and transformed into cells. Recombinant clones may be selected using ganciclovir, and the P1 virus will be harvested and used to transfect Sf-9 cells. The viral DNA may be extracted and the insert sequence confirmed by direct nucleotide sequencing. Transfected cells may be screened for recombinant protein production by Western analysis. Positive recombinant clones may then be maintained in insect cells. Baculovirus titers may be determined and the recombinant proteins (as IVPs) will be purified using standard protocols. The purity of the recombinant proteins may be determined using standard SDS-PAGE and Western blot analyses. Purified protein may also be analyzed by immune electron microscopy to confirm IVP assembly and for amino acid sequence by standard mass spectrometry analysis.

More specifically, Sf-9 cells may be prepared as spinner cultures using about 0.5

to $1x10^6$ cells/ml in about 300 mls total volume with media fortified with about 1% Pluronic F-68 (JRH) to prevent cell clumps, about 10% FBS, and about 1% antibiotics. The cells may be seeded in flasks when cell density approaches about $2xl0^6$ cells/ml but does not exceed about $3x10^6$ cells/ml. The cells should be sub-cultured every other day.

High-Five cells may be prepared as spinner cultures using about $0.5x10^6$ cells/ml in about 300 mls total volume. The media may be fortified with about 1% Pluronic F-68 (JRH) to prevent cell clumps, about 10% FBS and about 1% antibiotics. The cells may be seeded in flasks when the cell density approaches about $1.5x10^6$ cells/ml. Generally, H-5 cells will not grow to higher density than about $2x10^6$ cells/ml. These cells should be sub-cultured daily.

Stock virus may be prepared by seeding about 1.5 to $2x10^7$ cells in 8-10 ml volume in T-162 flasks. The cells may be allowed to attach for about 30 minutes, after which time the media may be removed and baculovirus added at an MOI of about 0.01. Media supplemented with about 10% FBS may then be added, followed by virus harvesting at about 10 days post-infection. The stock virus may be titrated, aliquoted, and stored at about -80°C.

For IVP production, about $3x10^7$ cells will be seeded in about 8-l0 ml volume in T-162 flasks. The cells may be allowed to attach for about 30 minutes. For H-5, about $2x10^7$ cells may be used. The media may be removed followed by the addition of recombinant baculoviruses RFVP2, VP6, VP4, and VP7 at MOI of about 5 each, diluted to a final volume of about 4 ml. The viruses may be attached to cells by rocking the flasks at low speed for about 3 hours. Media supplemented with about 1% FBS (final FBS should be about 2% - taking into account that stock viruses are in about 10% FBS) may be added, and virus harvested after about 7 days post infection.

For purification of IVPs, the supernatant may be centrifuged at about 2,000xg for about 30 minutes. The supernatant may be underlain with about 35% sucrose in TNC buffer followed by centrifugation at about 25,000 rpm for about 2 hours. The pellet from about 180-240 ml supernatant may be resuspended in about 4 ml of TNC buffer followed by the addition of about 1.89g $CsCl_2$. Since H-5 cells will have higher yield, the same volume of supernatant in H-5 may need to be resuspended in about 8 ml of TNC buffer. After centrifugation at about 35,000 rpm for about l8 hours, the bands may be collected and washed with TNC buffer at

about 25,000 rpm for about 1.5 hours. The pellet may be resuspended in about 1 ml of TNC buffer. The pellet may be stored at about 4°C prior to analysis by ELISA, immune electron microscopy, and western blot.

It is also contemplated that peptides of the present disclosure described above, particularly DIVPs, may be chemically synthesized. Such synthetic polypeptides may be prepared using standard techniques of solid phase, liquid phase, or peptide condensation techniques, or any combination thereof, and may include natural and/or unnatural amino acids. Amino acids used for peptide synthesis may be standard Boc (N^a-amino protected N^a-t-butyloxycarboyl) amino acid resin with the standard deprotecting, neutralization, coupling and wash protocols of the original solid phase procedure of Merrifield [J. Am. Chem. Soc., 85:2149-2154 (1963)], or the base-labile N^a-amino protected 9-fluorenylmethoxycarbonyl (Fmoc) amino acids first described by Carpino and Han [J. Ovg. Chem., 37:3403-3409 (1972)]. Both Fmoc and Boc N^a-amino protected amino acids may be obtained from Advanced Chemtech, Cambridge Research Biochemical, Fluka, Sigma or other chemical companies familiar to those of skill in the art. In addition, the method of the invention may be used with other N^a-protecting groups familiar to those skilled in the art. Solid phase peptide synthesis may be accomplished by standard techniques including, but not limited to, those described in Stewart and Young, 1984, Solid Phase Synthesis, Second Edition, Pierce Chemical Co., Rockford, Ill.; Fields *et al.*, Znt. J. Pept. Protein. 35:161-214 (1990), or by using automated synthesizers, such as those sold by ABI. Thus, polypeptides of the disclosure may comprise D-amino acids, a combination of D- and L-amino acids, and various "designer" amino acids (*e.g.*, -methyl amino acids, Ca-methyl amino acids, and N^a-methyl amino acids, *etc.*) to convey special properties. Synthetic amino acids may include ornithine for lysine, fluoro-phenylalanine for phenylalanine, and norleucine for leucine or isoleucine. Additionally, by assigning specific amino acids at specific coupling steps, α-helices, β-turns, β-sheets, and cyclic peptides may be generated, further increasing the potential immunogenicity of such DIVP peptides.

One of ordinary skill in the art may employ conventional biochemistry, molecular biology, microbiology, immunology, and recombinant DNA techniques to prepare the compositions of the current disclosure. Explanations

of such techniques may be found in standard laboratory reference materials including, but not limited to, the following: Molecular Cloning: A Laboratory Manual, Sambrook *et al*, (3rd edition, 2001); Current Protocols in Molecular Biology, Volumes I-III, Ausubel, R. M., ed., (1999 plus subsequent updates); Cell Biology: A Laboratory Handbook, Volumes I-III, J. E. Celis, ed., (1 994); Current Protocols in Immunology, Volumes I-IV, Coligan, J. E., ed. (1999 plus subsequent updates); Oligonucleotide Synthesis, M. J. Gait ed., (1984); Nucleic Acid Hybridization, B. D. Hames & S. J. Higgins eds., (1985); Transcription And Translation, B. D. Hames & S. J. Higgins, eds., (1984); Culture of Animal Cells (4th edition), R. I. Freshney, ed., (2000); Immobilized Cells And Enzymes, IRL Press, (1986); A Practical Guide To Molecular Cloning, B. Perbal, (1988); Using Antibodies: A Laboratory Manual, Harlow, E. and Lane, D. (Cold Spring Harbor Press, 1999), which are hereby incorporated by reference in their entirety.

The above described compositions of the disclosure preferably further comprise an adjuvant. For example, such adjuvants may include, but are not limited to, aluminum phosphate, aluminum hydroxide, Squalene, QS21, MF59 (Chiron), and complete Freund's adjuvant. Adjuvant properties may also be effected by the use of virosomes to deliver compositions of the disclosure. Many other adjuvants for use in vaccines are known in the art, and may be readily adapted for use with the compositions of the current disclosure by one of ordinary skill in the art. See, for example, Vaccine Adjuvants and Delivery Systems, Singh, M. ed., (2007); Vaccine Adjuvants: Immunological and Clinical Principals, Hackett, C. J. and Harn, D. A. ed., (2006); and Vaccine Adjuvants: Preparation Methods and Research Protocols, O'Hagan, D. T. ed., (2000). The vaccine may be prepared using any pharmaceutically acceptable carrier or vehicle, including, water, Hanks basic salt solution (HBSS) or phosphate buffered saline (PBS), with or without a preservative. The vaccine may be lyophilized for resuspension at the time of administration. Vaccines may be prepared for use in both active and passive immunizations. In the case of DIVP compositions, the antigenic material is preferably extensively dialyzed to remove undesired small molecular weight molecules. If desired, it may be lyophilized for more ready formulation into a desired vehicle.

The preparation of vaccines that contain peptide sequences as active ingredients is generally well understood in the art, as exemplified by U.S. Pat. Nos.

4,608,251; 4,601,903; 4,599,231; 4,599,230; 4,596,792; and 4,578,770 (all incorporated herein by reference in their entirety). Generally, such vaccines are prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for dissolution, or suspension, in liquid prior to injection may also be prepared. Such preparations may also be emulsified. The active immunogenic ingredient may be mixed with pharmaceutically acceptable excipients that are compatible with the NVCP, VLP, IVP, DIVP, and/or RFP containing compositions. Suitable excipients may include, but are not limited to, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. Additional examples of substances that may serve as pharmaceutically acceptably carriers are sugars (*e.g.* lactose, glucose, sucrose, and the like), starches (*e.g.* corn starch, potato starch, and the like), cellulose and its derivatives (*e.g.* sodium carboxymethylcellulose, ethylcellulose, cellulose acetate, and the like), powdered tragacanth, malt, gelatin, talc, stearic acid, magnesium stearate, calcium sulfate, vegetable oils (*e.g.* peanut oil, cottonseed oil, sesame oil, olive oil, corn oil, oil of theobroma, and the like); polyols (*e.g.* propylene glycol, glycerine, sorbitol, mannitol, polyethylene glycol, and the like), agar, alginic acid, pyrogen-free water, isotonic saline, and phosphate buffer solutions, as well as other non-toxic compatible substances used in pharmaceutical formulations. In addition, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or the above described adjuvants that enhance the efficacy of the vaccines.

Suitable methods of vaccine administration include, but are not limited to, topical (*e.g.* epidermal abrasion), oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, nasopharyngeal, and intradermal administration. Preferred routes of administration include intraperitoneal, intramuscular, subcutaneous, and intradermal. The most preferred route of administration is intradermal. In a preferred embodiment, a vaccine is packaged in a single dosage for immunization by intramuscular, intradermal, intraperitoneal, or nasopharyngeal administration. In the most preferred embodiment it is packaged in a single dosage for intradermal administration.

It will be recognized by those of skill in the art that an optimal dosing schedule of a vaccination regimen will vary according to the particular composition being used. For example, a dosing schedule may include as many as about five to six, but preferably about three to five, or even more preferably about one to three

administrations of the immunizing composition. Such administrations may be given at intervals of as short as about two to four weeks, or as long as about five to ten years. Such administration may occasionally be given at even longer intervals.

In yet another embodiment of the disclosure, a pharmaceutical composition is provided that comprises the NVCP, VLP, IVP, DIVP, and/or RFP containing compositions described above optionally in combination with pharmaceutically acceptable excipients, in a therapeutically effective amount to treat influenza infection.

Methods for preparing pharmaceutical compositions that contain polypeptides or peptide fragments as active ingredients are known in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for dissolution, or suspension, in liquid prior to injection may also be prepared. The preparation may also be emulsified, or encapsulated within liposome complexes. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. If desired, the composition may also contain minor amounts of auxiliary substances such as wetting agents, emulsifying agents, or pH buffering agents that enhance the effectiveness of the active ingredient.

A polypeptide or peptide fragment may be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule) that are formed with inorganic acids (*e.g.*, hydrochloric acid, phosphoric acids, or the like) or organic acids (*e.g.*, acetic, oxalic, tartaric, mandelic, and the like). Salts formed from the free carboxyl groups may also be derived from inorganic bases (*e.g.*, sodium, potassium, ammonium, calcium, ferric hydroxides, or the like) and/or organic bases (*e.g.*, isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like).

The therapeutic polypeptide or peptide fragment containing compositions are conventionally administered intravenously, for example by injection of a unit dose, which refers to physically discrete units suitable as unitary dosage for humans or animals, each unit containing a predetermined quantity of

active material calculated to produce the desired therapeutic effect in association with the required diluent.

Potential enveloped virus targets for these vaccination methods may be identified in the following virus families that include, but are not limited to, arenaviridae, arteriviridae, asfarviridae, baculoviridae, bornaviridae, bunyaviridae, coronaviridae, filoviridae, flaviviridae, hepadnaviridae, herpesviridae, orthomyxoviridae, paramyxoviridae, poxviridae, retroviridae, rhabdoviridae, and togaviridae. More preferred enveloped virus targets for these vaccination methods may be identified in the following virus families, herpesviridae, orthomyxoviridae, poxviridae, and retroviridae. The most preferred enveloped virus targets for these vaccination methods may be identified in the orthomyxoviridae and retroviridae virus families. In particular, preferred viral targets for vaccination may include influenza (orthomyxoviridae) and HIV (retroviridae).

The above detailed description presents one illustrative embodiment of the current disclosure in the form of compositions and methods for vaccinating humans and animals against the influenza virus. For the sake of clarity, terminology is used in this detailed description that is specific for this particular embodiment of the disclosure; however, this terminology is not intended to be limiting, and should not be construed as limiting insofar as one skilled in the art will recognize that many different forms and variations of the current disclosure will be generally applicable to all enveloped viruses, and are possible within the scope of the appended claims. All references cited in this disclosure are hereby incorporated by reference in their entirety.

Example 1

Studies at the University of Minnesota are evaluating protective immunity induced by vaccination with a commercially available, inactivated H1N1 and H3N2 bivalent SIV against challenge with recent field isolates of H1N1 or H3N2 swine influenza viruses (Gramer and Rossow, 2004; Lee, 2007). In both studies, pigs are vaccinated twice per label directions and then challenged intranasally with about 1 ml of about 10^6 TCID50/ml per nostril of heterologous H1NI virus (NSwine/MN/00040/2002) in trial 1 or with about 1 ml of about 10^6 TCID50/ml per nostril of heterologous H3N2 virus (A/Swine/Colorado/00294/2004) in trial 2. In both trials, pigs are evaluated daily

post challenge for clinical signs of respiratory disease or fever and nasal swabs are collected for virus detection. About five to seven days post-challenge, pigs are euthanized and necropsied for evaluation of pneumonia. Clinical signs and pneumonia lesions are reduced in the vaccinated groups when compared to the unvaccinated groups. However, virus is still detectable in nasal swabs taken from some vaccinated pigs.

Example 2

Characterization of influenza virus isolates are performed using our extensive collection of avian and swine influenza isolates. We are using strain A/Sw/NC/38448-1/2005 as a template for recombinant nucleoprotein production as well as in homologous challenge experiments. Beginning in June 2005, H1N1 viruses of human-like HA genotype were isolated from swine. A/Swine/North Carolina/38448-1/2005 (ISDN126735), is typical of the viruses isolated from NC during that time period. It was isolated from 9 week old pigs with clinical signs of respiratory disease including coughing and nasal discharge. This virus and the others isolated from NC in 2005 are human-like H1N1 viruses that share >98% similarity to H1 human influenza viruses circulating in 2004 such as A/Poitiers/2168/2003 and <75% similarity with swine influenza reference strains. The 2005 swine influenza viruses with human-like H1 HA sequences share >97% similarity to each other but <95% similarity with the 2003 human/swine HIN2 reassortant viruses. Phylogenetic analyses suggest that the 2005 strains is the result of separate reassortment events between human and swine influenza viruses and is not a direct descendent of the 2003 strains (Figure 1). Furthermore, the NA genotype of the 2005 humanlike H1N1 is an N1 of human origin. Genetic analyses of the internal genes of both the 2003 H1N2 and the 2005 H1N1 human/swine reassortant viruses reveals M, NP, NS, PA, PB1 and PB2 genes similar to those of the triple reassortant swine H3N2 viruses. Triple reassortant H3N2 viruses are the dominant H3N2 strain circulating in pigs in the U.S. (Webby et al, 2000).

Example 3

The immune-stimulatory effect and protective ability of VLP-NP given either transdermally or transmuscularly is evaluated in pigs. Two-week-old piglets are divided into groups of 10 pigs per challenge group and 5 pigs per

control group (Table 1, Figure 1). Pigs receive the vaccine at days 0 and 14 of the study. They are then challenged intranasally on day 28 of the study with homologous influenza strain: A /Swine/North Carolina/38448- 1/2005 human-like H1N1. Blood samples are drawn prior to injection on days 3, 7, 14, 28, 35, and 42. Serum levels of anti antibodies (via HA inhibition against vaccine and challenge strains) are measured. Any concomitant treatment, morbidity, or mortality will be recorded. To assess potential immune stimulation afforded by IVP-NP, blood collected from pigs are evaluated for the following: (1) the numbers of circulating immune cells (macrophage, B cell, CD4, CD8 and T cell populations); (2) concentration of activated lymphocytes using CD45 and CD44 markers and (3) natural killer immune cell activity. All immune measures are assessed on peripheral blood mononuclear cells using flow cytometry. Half of the pigs (n=5) are euthanized at 5 days post challenge (dpc) and the remaining pigs are euthanized at 14 dpc. Clinical disease are measured and assessed on a daily basis using body temperature, respiratory signs, and weight gain as criteria. Lesions are assessed post-mortem. Virological measures are assessed both qualitatively and quantitatively using virus isolation and titration from swabs and tissue homogenates, with virus detection and subtype confirmed by RT-PCR. The optimum route is defined as the route that assures physiologic stimulation of the immune system to levels no more than 30% above control levels and a physiological increase in activated lymphocytes and/or natural killer cells.

Table 1. Experimental Design for Specific Aim 2

Group #	Vaccine dose	Vaccination Route	# Animals	Challenge Strain
1	Maximum, 2X 2 weeks	Transdermal	10	Homologous: human-like
2	Maximum, 2X 2 weeks	Intramuscular	10	Homologous: human-like
3	None	N/A	10	Homologous: human-like
4	None	N/A	5	None

Example 4

Pigs are divided into 5 groups (Table 2, Fig. 1). Pigs of 14 days of age remain in

facilities from study day -3 to 21. Pigs are weighed on study days 3, 7, 14, 21, 28 and 35 to evaluate both weight gain and weight differences by group. They are bled on days 3, 14, 28, 35, and 42. IVP-NP treated pigs receive the vaccine via the optimum route determined on days 0 and 14 of the study. Pigs are intranasally challenged on day 28 of the study with one of two virulent SIV strains: heterosubtypic A/Swine/Colorado/00294/2001 H3N2 or heterologous A/Swine/Minnesota/00040/2002 reassortant H1N1 (rH1N1). Pigs in all groups are evaluated daily post-challenge or post-exposure until humane euthanasia. Blood samples are drawn prior to injection on day 3, 7, 14, 28, 35, and 42. Serum levels of anti-influenza antibodies are determined via HA inhibition against vaccine and challenge strains. Any concomitant treatment, morbidity, or mortality is recorded. To assess potential immune stimulation afforded by IVP-NP, blood collected from pigs is evaluated for the following: (1) the numbers of circulating immune cells (macrophage, B cell, CD4, CD8 and yo-T cell populations); (2) concentration of activated lymphocytes using CD45 and CD44 markers; and (3) natural killer immune cell activity. All immune measures are assessed on peripheral blood mononuclear cells using flow cytometry. At day 5 post-challenge, 5 pigs in each group (n=5) are euthanized to evaluate viral load in lungs and lesions. The remaining pigs are euthanized at 14 days post-challenge to evaluate the immune response to SIV post-challenge. Lesions are assessed post-mortem. Virological measures are assessed both qualitatively and quantitatively using virus isolation and titration from swabs and tissues homogenates, with virus detection and subtype confirmed by RT-PCR.

Table 2 Experimental Design Specific Aim 3

Group	Vaccine Dose	N per group	Challenge strain
1	Maximum, 2X, 2 weeks apart hu-like H1N1 IVP-NP vaccine	10	Heterologous rH1N1
2	Maximum, twice, 2 weeks apart hu-like H1N1 VLP-NP vaccine	10	Heterosubtypic H3N2
3	None	10	rH1N1
4	None	10	H3N2
5	None	5	None

CLAIMS

What is claimed is:

1. An immunogenic composition comprising an effective amount of at least one internal moiety of an enveloped virus, or antigenic fragment thereof, and a pharmaceutically acceptable carrier or excipient.

2. The immunogenic composition of claim 1, wherein the internal moiety is any one or more viral polypeptides or polynucleotides selected from the group consisting of the RNA genome (RNAg), nucleoprotein (NP), RNA polymerase (PA), RNA polymerase 1 (PB1), RNA polymerase 2 (PB2), non-structural protein 1(NS1), non-structural protein 2 (NS2), and matrix protein 1 (M1).

3. The immunogenic composition of claim 1, wherein the enveloped virus is selected from the group consisting of arenaviridae, arteriviridae, asfarviridae, baculoviridae, bornaviridae, bunyaviridae, coronaviridae, filoviridae, flaviviridae, hepadnaviridae, herpesviridae, orthomyxoviridae, paramyxoviridae, poxviridae, retroviridae, rhabdoviridae, and togaviridae.

4. The immunogenic composition of claim 3, wherein the enveloped virus is influenza.

5. The immunogenic composition of claim 1, wherein the at least one internal moiety comprises NP.

6. The immunogenic composition of claim 1, wherein the at least one internal moiety comprises NP and RNAg.

7. The immunogenic composition of claim 1, wherein the at least one internal moiety consists of NP.

8. The immunogenic composition of claim 1, wherein the at least one internal moiety consists of NP and RNAg.

9. A immunogenic composition comprising an effective amount of a naked viral core particle of an enveloped virus, and a pharmaceutically acceptable carrier or excipient.

10. The immunogenic composition of claim 9, wherein the enveloped virus is selected from the group consisting of arenaviridae, arteriviridae, asfarviridae, baculoviridae, bornaviridae, bunyaviridae, coronaviridae, filoviridae, flaviviridae, hepadnaviridae, herpesviridae, orthomyxoviridae, paramyxoviridae, poxviridae, retroviridae, rhabdoviridae, and togaviridae.

11. The immunogenic composition of claim 10, wherein the enveloped virus is influenza.

12. The immunogenic composition of claim 9, wherein the naked viral core particle is any one or more viral polypeptides or polynucleotides selected from the group consisting of the RNA genome (RNAg), nucleoprotein (NP), RNA polymerase (PA), RNA polymerase 1 (PB1), RNA polymerase 2 (PB2), non-structural protein 1(NS1), and non-structural protein 2 (NS2).

13. The immunogenic composition of claim 12, wherein the naked viral core particle comprises at least three viral polypeptides or polynucleotides selected from the group consisting of RNAg, NP, PA, PB1, PB2, NS1, and NS2.

14. The immunogenic composition of claim 12, wherein the naked viral core particle consists of NP, NS1, and RNAg.

15. The immunogenic composition of claim 12, wherein the naked viral core particle consists of NP, NS2, and RNAg.

16. The immunogenic composition of claim 12, wherein the naked viral core particle consists of NP, NS1, NS2, and RNAg.

17. An immunogenic composition comprising an effective amount of at least one virus like particle (VLP) of an enveloped virus and a pharmaceutically acceptable

carrier or excipient.

18. The immunogenic composition of claim 17, wherein the enveloped virus is selected from the group consisting of arenaviridae, arteriviridae, asfarviridae, baculoviridae, bornaviridae, bunyaviridae, coronaviridae, filoviridae, flaviviridae, hepadnaviridae, herpesviridae, orthomyxoviridae, paramyxoviridae, poxviridae, retroviridae, rhabdoviridae, and togaviridae.

19. The immunogenic composition of claim 18, wherein the enveloped virus is influenza.

20. The immunogenic composition of claim 17, wherein the VLP comprises any one or more viral polypeptides or polynucleotides, or antigenic fragments thereof, selected from the group consisting of RNA genome (RNAg), nucleoprotein (NP), RNA polymerase (PA), RNA polymerase 1 (PB1), RNA polymerase 2 (PB2), non-structural protein 1(NS1), and non-structural protein 2 (NS2).

21. The immunogenic composition according to claim 20, wherein the antigenic fragment is selected from the group consisting of a N-terminal, a C-terminal, and an internal polypeptide fragment.

22. An immunogenic composition comprising an effective amount of at least one recombinant fusion protein of an enveloped virus and a pharmaceutically acceptable carrier or excipient.

23. The immunogenic composition according to claim 22, wherein the at least one recombinant fusion protein comprises a protein, or antigenic fragment thereof, selected from the group consisting of nucleoprotein (NP), RNA polymerase (PA), RNA polymerase 1 (PB1), RNA polymerase 2 (PB2), non-structural protein 1(NS1), non-structural protein 2 (NS2), and matrix protein 1 (M1).

24. The immunogenic composition according to claim 23, wherein the at least one recombinant fusion protein is joined with a viral polypeptide, or antigenic fragment thereof, selected from the group consisting of hemagglutinin (HA), neuraminidase

(NA), and matrix protein 2 (M2).

25. The immunogenic composition according to claim 23, wherein the at least one recombinant fusion protein consists of NP, or an antigenic fragment thereof, joined with HA, or an antigenic fragment thereof.

26. A method of synthesizing naked viral core particles from enveloped viruses, said method comprising the production of live virus from a host, purification of the live virus from the host, and removal of the envelope from the purified live virus.

27. A method of synthesizing naked viral core particles from enveloped viruses, said method comprising the production of live virus from a cell culture system, purification of the live virus from the cell culture system, and removal of the envelope from the purified live virus.

28. A method of synthesizing naked viral core particles, said method comprising the *in vitro* production of genome and protein components corresponding to internal moieties.

29. A method of inducing immunity against an enveloped virus in a mammal, said method comprising administering said mammal with an immunologically effective amount of an immunogenic composition according to claim 1.

30. The method of claim 29, wherein said method of administration is selected from the group consisting of topical, oral, anal, vaginal, mucosal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal, nasopharyngeal, buccal, and intradermal administration.

31. A method of generating an immune response in a subject, the method comprising administering to said mammal an antigenic amount of an immunogenic composition of any of claims 1-25.

32. An influenza vaccine comprising:

a therapeutically or prophylactically effective amount of a naked viral core

particle, wherein the naked viral core particle is comprised of any combination of one or more of the RNA genome (RNAg), nucleoprotein (NP), RNA polymerase (PA), RNA polymerase 1 (PB1), RNA polymerase 2 (PB2), non-structural protein 1(NS1), and non-structural protein 2 (NS2); a pharmaceutically acceptable carrier or excipient; and, a pharmaceutically acceptable adjuvant.

33. A method of vaccinating a mammal against influenza, said method comprising administering to the mammal intradermally a vaccine comprising:

a therapeutically or prophylactically effective amount of a naked viral core particle, wherein the naked viral core particle is comprised of any combination of one or more of the RNA genome (RNAg), nucleoprotein (NP), RNA polymerase (PA), RNA polymerase 1 (PB1), RNA polymerase 2 (PB2), non-structural protein 1(NS1), and non-structural protein 2 (NS2);

a pharmaceutically acceptable carrier or excipient; and,

a pharmaceutically acceptable adjuvant.

Figure 1

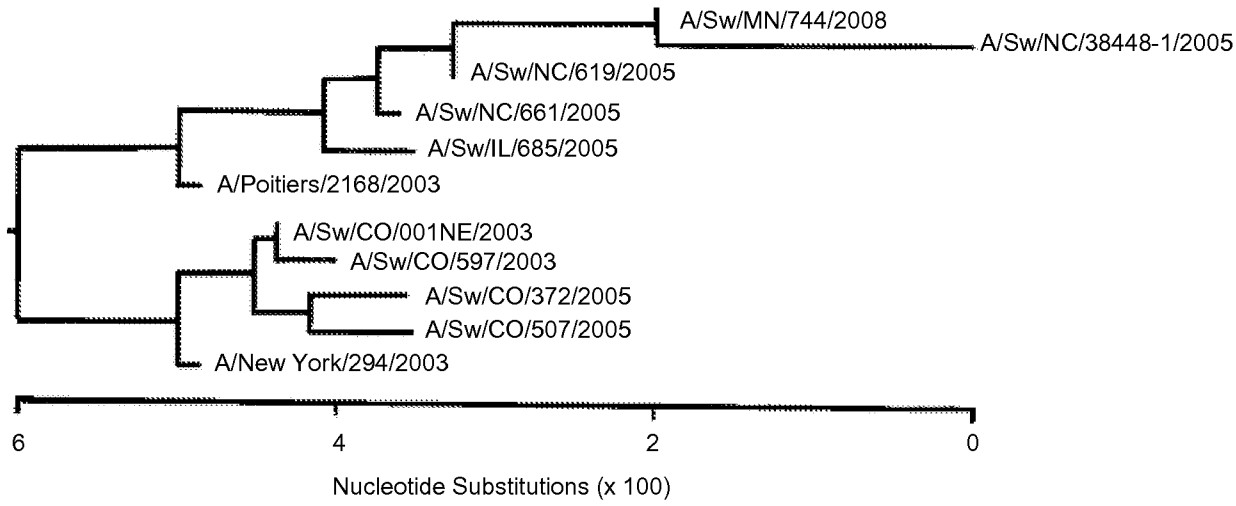

A/Sw/MN/744/2008
A/Sw/NC/38448-1/2005
A/Sw/NC/619/2005
A/Sw/NC/661/2005
A/Sw/IL/685/2005
A/Poitiers/2168/2003
A/Sw/CO/001NE/2003
A/Sw/CO/597/2003
A/Sw/CO/372/2005
A/Sw/CO/507/2005
A/New York/294/2003
6
4
2
0
Nucleotide Substitutions (x 100)

Figure 2

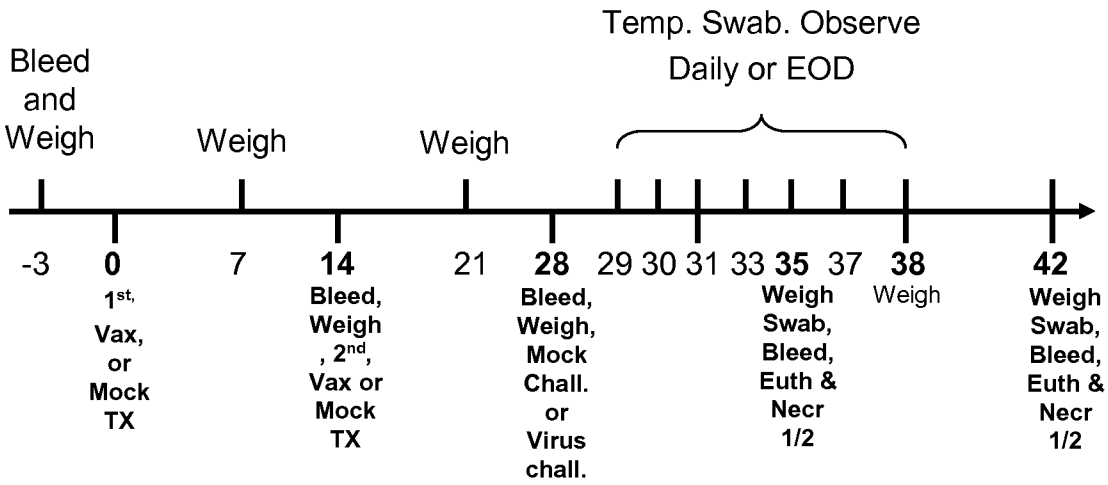

Temp. Swab. Observe
Daily or EOD
Bleed and Weigh
Weigh
Weigh
-3
0
1st, Vax, or Mock TX
7
14
Bleed, Weigh, 2nd, Vax or Mock TX
21
28
Bleed, Weigh, Mock Chall. or Virus chall.
29 30 31 33 35
Weigh Swab, Bleed, Euth & Necr 1/2
37
38
Weigh
42
Weigh Swab, Bleed, Euth & Necr 1/2

Figure 3A

NC_002019.1

AGCAAAAGCAGGGTAGATAATCACTCACTGAGTGACATCAAAATCATGGCGTCCCAAGG
CACCAAACGGTCTTACGAACAGATGGAGACTGATGGAGAACGCCAGAATGCCACTGAAA
TCAGAGCATCCGTCGGAAAAATGATTGGTGGAATTGGACGATTCTACATCCAAATGTGC
ACAGAACTTAAACTCAGTGATTATGAGGGACGGTTGATCCAAAACAGCTTAACAATAGA
GAGAATGGTGCTCTCTGCTTTTGACGAAAGGAGAAATAAATACCTGGAAGAACATCCCA
GTGCGGGGAAGGATCCTAAGAAAACTGGAGGACCTATATACAGAAGAGTAAACGGAAAG
TGGATGAGAGAACTCATCCTTTATGACAAAGAAGAAATAAGGCGAATCTGGCGCCAAGC
TAATAATGGTGACGATGCAACGGCTGGTCTGACTCACATGATGATCTGGCATTCCAATT
TGAATGATGCAACTTATCAGAGGACAAGGGCTCTTGTTCGCACCGGAATGGATCCCAGG
ATGTGCTCTCTGATGCAAGGTTCAACTCTCCCTAGGAGGTCTGGAGCCGCAGGTGCTGC
AGTCAAAGGAGTTGGAACAATGGTGATGGAATTGGTCAGGATGATCAAACGTGGGATCA
ATGATCGGAACTTCTGGAGGGGTGAGAATGGACGAAAAACAAGAATTGCTTATGAAAGA
ATGTGCAACATTCTCAAAGGGAAATTTCAAACTGCTGCACAAAAAGCAATGATGGATCA
AGTGAGAGAGAGCCGGGACCCAGGGAATGCTGAGTTCGAAGATCTCACTTTTCTAGCAC
GGTCTGCACTCATATTGAGAGGGTCGGTTGCTCACAAGTCCTGCCTGCCTGCCTGTGTG
TATGGACCTGCCGTAGCCAGTGGGTACGACTTTGAAAGAGAGGGATACTCTCTAGTCGG
AATAGACCCTTTCAGACTGCTTCAAAACAGCCAAGTGTACAGCCTAATCAGACCAAATG
AGAATCCAGCACACAAGAGTCAACTGGTGTGGATGGCATGCCATTCTGCCGCATTTGAA
GATCTAAGAGTATTGAGCTTCATCAAAGGGACGAAGGTGGTCCCAAGAGGGAAGCTTTC
CACTAGAGGAGTTCAAATTGCTTCCAATGAAAATATGGAGACTATGGAATCAAGTACAC
TTGAACTGAGAAGCAGGTACTGGGCCATAAGGACCAGAAGTGGAGGAAACACCAATCAA
CAGAGGGCATCTGCGGGCCAAATCAGCATACAACCTACGTTCTCAGTACAGAGAAATCT
CCCTTTTGACAGAACAACCGTTATGGCAGCATTCACTGGGAATACAGAGGGGAGAACAT
CTGACATGAGGACCGAAATCATAAGGATGATGGAAAGTGCAAGACCAGAAGATGTGTCT
TTCCAGGGGCGGGGAGTCTTCGAGCTCTCGGACGAAAAGGCAGCGAGCCCGATCGTGCC
TTCCTTTGACATGAGTAATGAAGGATCTTATTTCTTCGGAGACAATGCAGAGGAGTACG
ACAATTAAAGAAAAATACCCTTGTTTCTACT

Figure 3B

NP_040982.1

MASQGTKRSYEQMETDGERQNATEIRASVGKMIGGIGRFYIQMCTELKLSDYEGRLIQN
SLTIERMVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRRVNGKWMRELILYDKEEIRR
IWRQANNGDDATAGLTHMMIWHSNLNDATYQRTRALVRTGMDPRMCSLMQGSTLPRRSG
AAGAAVKGVGTMVMELVRMIKRGINDRNFWRGENGRKTRIAYERMCNILKGKFQTAAQK
AMMDQVRESRDPGNAEFEDLTFLARSALILRGSVAHKSCLPACVYGPAVASGYDFEREG
YSLVGIDPFRLLQNSQVYSLIRPNENPAHKSQLVWMACHSAAFEDLRVLSFIKGTKVVP
RGKLSTRGVQIASNENMETMESSTLELRSRYWAIRTRSGGNTNQQRASAGQISIQPTFS
VQRNLPFDRTTVMAAFTGNTEGRTSDMRTEIIRMMESARPEDVSFQGRGVFELSDEKAA
SPIVPSFDMSNEGSYFFGDNAEEYDN

Figure 4A

NC_007360.1

AGCAAAAGCAGGGTAGATAATCACTCACTGAGTGACATCAACATCATGGCGTCTCAGGG
CACCAAACGATCTTATGAACAGATGGAAACTGGTGGAGAACGCCAGAATGCTACTGAGA
TCAGAGCATCTGTTGGAAGAATGGTTGGTGGAATTGGGAGGTTTTATATACAGATGTGC
ACTGAACTCAAACTCAGCGACTATGAAGGAAGGCTGATTCAGAACAGCATAACAATAGA
GAGAATGGTTCTCTCTGCATTTGATGAAAGGAGGAACAAATACCTGGAAGAACATCCCA
GTGCGGGGAAGGACCCAAAGAAAACTGGAGGTCCAATCTACCGAAGAAGAGACGGAAAA
TGGGTGAGAGAGCTGATTCTGTATGACAAAGAGGAGATCAGGAGAATTTGGCGTCAAGC
GAACAATGGAGAAGATGCAACTGCTGGTCTCACTCACATGATGATCTGGCATTCCAATC
TAAATGATGCCACATACCAGAGAACAAGAGCTCTCGTGCGTACTGGGATGGACCCTAGA
ATGTGCTCTCTGATGCAAGGATCAACTCTCCCGAGGAGATCTGGAGCTGCTGGTGCGGC
AGTAAAGGGAGTCGGAACGATGGTGATGGAACTAATTCGGATGATAAAGCGAGGGATTA
ACGATCGGAATTTCTGGAGAGGTGAAAATGGGCGAAGAACAAGAATTGCATATGAGAGA
ATGTGCAACATCCTCAAAGGGAAATTCCAAACAGCAGCACAAAGAGCAATGATGGATCA
GGTACGGGAAAGCAGAAATCCTGGGAATGCTGAGATTGAAGATCTCATATTTCTGGCAC
GGTCTGCACTCATCCTGAGAGGATCAGTGGCCCACAAGTCCTGCTTGCCTGCTTGTGTG
TACGGGCTTGCCGTGGCCAGTGGATATGACTTTGAGAGAGAAGGGTACTCTCTGGTCGG
GATTGATCCTTTCCGTCTGCTGCAAAACAGCCAGGTCTTTAGTCTAATTAGACCAAATG
AGAATCCAGCACATAAAAGTCAATTGGTGTGGATGGCATGCCATTCTGCAGCATTTGAA
GATCTGAGAGTCTCAAGCTTCATCAGAGGGACAAGAGTGGCCCCAAGGGGACAACTATC
TACTAGAGGAGTTCAAATTGCTTCAAATGAGAACATGGAAACAATGGACTCCAGCACTC
TTGAACTGAGAAGCAGATATTGGGCTATAAGGACCAGGAGTGGAGGAAACACCAACCAG
CAGAGAGCATCTGCAGGACAAATCAGTGTGCAGCCTACTTTCTCGGTACAGAGAAATCT
TCCCTTCGAAAGAGCGACCATTATGGCGGCATTCACAGGGAATACAGAGGGCAGAACAT
CTGACATGAGGACTGAAATCATAAGGATGATGGAAAGCTCCAGACCAGAAGATGTGTCT
TTCCAGGGGCGGGGAGTCTTCGAGCTCTCGGACGAAAAGGCAACGAACCCGATCGTGCC
TTCCTTTGACATGAGTAATGAAGGATCTTATTTCTTCGGAGACAATGCAGAGGAATATG
ACAATTGAAGAAAAATACCCTTGTTTCTACT

Figure 4B

YP_308667.1

MASQGTKRSYEQMETGGERQNATEIRASVGRMVGGIGRFYIQMCTELKLSDYEGRLIQN
SITIERMVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRRRDGKWVRELILYDKEEIRR
IWRQANNGEDATAGLTHMMIWHSNLNDATYQRTRALVRTGMDPRMCSLMQGSTLPRRSG
AAGAAVKGVGTMVMELIRMIKRGINDRNFWRGENGRRTRIAYERMCNILKGKFQTAAQR
AMMDQVRESRNPGNAEIEDLIFLARSALILRGSVAHKSCLPACVYGLAVASGYDFEREG
YSLVGIDPFRLLQNSQVFSLIRPNENPAHKSQLVWMACHSAAFEDLRVSSFIRGTRVAP
RGQLSTRGVQIASNENMETMDSSTLELRSRYWAIRTRSGGNTNQQRASAGQISVQPTFS
VQRNLPFERATIMAAFTGNTEGRTSDMRTEIIRMMESSRPEDVSFQGRGVFELSDEKAT
NPIVPSFDMSNEGSYFFGDNAEEYDN

Figure 5A

NC_007369.1

AGCAAAAGCAGGGTTAATAATCACTCACCGAGTGACATCAAAATCATGGCGTCCCAAGG
CACCAAACGGTCTTATGAACAGATGGAAACTGATGGGGATCGCCAGAATGCAACTGAGA
TTAGGGCATCCGTCGGGAAGATGATTGATGGAATTGGGAGATTCTACATCCAAATGTGC
ACTGAACTTAAACTCAGTGATCATGAAGGGCGGTTGATCCAGAACAGCTTGACAATAGA
GAAAATGGTGCTCTCTGCTTTTGATGAAAGAAGGAATAAATACCTGGAAGAACACCCCA
GCGCGGGGAAAGATCCCAAGAAAACTGGGGGGCCCATATACAGGAGAGTAGATGGAAAA
TGGATGAGGGAACTCGTCCTTTATGACAAAGAAGAGATAAGGCGAATCTGGCGCCAAGC
CAACAATGGTGAGGATGCGACAGCTGGTCTAACTCACATAATGATCTGGCATTCCAATT
TGAATGATGCAACATACCAGAGGACAAGAGCTCTTGTTCGAACTGGAATGGATCCCAGA
ATGTGCTCTCTGATGCAGGGCTCGACTCTCCCTAGAAGGTCCGGAGCTGCAGGTGCTGC
AGTCAAAGGAATCGGGACAATGGTGATGGAACTGATCAGAATGGTCAAACGGGGGATCA
ACGATCGAAATTTCTGGAGAGGTGAGAATGGGCGGAAAACAAGAAGTGCTTATGAGAGA
ATGTGCAACATTCTTAAAGGAAAATTTCAAACAGCTGCACAAAGAGCAATGGTGGATCA
AGTGAGAGAAAGTCGGAACCCAGGAAATGCTGAGATCGAAGATCTCATATTTTTGGCAA
GATCTGCATTGATATTGAGAGGGTCAGTTGCTCACAAATCTTGCCTACCTGCCTGTGCG
TATGGACCTGCAGTATCCAGTGGGTACGACTTCGAAAAAGAGGGATATTCCTTGGTGGG
AATAGACCCTTTCAAACTACTTCAAAATAGCCAAATATACAGCCTAATCAGACCTAACG
AGAATCCAGCACACAAGAGTCAGCTGGTGTGGATGGCATGCCATTCTGCTGCATTTGAA
GATTTAAGATTGTTAAGCTTCATCAGAGGGACAAAAGTATCTCCGCGGGGGAAACTGTC
AACTAGAGGAGTACAAATTGCTTCAAATGAGAACATGGATAATATGGGATCGAGCACTC
TTGAACTGAGAAGCGGGTACTGGGCCATAAGGACCAGGAGTGGAGGAAACACTAATCAA
CAGAGGGCCTCCGCAGGCCAAACCAGTGTGCAACCTACGTTTTCTGTACAAAGAAACCT
CCCATTTGAAAAGTCAACCATCATGGCAGCATTCACTGGAAATACGGAGGGAAGGACTT
CAGACATGAGGGCAGAAATCATAAGAATGATGGAAGGTGCAAAACCAGAAGAAGTGTCA
TTCCGGGGGAGGGGAGTTTTCGAGCTCTCAGACGAGAAGGCAACGAACCCGATCGTGCC
CTCTTTTGATATGAGTAATGAAGGATCTTATTTCTTCGGAGACAATGCAGAAGAGTACG
ACAATTAAGGAAAAAATACCCTTGTTTCTACT

Figure 5B

YP_308843.1

MASQGTKRSYEQMETDGDRQNATEIRASVGKMIDGIGRFYIQMCTELKLSDHEGRLIQN
SLTIEKMVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRRVDGKWMRELVLYDKEEIRR
IWRQANNGEDATAGLTHIMIWHSNLNDATYQRTRALVRTGMDPRMCSLMQGSTLPRRSG
AAGAAVKGIGTMVMELIRMVKRGINDRNFWRGENGRKTRSAYERMCNILKGKFQTAAQR
AMVDQVRESRNPGNAEIEDLIFLARSALILRGSVAHKSCLPACAYGPAVSSGYDFEKEG
YSLVGIDPFKLLQNSQIYSLIRPNENPAHKSQLVWMACHSAAFEDLRLLSFIRGTKVSP
RGKLSTRGVQIASNENMDNMGSSTLELRSGYWAIRTRSGGNTNQQRASAGQTSVQPTFS
VQRNLPFEKSTIMAAFTGNTEGRTSDMRAEIIRMMEGAKPEEVSFRGRGVFELSDEKAT
NPIVPSFDMSNEGSYFFGDNAEEYDN

Figure 6A

NC_002021.1

AGCGAAAGCAGGCAAACCATTTGAATGGATGTCAATCCGACCTTACTTTTCTTAAAAGTGCCAGCACAAAA
TGCTATAAGCACAACTTTCCCTTATACCGGAGACCCTCCTTACAGCCATGGGACAGGAACAGGATACACCA
TGGATACTGTCAACAGGACACATCAGTACTCAGAAAAGGCAAGATGGACAACAAACACCGAAACTGGAGCA
CCGCAACTCAACCCGATTGATGGGCCACTGCCAGAAGACAATGAACCAAGTGGTTATGCCCAAACAGATTG
TGTATTGGAAGCAATGGCTTTCCTTGAGGAATCCCATCCTGGTATTTTTGAAAACTCGTGTATTGAAACGA
TGGAGGTTGTTCAGCAAACACGAGTAGACAAGCTGACACAAGGCCGACAGACCTATGACTGGACTTTAAAT
AGAAACCAGCCTGCTGCAACAGCATTGGCCAACACAATAGAAGTGTTCAGATCAAATGGCCTCACGGCCAA
TGAGTCTGGAAGGCTCATAGACTTCCTTAAGGATGTAATGGAGTCAATGAAAAAAGAAGAAATGGGGATCA
CAACTCATTTTCAGAGAAAGAGACGGGTGAGAGACAATATGACTAAGAAAATGATAACACAGAGAACAATA
GGTAAAAGGAAACAGAGATTGAACAAAAGGAGTTATCTAATTAGAGCATTGACCCTGAACACAATGACCAA
AGATGCTGAGAGAGGGAAGCTAAAACGGAGAGCAATTGCAACCCCAGGGATGCAAATAAGGGGGTTTGTAT
ACTTTGTTGAGACACTGGCAAGGAGTATATGTGAGAAACTTGAACAATCAGGGTTGCCAGTTGGAGGCAAT
GAGAAGAAAGCAAAGTTGGCAAATGTTGTAAGGAAGATGATGACCAATTCTCAGGACACCGAACTTTCTTT
GACCATCACTGGAGATAACACCAAATGGAACGAAAATCAGAATCCTCGGATGTTTTTGGCCATGATCACAT
ATATGACCAGAAATCAGCCCGAATGGTTCAGAAATGTTCTAAGTATTGCTCCAATAATGTTCTCAAACAAA
ATGGCGAGACTGGGAAAAGGGTATATGTTTGAGAGCAAGAGTATGAAACTTAGAACTCAAATACCTGCAGA
AATGCTAGCAAGCATTGATTTGAAATATTTCAATGATTCAACAAGAAAGAAGATTGAAAAAATCCGACCGC
TCTTAATAGAGGGGACTGCATCATTGAGCCCTGGAATGATGATGGGCATGTTCAATATGTTAAGCACTGTA
TTAGGCGTCTCCATCCTGAATCTTGGACAAAAGAGATACACCAAGACTACTTACTGGTGGGATGGTCTTCA
ATCCTCTGACGATTTTGCTCTGATTGTGAATGCACCCAATCATGAAGGGATTCAAGCCGGAGTCGACAGGT
TTTATCGAACCTGTAAGCTACATGGAATCAATATGAGCAAGAAAAAGTCTTACATAAACAGAACAGGTACA
TTTGAATTCACAAGTTTTTTCTATCGTTATGGGTTTGTTGCCAATTTCAGCATGGAGCTTCCCAGTTTTGG
TGTGTCTGGGAGCAACGAGTCAGCGGACATGAGTATTGGAGTTACTGTCATCAAAAACAATATGATAAACA
ATGATCTTGGTCCAGCAACAGCTCAAATGGCCCTTCAGTTGTTCATCAAAGATTACAGGTACACGTACCGA
TGCCATAGAGGTGACACACAAATACAAACCCGAAGATCATTTGAAATAAAGAAACTGTGGGAGCAAACCCG
TTCCAAAGCTGGACTGCTGGTCTCCGACGGAGGCCCAAATTTATACAACATTAGAAATCTCCACATTCCTG
AAGTCTGCCTAAAATGGGAATTGATGGATGAGGATTACCAGGGGCGTTTATGCAACCCACTGAACCCATTT
GTCAGCCATAAAGAAATTGAATCAATGAACAATGCAGTGATGATGCCAGCACATGGTCCAGCCAAAAACAT
GGAGTATGATGCTGTTGCAACAACACACTCCTGGATCCCCAAAAGAAATCGATCCATCTTGAATACAAGTC
AAAGAGGAGTACTTGAAGATGAACAAATGTACCAAAGGTGCTGCAATTTATTTGAAAAATTCTTCCCCAGC
AGTTCATACAGAAGACCAGTCGGGATATCCAGTATGGTGGAGGCTATGGTTTCCAGAGCCCGAATTGATGC
ACGGATTGATTTCGAATCTGGAAGGATAAAGAAAGAAGAGTTCACTGAGATCATGAAGATCTGTTCCACCA
TTGAAGAGCTCAGACGGCAAAAATAGTGAATTTAGCTTGTCCTTCATGAAAAAATGCCTTGTTCCTACT

Figure 6B

NP_040985.1

MDVNPTLLFLKVPAQNAISTTFPYTGDPPYSHGTGTGYTMDTVNRTHQYSEKARWTTNTETGAPQLNPIDG
PLPEDNEPSGYAQTDCVLEAMAFLEESHPGIFENSCIETMEVVQQTRVDKLTQGRQTYDWTLNRNQPAATA
LANTIEVFRSNGLTANESGRLIDFLKDVMESMKKEEMGITTHFQRKRRVRDNMTKKMITQRTIGKRKQRLN
KRSYLIRALTLNTMTKDAERGKLKRRAIATPGMQIRGFVYFVETLARSICEKLEQSGLPVGGNEKKAKLAN
VVRKMMTNSQDTELSLTITGDNTKWNENQNPRMFLAMITYMTRNQPEWFRNVLSIAPIMFSNKMARLGKGY
MFESKSMKLRTQIPAEMLASIDLKYFNDSTRKKIEKIRPLLIEGTASLSPGMMMGMFNMLSTVLGVSILNL
GQKRYTKTTYWWDGLQSSDDFALIVNAPNHEGIQAGVDRFYRTCKLHGINMSKKKSYINRTGTFEFTSFFY
RYGFVANFSMELPSFGVSGSNESADMSIGVTVIKNNMINNDLGPATAQMALQLFIKDYRYTYRCHRGDTQI
QTRRSFEIKKLWEQTRSKAGLLVSDGGPNLYNIRNLHIPEVCLKWELMDEDYQGRLCNPLNPFVSHKEIES
MNNAVMMPAHGPAKNMEYDAVATTHSWIPKRNRSILNTSQRGVLEDEQMYQRCCNLFEKFFPSSSYRRPVG
ISSMVEAMVSRARIDARIDFESGRIKKEEFTEIMKICSTIEELRRQK

Figure 7A

NC_007358.1

AGCAAAAGCAGGCAAACCATTTGAATGGATGTCAATCCGACTTTACTTTTCTTAAAAGTGCCAGCGCAAAA
TGCTATAAGTACCACATTCCCTTATACTGGAGATCCTCCATACAGCCATGGAACAGGAACAGGATACACCA
TGGACACAGTCAACAGAACACATCAATATTCAGAAAAGGGGAAATGGACAACGAACACAGAGACTGGAGCA
CCCCAACTCAATCCGATTGATGGACCACTACCTGAGGATAATGAGCCGAGTGGGTATGCACAAACAGATTG
TGTATTGGAAGCAATGGCTTTCCTTGAAGAATCCCACCCAGGGATCTTTGAAAACTCGTGTCTTGAAACGA
TGGAAGTTGTTCAGCAAACAAGAGTGGATAAGCTGACCCAAGGTCGCCAAACCTATGACTGGACATTGAAA
AGAAACCAGCCGGCTGCAACCGCTTTGGCCAACACTATAGAGGTCTTCAGATCGAATGGTCTAACAGCCAA
TGAATCGGGAAGGCTAATAGATTTCCTCAAAGACGTGATGGAATCAATGGATAAGGGAGAAATGGAAATAA
TAACACATTTCCAGAGAAAGAGAAGAGTGAGGGACAACATGACCAAGAAAATGGTCACACAAAGAACAATA
GGGAAGAAAAAACAAAGGCTGAACAAAAGGAGCTACCTAATAAGAGCACTGACACTGAACACAATGACAAA
AGACGCAGAAAGAGGCAAATTGAAGAGGCGGGCAATTGCAACACCCGGGATGCAAATCAGAGGATTCGTGT
ACTTTGTCGAAACACTAGCGAGGAGTATCTGTGAGAAACTTGAGCAATCTGGACTCCCCGTCGGAGGGAAT
GAAAAGAAGGCTAAATTGGCAAATGTCGTGAGGAAGATGATGACTAACTCACAAGATACAGAGCTCTCTTT
TACAATTACTGGAGACAACACCAAATGGAATGAGAATCAGAACCCTCGGATGTTTCTAGCAATGATAACAT
ACATCACAAGGAACCAACCTGAATGGTTTAGAAATGTCTTAAGCATTGCTCCTATAATGTTCTCAAACAAG
ATGGCAAGATTAGGGAAAGGATACATGTTCGAAAGTAAGAGCATGAAGCTACGGACACAAATACCAGCAGA
AATGCTTGCAAGCATTGACTTGAAATACTTCAACGAATCAACGAGAAAGAAAATCGAGAAAATAAGACCTC
TACTAATAGATGGCACAGCCTCATTGAGTCCTGGAATGATGATGGGCATGTTCAATATGCTGAGTACAGTC
TTAGGAGTTTCAATCCTGAATCTTGGGCAGAAGAGGTACACCAAAACCACATACTGGTGGGACGGACTCCA
ATCCTCTGATGATTTCGCTCTCATAGTGAATGCACCAAATCATGAGGGAATAGAAGCAGGGGTGGATAGGT
TCTATAGGACTTGCAAACTAGTTGGAATCAATATGACCAAGAAGAAGTCTTACATAAATCGGACAGGAACA
TGTGAATTCACAAGCTTCTTCTACCGCTATGGGTTCGTAGCCAACTTCAGTATGGAGCTGCCCAGCTTTGG
AGTGTCTGGGATTAATGAATCGGCTGACATGAGCATTGGTGTTACAGTGATAAAGAACAATATGATGGACA
ACGACCTTGGACCAGCAACAGCTCAGATGGCTCTTCAGCTATTCATTAAGGACTACAGATACCCATACCGA
TGCCACAGGGGGGATACACAAATCCAAACGAGGAGATCATTCGAGCTGAAGAAGCTGTGGGAGCAGACCCG
CTCAAAGGCAGGACTGTTGGTTTCAGATGGAGGACCAAACCCATACAATATCCGGAATCTCCACATTCCGG
AGGCTGGCTTGAAGTGGGAATTGATGGATGAAGACTACCAGGGCAGACTGTGTAATCCTCTGAACCCGTTT
GTTAGTCATAAGGAAATTGAGTCTGTCAACAATGCTGTGGTAATGCCAGCTCATGGCCCAGCCAAGAGCAT
GGAATATGATGCAGTTGCGACTACACATTCATGGATTCCCAAGAGGAATCGTTCCATTCTCAACACCAGCC
AAAGGGGGATTCTTGAGGATGAACAGATGTATCAGAAGTGCTGCAATCTATTCGAGAAATTCTTCCCTAGC
AGTTCATATCGGAGGCCAGTTGGAATTTCCAGCATGGTGGAGGCCATGGTGTCTAGGGCCCGAATTGATGC
ACGAATTGACTTCGAGTCTGGAAGGATTAAGAAAGAAGAGTTTGCTGAGATCATGAAGATCTGTTCCACCA
TTGAAGAGCTCGGACGGCAAAAATAGTGAATTTAGCTTGTCCTTCATGAAAAAATGCCTTGTTTCTACT

Figure 7B

YP_308665.1

MDVNPTLLFLKVPAQNAISTTFPYTGDPPYSHGTGTGYTMDTVNRTHQYSEKGKWTTNTETGAPQLNPIDG
PLPEDNEPSGYAQTDCVLEAMAFLEESHPGIFENSCLETMEVVQQTRVDKLTQGRQTYDWTLKRNQPAATA
LANTIEVFRSNGLTANESGRLIDFLKDVMESMDKGEMEIITHFQRKRRVRDNMTKKMVTQRTIGKKKQRLN
KRSYLIRALTLNTMTKDAERGKLKRRAIATPGMQIRGFVYFVETLARSICEKLEQSGLPVGGNEKKAKLAN
VVRKMMTNSQDTELSFTITGDNTKWNENQNPRMFLAMITYITRNQPEWFRNVLSIAPIMFSNKMARLGKGY
MFESKSMKLRTQIPAEMLASIDLKYFNESTRKKIEKIRPLLIDGTASLSPGMMMGMFNMLSTVLGVSILNL
GQKRYTKTTYWWDGLQSSDDFALIVNAPNHEGIEAGVDRFYRTCKLVGINMTKKKSYINRTGTCEFTSFFY
RYGFVANFSMELPSFGVSGINESADMSIGVTVIKNNMMDNDLGPATAQMALQLFIKDYRYPYRCHRGDTQI
QTRRSFELKKLWEQTRSKAGLLVSDGGPNPYNIRNLHIPEAGLKWELMDEDYQGRLCNPLNPFVSHKEIES
VNNAVVMPAHGPAKSMEYDAVATTHSWIPKRNRSILNTSQRGILEDEQMYQKCCNLFEKFFPSSSYRRPVG
ISSMVEAMVSRARIDARIDFESGRIKKEEFAEIMKICSTIEELGRQK

Figure 8A

NC_007375.1

AGCAAAAGCAGGCAAACCATTTGAATGGATGTCAATCCGACCTTACTTTTCTTGAAAGTTCCAGCGCAAAA
TGCCATAAGTACTACATTCCCTTATACTGGAGATCCTCCATACAGCCATGGGACAGGAACAGGATACACCA
TGGACACAGTCAACAGAACACATCAATATTCAGAAAAGGGGAAGTGGACAACAAACACGGAAACTGGAGCG
CCCCAACTTAACCCAATTGATGGACCACTACCTGAGGACAATGAACCAAGTGGATATGCACAAACAGACTG
CGTCCTGGAAGCAATGGCTTTCCTTGAGGAATCACACCCAGGAATCTTTGAAAATTCGTGTCTTGAAACGA
TGGAAGTTATTCAACAAACAAGAGTGGACAAACTGACCCAAGGTCGTCAGACCTATGACTGGACATTGAAC
AGAAATCAGCCGGCTGCAACTGCGCTAGCCAACACTATAGAGGTCTTCAGATCGAATGGACTGACAGCTAA
TGAGTCGGGAAGGCTAATAGATTTCCTCAAGGATGTGATAGAATCAATGGATAAAGAGGAGATGGAAATAA
CAACACACTTCCAAAGAAAAAGAAGAGTAAGAGACAACATGACCAAGAAAATGGTCACACAACGAACAATA
GGAAAGAAGAAGCAAAGATTGAACAAGAGAAGCTATCTGATAAGAGCACTGACATTGAACACAATGACTAA
AGATGCAGAGAGAGGTAAATTAAAAAGAAGAGCAATTGCAACACCCGGTATGCAGATCAGAGGGTTCGTGC
ACTTTGTCGAAACACTAGCGAGAAATATTTGTGAGAAACTTGAACAGTCTGGGCTTCCGGTTGGAGGTAAT
GAAAAGAAGGCTAAACTAGCAAATGTTGTTAGAAAAATGATGACTAATTCACAAGACACAGAGCTCTCTTT
CACAATTACTGGAGACAACACCAAATGGAATGAGAATCAAAATCCTCGAGTGTTTCTGGCGATGATAACAT
ACATCACAAGAAATCAACCTGAATGGTTTAGAAACGTCCTGAGCATTGCACCCATAATGTTCTCAAATAAA
ATGGCTAGACTAGGGAAAGGTTACATGTTCGAAAGCAAGAGCATGAAGCTCCGAACACAAATACCAGCAGA
AATGCTAGCAAGTATTGACCTGAAATACTTTAATGAATCAACCAGAAAGAAAATTGAGAAAATAAGGCCTC
TCCTAATAGATGGCACAGTCTCATTGAGTCCTGGAATGATGATGGGCATGTTCAACATGCTAAGTACAGTC
TTAGGAGTCTCAATCCTGAATCTCGGGCAAAAGAAATACACCAAAACNACATACTGGTGGGACGGACTCCA
ATCCTCTGATGACTTCGCTCTCATAGTGAATGCACCAAATCATGAGGGAATACAAGCAGGGGTGAATAGAT
TCTACAGAACCTGCAAGCTAGTCGGAATCAATATGAGCAAAAAGAAGTCCTACATAAATAGGACAGGGACA
TTTGAATTCACAAGCTTTTTCTATCGCTATGGATTTGTAGCCAATTTTAGCATGGAGCTGCCCAGCTTTGG
AGTGTCTGGAATTAATGAATCGGCTGATATGAGCATTGGGGTAACAGTGATAAAGAACAATATGATAAATA
ATGACCTTGGGCCAGCAACAGCCCAAATGGCTCTTCAACTATTCATCAAAGACTACAGATACACGTACCGG
TGCCACAGAGGGGACACACAAATTCAGACAAGGAGATCATTCGAGCTAAAGAAGCTGTGGGAGCAAACCCG
CTCAAAGGCAGGACTTTTGGTGTCGGATGGAGGATCAAACTTATACAATATCCGGAATCTCCACATTCCAG
AAGTCTGCTTGAAATGGGAGCTAATGGATGAAGACTATCAGGGGAGGCTTTGTAATCCCCTGAATCCATTT
GTCAGTCATAAGGAAATTGAGTCTGTAAACAATGCTGTGGTAATGCCAGCTCACGGTCCAGCCAAGAGCAT
GGAATATGATGCTGTTGCTACTACACACTCCTGGACCCCTAAGAGGAACCGCTCCATTCTCAACACAAGCC
AAAGGGGAATTCTTGAAGATGAACAGATGTATCAGAAGTGTTGCAATCTATTTGAGAAATTCTTCCCTAGC
AGTTCGTACAGGAGACCAGTTGGAATTTCCAGCATGGTGGAGGCCATGGTGTCTAGGGCTCGGATTGATGC
ACGGATTGACTTCGAGTCTGGACGGATTAAGAAAGAGGAGTTCGCTGAGATCATGAAGATCTGTTCCACCA
TTGAAGAGCTCAGACGGCAAAAATAGTGAATTTAGCTTGTCCTTCATGAAAAAATGCCTTGTTTCTACT

Figure 8B

YP_308851.1

MDVNPTLLFLKVPAQNAISTTFPYTGDPPYSHGTGTGYTMDTVNRTHQYSEKGKWTTNTETGAPQLNPIDG
PLPEDNEPSGYAQTDCVLEAMAFLEESHPGIFENSCLETMEVIQQTRVDKLTQGRQTYDWTLNRNQPAATA
LANTIEVFRSNGLTANESGRLIDFLKDVIESMDKEEMEITTHFQRKRRVRDNMTKKMVTQRTIGKKKQRLN
KRSYLIRALTLNTMTKDAERGKLKRRAIATPGMQIRGFVHFVETLARNICEKLEQSGLPVGGNEKKAKLAN
VVRKMMTNSQDTELSFTITGDNTKWNENQNPRVFLAMITYITRNQPEWFRNVLSIAPIMFSNKMARLGKGY
MFESKSMKLRTQIPAEMLASIDLKYFNESTRKKIEKIRPLLIDGTVSLSPGMMMGMFNMLSTVLGVSILNL
GQKKYTKTTYWWDGLQSSDDFALIVNAPNHEGIQAGVNRFYRTCKLVGINMSKKKSYINRTGTFEFTSFFY
RYGFVANFSMELPSFGVSGINESADMSIGVTVIKNNMINNDLGPATAQMALQLFIKDYRYTYRCHRGDTQI
QTRRSFELKKLWEQTRSKAGLLVSDGGSNLYNIRNLHIPEVCLKWELMDEDYQGRLCNPLNPFVSHKEIES
VNNAVVMPAHGPAKSMEYDAVATTHSWTPKRNRSILNTSQRGILEDEQMYQKCCNLFEKFFPSSSYRRPVG
ISSMVEAMVSRARIDARIDFESGRIKKEEFAEIMKICSTIEELRRQK

Figure 9A

NC_002023.1

AGCGAAAGCAGGTCAATTATATTCAATATGGAAAGAATAAAAGAACTAAGAAATCTAATGTCGCAGTCTCG
CACCCGCGAGATACTCACAAAAACCACCGTGGACCATATGGCCATAATCAAGAAGTACACATCAGGAAGAC
AGGAGAAGAACCCAGCACTTAGGATGAAATGGATGATGGCAATGAAATATCCAATTACAGCAGACAAGAGG
ATAACGGAAATGATTCCTGAGAGAAATGAGCAAGGACAAACTTTATGGAGTAAAATGAATGATGCCGGATC
AGACCGAGTGATGGTATCACCTCTGGCTGTGACATGGTGGAATAGGAATGGACCAATGACAAATACAGTTC
ATTATCCAAAAATCTACAAAACTTATTTTGAAAGAGTCGAAAGGCTAAAGCATGGAACCTTTGGCCCTGTC
CATTTTAGAAACCAAGTCAAAATACGTCGGAGAGTTGACATAAATCCTGGTCATGCAGATCTCAGTGCCAA
GGAGGCACAGGATGTAATCATGGAAGTTGTTTTCCCTAACGAAGTGGGAGCCAGGATACTAACATCGGAAT
CGCAACTAACGATAACCAAAGAGAAGAAAGAAGAACTCCAGGATTGCAAAATTTCTCCTTTGATGGTTGCA
TACATGTTGGAGAGAGAACTGGTCCGCAAAACGAGATTCCTCCCAGTGGCTGGTGGAACAAGCAGTGTGTA
CATTGAAGTGTTGCATTTGACTCAAGGAACATGCTGGGAACAGATGTATACTCCAGGAGGGGAAGTGAAGA
ATGATGATGTTGATCAAAGCTTGATTATTGCTGCTAGGAACATAGTGAGAAGAGCTGCAGTATCAGCAGAC
CCACTAGCATCTTTATTGGAGATGTGCCACAGCACACAGATTGGTGGAATTAGGATGGTAGACATCCTTAA
GCAGAACCCAACAGAAGAGCAAGCCGTGGGTATATGCAAGGCTGCAATGGGACTGAGAATTAGCTCATCCT
TCAGTTTTGGTGGATTCACATTTAAGAGAACAAGCGGATCATCAGTCAAGAGAGAGGAAGAGGTGCTTACG
GGCAATCTTCAAACATTGAAGATAAGAGTGCATGAGGGATATGAAGAGTTCACAATGGTTGGGAGAAGAGC
AACAGCCATACTCAGAAAAGCAACCAGGAGATTGATTCAGCTGATAGTGAGTGGGAGAGACGAACAGTCGA
TTGCCGAAGCAATAATTGTGGCCATGGTATTTTCACAAGAGGATTGTATGATAAAAGCAGTTAGAGGTGAT
CTGAATTTCGTCAATAGGGCGAATCAGCGACTGAATCCTATGCATCAACTTTTAAGACATTTTCAGAAGGA
TGCGAAAGTGCTTTTTCAAAATTGGGGAGTTGAACCTATCGACAATGTGATGGGAATGATTGGGATATTGC
CCGACATGACTCCAAGCATCGAGATGTCAATGAGAGGAGTGAGAATCAGCAAAATGGGTGTAGATGAGTAC
TCCAGCACGGAGAGGGTAGTGGTGAGCATTGACCGGTTCTTGAGAGTCCGGGACCAACGAGGAAATGTACT
ACTGTCTCCCGAGGAGGTCAGTGAAACACAGGGAACAGAGAAACTGACAATAACTTACTCATCGTCAATGA
TGTGGGAGATTAATGGTCCTGAATCAGTGTTGGTCAATACCTATCAATGGATCATCAGAAACTGGGAAACT
GTTAAAATTCAGTGGTCCCAGAACCCTACAATGCTATACAATAAAATGGAATTTGAACCATTTCAGTCTTT
AGTACCTAAGGCCATTAGAGGCCAATACAGTGGGTTTGTGAGAACTCTGTTCCAACAAATGAGGGATGTGC
TTGGGACATTTGATACCGCACAGATAATAAAACTTCTTCCCTTCGCAGCCGCTCCACCAAAGCAAAGTAGA
ATGCAGTTCTCCTCATTTACTGTGAATGTGAGGGGATCAGGAATGAGAATACTTGTAAGGGGCAATTCTCC
TGTATTCAACTACAACAAGGCCACGAAGAGACTCACAGTTCTCGGAAAGGATGCTGGCACTTTAACCGAAG
ACCCAGATGAAGGCACAGCTGGAGTGGAGTCCGCTGTTCTGAGGGGATTCCTCATTCTGGGCAAAGAAGAC
AGGAGATATGGGCCAGCATTAAGCATCAATGAACTGAGCAACCTTGCGAAAGGAGAGAAGGCTAATGTGCT
AATTGGGCAAGGAGACGTGGTGTTGGTAATGAAACGAAAACGGGACTCTAGCATACTTACTGACAGCCAGA
CAGCGACCAAAAGAATTCGGATGGCCATCAATTAGTGTCGAATAGTTTAAAAACGACCTTGTTTCTACT

Figure 9B

NP_040987.1

MERIKELRNLMSQSRTREILTKTTVDHMAIIKKYTSGRQEKNPALRMKWMMAMKYPITADKRITEMIPERN
EQGQTLWSKMNDAGSDRVMVSPLAVTWWNRNGPMTNTVHYPKIYKTYFERVERLKHGTFGPVHFRNQVKIR
RRVDINPGHADLSAKEAQDVIMEVVFPNEVGARILTSESQLTITKEKKEELQDCKISPLMVAYMLERELVR
KTRFLPVAGGTSSVYIEVLHLTQGTCWEQMYTPGGEVKNDDVDQSLIIAARNIVRRAAVSADPLASLLEMC
HSTQIGGIRMVDILKQNPTEEQAVGICKAAMGLRISSSFSFGGFTFKRTSGSSVKREEEVLTGNLQTLKIR
VHEGYEEFTMVGRRATAILRKATRRLIQLIVSGRDEQSIAEAIIVAMVFSQEDCMIKAVRGDLNFVNRANQ
RLNPMHQLLRHFQKDAKVLFQNWGVEPIDNVMGMIGILPDMTPSIEMSMRGVRISKMGVDEYSSTERVVVS
IDRFLRVRDQRGNVLLSPEEVSETQGTEKLTITYSSSMMWEINGPESVLVNTYQWIIRNWETVKIQWSQNP
TMLYNKMEFEPFQSLVPKAIRGQYSGFVRTLFQQMRDVLGTFDTAQIIKLLPFAAAPPKQSRMQFSSFTVN
VRGSGMRILVRGNSPVFNYNKATKRLTVLGKDAGTLTEDPDEGTAGVESAVLRGFLILGKEDRRYGPALSI
NELSNLAKGEKANVLIGQGDVVLVMKRKRDSSILTDSQTATKRIRMAIN

Figure 10A

NC_007357.1

AGCAAAAGCAGGTCAATTATATTCAATATGGAAAGAATAAAAGAACTAAGAGATCTAATGTCGCAGTCCCG
CACTCGCGAGATACTAACAAAAACCACTGTGGATCATATGGCCATAATCAAGAAATACACATCAGGAAGAC
AAGAGAAGAACCCTGCTCTCAGAATGAAATGGATGATGGCAATGAAATATCCAATCACAGCAGACAAGAGA
ATAATGGAGATGATTCCTGAAAGGAATGAGCAAGGACAAACGCTTTGGAGCAAGACAAATGATGCTGGGTC
GGACAGAGTGATGGTGTCTCCCCTAGCTGTAACTTGGTGGAACAGGAATGGGCCGACAACAAGTACAGTCC
ATTATCCAAAGGTTTACAAAACATACTTTGAGAAGGTTGAAAGGTTAAAACATGGAACCTTCGGTCCCGTT
CATTTCCGAAACCAAGTTAAAATACGTCGCCGGGTGGATATAAACCCGGGCCATGCAGATCTCAGTGCTAA
AGAAGCACAAGATGTTATCATGGAGGTCGTTTTCCCAAATGAAGTGGGAGCTAGAATATTGACATCAGAGT
CGCAATTGACAATAACAAAAGAGAAGAAAGAAGAGCTCCAGGATTGTAAAATTGCTCCTTTAATGGTGGCA
TACATGTTGGAAAGAGAACTGGTCCGCAAAACCAGATTTCTACCGGTAGCAGGCGGAACAAGCAGTGTGTA
CATTGAGGTATTGCATTTGACTCAAGGGACCTGTTGGGAACAGATGTACACTCCCGGCGGAGAAGTAAGAA
ATGATGATGTTGACCAGAGTTTGATCATCGCTGCCAGAAACATTGTTAGGAGAGCAACAGTATCAGCGGAC
CCACTGGCATCACTCTTGGAGATGTGTCACAGCACACAAATTGGGGGAATAAGGATGGTGGACATCCTTAG
GCAAAACCCAACTGAGGAGCAAGCTGTGGATATATGCAAAGCAGCAATGGGTTTGAGGATCAGTTCATCCT
TTAGCTTTGGAGGCTTCACTTTCAAAAGAACAAATGGATCATCCGTCAAGAAGGAAGAGGAAGTGCTTACA
GGCAACCTCCAAACATTGAAAATAAAAGTACATGAGGGGTATGAAGAATTCACAATGGTTGGGCGGAGAGC
AACAGCTATCCTGAGGAAAGCAACTAGAAGGCTGATTCAGTTGATAGTAAGTGGAAGAGATGAACAATCAA
TCGCTGAAGCGATCATTGTAGCAATGGTGTTCTCACAGGAGGATTGCATGATAAAGGCAGTCCGAGGCGAT
CTGAATTTCGTGAACAGAGCAAACCAAAGATTGAACCCCATGCATCAACTCCTGAGGCACTTCCAAAAAGA
TGCAAAAGTGCTGTTTCAGAACTGGGGAATTGAACCTATTGACAATGTCATGGGGATGATCGGAATATTAC
CTGACATGACTCCAAGCGCAGAGATGTCACTGAGAGGAGTGAGAGTTAGTAAGATGGGAGTAGATGAATAT
TCCAGCACGGAGAGAGTGGTGGTGAGTATTGACCGTTTCTTGAGGGTCCGAGATCAGCAGGGGAACGTACT
CTTATCTCCTGAAGAGGTTAGTGAAACACAGGGAACAGAGAAGTTGACAATAACATATTCATCCTCAATGA
TGTGGGAAATCAACGGTCCTGAGTCAGTGCTTGTTAACACTTATCAATGGATCATCAGGAATTGGGAGACT
GTAAAGATTCAATGGTCTCAAGATCCCACAATGCTGTACAATAAGATGGAGTTTGAATCGTTCCAATCCTT
GGTGCCAAAGGCTGCCAGAAGCCAATATAGTGGATTTGTGAGAACACTATTCCAACAGATGCGTGATGTTT
TGGGGACATTTGATACTGTCCAAATAATCAAGCTGCTACCATTTGCAGCAGCCCCACCGGAGCCGAGCAGA
ATGCAGTTTTCTTCTCTAACTGTGAATGTGAGAGGCTCAGGAATGAGAATACTCGTGAGGGGTAACTCCCC
CGTGTTCAACTACAACAAGGCAACCAAAAGGCTTACAGTCCTCGGAAAGGACGCAGGTGCATTAACAGAAG
ATCCAGACGAGGGAACAGCCGGGGTGGAATCTGCAGTATTGAGGGGATTCCTAATTCTAGGCAGAGAGGAC
AAAAGATATGGACCCGCATTGAGCATCAATGAACTGAGCAATCTTGCAAAAGGGGAGAAGGCTAATGTATT
GATAATGCAAGGAGACGTGGTGTTGGTAATGAAACGGAAACGGGACTTTAGCATACTTACTGACAGCCAGA
CAGCGACCAAAAGAATTCGGATGGCCATCAATTAGTGTTGAATAGTTTAAAAACGACCTTGTTTCTACT

Figure 10B

YP_308664.1

MERIKELRDLMSQSRTREILTKTTVDHMAIIKKYTSGRQEKNPALRMKWMMAMKYPITADKRIMEMIPERN
EQGQTLWSKTNDAGSDRVMVSPLAVTWWNRNGPTTSTVHYPKVYKTYFEKVERLKHGTFGPVHFRNQVKIR
RRVDINPGHADLSAKEAQDVIMEVVFPNEVGARILTSESQLTITKEKKEELQDCKIAPLMVAYMLERELVR
KTRFLPVAGGTSSVYIEVLHLTQGTCWEQMYTPGGEVRNDDVDQSLIIAARNIVRRATVSADPLASLLEMC
HSTQIGGIRMVDILRQNPTEEQAVDICKAAMGLRISSSFSFGGFTFKRTNGSSVKKEEEVLTGNLQTLKIK
VHEGYEEFTMVGRRATAILRKATRRLIQLIVSGRDEQSIAEAIIVAMVFSQEDCMIKAVRGDLNFVNRANQ
RLNPMHQLLRHFQKDAKVLFQNWGIEPIDNVMGMIGILPDMTPSAEMSLRGVRVSKMGVDEYSSTERVVVS
IDRFLRVRDQQGNVLLSPEEVSETQGTEKLTITYSSSMMWEINGPESVLVNTYQWIIRNWETVKIQWSQDP
TMLYNKMEFESFQSLVPKAARSQYSGFVRTLFQQMRDVLGTFDTVQIIKLLPFAAAPPEPSRMQFSSLTVN
VRGSGMRILVRGNSPVFNYNKATKRLTVLGKDAGALTEDPDEGTAGVESAVLRGFLILGREDKRYGPALSI
NELSNLAKGEKANVLIMQGDVVLVMKRKRDFSILTDSQTATKRIRMAIN

Figure 11A

NC_007373.1

AGCAAAAGCAGGTCAATTATATTCAGTATGGAAAGAATAAAAGAACTACGGAACCTGATGTCGCAGTCTCG
CACTCGCGAGATACTGACAAAAACCACAGTGGACCATATGGCCATAATTAAGAAGTACACATCGGGGAGAC
AGGAAAAGAACCCGTCACTTAGGATGAAATGGATGATGGCAATGAAATACCCAATCACTGCTGACAAAAGG
ATAACAGAAATGGTTCCGGAGAGAAATGAACAAGGACAAACTCTATGGAGTAAAATGAGTGATGCTGGATC
AGATCGAGTGATGGTATCACCTTTGGCTGTAACATGGTGGAATAGAAATGGACCCGTGGCAAGTACGGTCC
ATTACCCAAAAGTATACAAGACTTATTTTGACAAAGTCGAAAGGTTAAAACATGGAACCTTTGGCCCTGTT
CATTTTAGAAATCAAGTCAAGATACGCAGAAGAGTAGACATAAACCCTGGTCATGCAGACCTCAGTGCCAA
AGAGGCACAAGATGTAATTATGGAAGTTGTTTTTCCCAATGAAGTGGGAGCCAGGATACTAACATCAGAAT
CGCAATTAACAATAACTAAAGAGAAAAAAGAAGAACTCCGAGATTGCAAAATTTCTCCCTTGATGGTTGCA
TACATGTTAGAGAGAGAACTTGTCCGAAAAACAAGATTTCTCCCAGTTGCTGGCGGAACAAGCAGTATATA
CATTGAAGTCTTACATTTGACTCAAGGAACGTGTTGGGAACAAATGTACACTCCAGGTGGAGAAGTGAGGA
ATGACGATGTTGACCAAAGCCTAATTATTGCGGCCAGGAACATAGTAAGAAGAGCTGCAGTATCAGCAGAT
CCACTAGCATCTTTATTGGAGATGTGCCACAGCACACAAATTGGCGGGACAAGGATGGTGGACATTCTTAG
ACAGAACCCGACTGAAGAACAAGCTGTGGATATATGCAAGGCTGCAATGGGATTGAGAATCAGCTCATCCT
TCAGCTTTGGTGGGTTTACATTTAAAAGAACAAGCGGGTCATCAGTCAAAAAAGAGGAAGAAGTGCTTACA
GGCAATCTCCAAACATTGAAGATAAGAGTACATGAGGGGTATGAGGAGTTCACAATGGTGGGGAAAAGAGC
AACAGCTATACTCAGAAAAGCAACCAGAAGATTGGTTCAGCTCATAGTGAGTGGAAGAGACGAACAGTCAA
TAGCCGAAGCAATAATCGTGGCCATGGTGTTTTCACAAGAGGATTGCATGATAAAAGCAGTTAGAGGTGAC
CTGAATTTCGTCAACAGAGCAAATCAACGGTTGAACCCCATGCATCAGCTTTTAAGGCATTTTCAGAAAGA
TGCGAAAGTGCTTTTTCAAAATTGGGGAATTGAACACATCGACAGTGTGATGGGAATGGTTGGAGTATTAC
CAGATATGACTCCAAGCACAGAGATGTCAATGAGAGGAATAAGAGTCAGCAAAATGGGTGTGGATGAATAC
TCCAGTACAGAGAGGGTGGTGGTTAGCATTGATCGGTTTTTGAGAGTTCGAGACCAACGCGGGAATGTATT
ATTGTCTCCTGAGGAGGTCAGTGAAACACAGGGAACTGAAAGATTGACAATAACATATTCATCGTCGATGA
TGTGGGAGATTAACGGTCCTGAGTCGGTTTTGGTCAATACCTATCAATGGATCATCAGAAATTGGGAAGCT
GTCAAAATTCAATGGTCTCAGAATCCTGCAATGTTGTACAACAAAATGGAATTTGAACCATTTCAATCTTT
AGTCCCCAAGGCCATTAGAAGCCAATACAGTGGGTTTGTCAGAACTCTATTCCAACAAATGAGAGACGTAC
TTGGGACATTTGACACCACCCAGATAATAAAGCTTCTCCCTTTTGCAGCCGCTCCACCAAAGCAAAGCAGA
ATGCAGTTCTCTTCACTGACTGTAAATGTGAGGGGATCAGGGATGAGAATACTTGTAAGGGGCAATTCTCC
TGTATTCAACTACAACAAGACCACTAAAAGACTAACAATTCTCGGAAAAGATGCCGGCACTTTAATTGAAG
ACCCAGATGAAAGCACATCCGGAGTGGAGTCCGCCGTCTTGAGAGGGTTTCTCATTATAGGTAAGGAAGAC
AGAAGATACGGACCAGCATTAAGCATCAATGAACTGAGTAACCTTGCAAAAGGGGAAAAGGCTAATGTGCT
AATCGGGCAAGGAGACGTGGTGTTGGTAATGAAACGAAAACGGGACTCTAGCATACTTACTGACAGCCAGA
CAGCGACCAAAAGAATTCGGATGGCCATCAATTAATGTTGAATAGTTTAAAAACGACCTTGTTTCTACT

Figure 11B

YP_308849.1

MERIKELRNLMSQSRTREILTKTTVDHMAIIKKYTSGRQEKNPSLRMKWMMAMKYPITADKRITEMVPERN
EQGQTLWSKMSDAGSDRVMVSPLAVTWWNRNGPVASTVHYPKVYKTYFDKVERLKHGTFGPVHFRNQVKIR
RRVDINPGHADLSAKEAQDVIMEVVFPNEVGARILTSESQLTITKEKKEELRDCKISPLMVAYMLERELVR
KTRFLPVAGGTSSIYIEVLHLTQGTCWEQMYTPGGEVRNDDVDQSLIIAARNIVRRAAVSADPLASLLEMC
HSTQIGGTRMVDILRQNPTEEQAVDICKAAMGLRISSSFSFGGFTFKRTSGSSVKKEEEVLTGNLQTLKIR
VHEGYEEFTMVGKRATAILRKATRRLVQLIVSGRDEQSIAEAIIVAMVFSQEDCMIKAVRGDLNFVNRANQ
RLNPMHQLLRHFQKDAKVLFQNWGIEHIDSVMGMVGVLPDMTPSTEMSMRGIRVSKMGVDEYSSTERVVVS
IDRFLRVRDQRGNVLLSPEEVSETQGTERLTITYSSSMMWEINGPESVLVNTYQWIIRNWEAVKIQWSQNP
AMLYNKMEFEPFQSLVPKAIRSQYSGFVRTLFQQMRDVLGTFDTTQIIKLLPFAAAPPKQSRMQFSSLTVN
VRGSGMRILVRGNSPVFNYNKTTKRLTILGKDAGTLIEDPDESTSGVESAVLRGFLIIGKEDRRYGPALSI
NELSNLAKGEKANVLIGQGDVVLVMKRKRDSSILTDSQTATKRIRMAIN

Figure 12A

NC_002022.1

AGCGAAAGCAGGTACTGATCCAAAATGGAAGATTTTGTGCGACAATGCTTCAATCCGATGATTGTCGA
GCTTGCGGAAAAAACAATGAAAGAGTATGGGGAGGACCTGAAAATCGAAACAAACAAATTTGCAGCAA
TATGCACTCACTTGGAAGTATGCTTCATGTATTCAGATTTCCACTTCATCAATGAGCAAGGCGAGTCA
ATAATCGTAGAACTTGGTGATCCTAATGCACTTTTGAAGCACAGATTTGAAATAATCGAGGGAAGAGA
TCGCACAATGGCCTGGACAGTAGTAAACAGTATTTGCAACACTACAGGGGCTGAGAAACCAAAGTTTC
TACCAGATTTGTATGATTACAAGGAAAATAGATTCATCGAAATTGGAGTAACAAGGAGAGAAGTTCAC
ATATACTATCTGGAAAAGGCCAATAAAATTAAATCTGAGAAAACACACATCCACATTTTCTCGTTCAC
TGGGGAAGAAATGGCCACAAAGGCCGACTACACTCTCGATGAAGAAAGCAGGGCTAGGATCAAAACCA
GGCTATTCACCATAAGACAAGAAATGGCCAGCAGAGGCCTCTGGGATTCCTTTCGTCAGTCCGAGAGA
GGAGAAGAGACAATTGAAGAAAGGTTTGAAATCACAGGAACAATGCGCAAGCTTGCCGACCAAAGTCT
CCCGCCGAACTTCTCCAGCCTTGAAAATTTTAGAGCCTATGTGGATGGATTCGAACCGAACGGCTACA
TTGAGGGCAAGCTGTCTCAAATGTCCAAAGAAGTAAATGCTAGAATTGAACCTTTTTTGAAAACAACA
CCACGACCACTTAGACTTCCGAATGGGCCTCCCTGTTCTCAGCGGTCCAAATTCCTGCTGATGGATGC
CTTAAAATTAAGCATTGAGGACCCAAGTCATGAAGGAGAGGGAATACCGCTATATGATGCAATCAAAT
GCATGAGAACATTCTTTGGATGGAAGGAACCCAATGTTGTTAAACCACACGAAAAGGGAATAAATCCA
AATTATCTTCTGTCATGGAAGCAAGTACTGGCAGAACTGCAGGACATTGAGAATGAGGAGAAAATTCC
AAAGACTAAAAATATGAAAAAAACAAGTCAGCTAAAGTGGGCACTTGGTGAGAACATGGCACCAGAAA
AGGTAGACTTTGACGACTGTAAAGATGTAGGTGATTTGAAGCAATATGATAGTGATGAACCAGAATTG
AGGTCGCTTGCAAGTTGGATTCAGAATGAGTTCAACAAGGCATGCGAACTGACAGATTCAAGCTGGAT
AGAGCTTGATGAGATTGGAGAAGATGTGGCTCCAATTGAACACATTGCAAGCATGAGAAGGAATTATT
TCACATCAGAGGTGTCTCACTGCAGAGCCACAGAATACATAATGAAGGGGGTGTACATCAATACTGCC
TTACTTAATGCATCTTGTGCAGCAATGGATGATTTCCAATTAATTCCAATGATAAGCAAGTGTAGAAC
TAAGGAGGGAAGGCGAAAGACCAACTTGTATGGTTTCATCATAAAAGGAAGATCCCACTTAAGGAATG
ACACCGACGTGGTAAACTTTGTGAGCATGGAGTTTTCTCTCACTGACCCAAGACTTGAACCACACAAA
TGGGAGAAGTACTGTGTTCTTGAGATAGGAGATATGCTTCTAAGAAGTGCCATAGGCCAGGTTTCAAG
GCCCATGTTCTTGTATGTGAGGACAAATGGAACCTCAAAAATTAAAATGAAATGGGGAATGGAGATGA
GGCGTTGTCTCCTCCAGTCACTTCAACAAATTGAGAGTATGATTGAAGCTGAGTCCTCTGTCAAAGAG
AAAGACATGACCAAAGAGTTCTTTGAGAACAAATCAGAAACATGGCCCATTGGAGAGTCTCCCAAAGG
AGTGGAGGAAAGTTCCATTGGGAAGGTCTGCAGGACTTTATTAGCAAAGTCGGTATTTAACAGCTTGT
ATGCATCTCCACAACTAGAAGGATTTTCAGCTGAATCAAGAAAACTGCTTCTTATCGTTCAGGCTCTT
AGGGACAATCTGGAACCTGGGACCTTTGATCTTGGGGGGCTATATGAAGCAATTGAGGAGTGCCTAAT
TAATGATCCCTGGGTTTTGCTTAATGCTTCTTGGTTCAACTCCTTCCTTACACATGCATTGAGTTAGT
TGTGGCAGTGCTACTATTTGCTATCCATACTGTCCAAAAAAGTACCTTGTTTCTACT

Figure 12B

NP_040986.1

MEDFVRQCFNPMIVELAEKTMKEYGEDLKIETNKFAAICTHLEVCFMYSDFHFINEQGESIIVELGDP
NALLKHRFEIIEGRDRTMAWTVVNSICNTTGAEKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKAN
KIKSEKTHIHIFSFTGEEMATKADYTLDEESRARIKTRLFTIRQEMASRGLWDSFRQSERGEETIEER
FEITGTMRKLADQSLPPNFSSLENFRAYVDGFEPNGYIEGKLSQMSKEVNARIEPFLKTTPRPLRLPN
GPPCSQRSKFLLMDALKLSIEDPSHEGEGIPLYDAIKCMRTFFGWKEPNVVKPHEKGINPNYLLSWKQ
VLAELQDIENEEKIPKTKNMKKTSQLKWALGENMAPEKVDFDDCKDVGDLKQYDSDEPELRSLASWIQ
NEFNKACELTDSSWIELDEIGEDVAPIEHIASMRRNYFTSEVSHCRATEYIMKGVYINTALLNASCAA
MDDFQLIPMISKCRTKEGRRKTNLYGFIIKGRSHLRNDTDVVNFVSMEFSLTDPRLEPHKWEKYCVLE
IGDMLLRSAIGQVSRPMFLYVRTNGTSKIKMKWGMEMRRCLLQSLQQIESMIEAESSVKEKDMTKEFF
ENKSETWPIGESPKGVEESSIGKVCRTLLAKSVFNSLYASPQLEGFSAESRKLLLIVQALRDNLEPGT
FDLGGLYEAIEECLINDPWVLLNASWFNSFLTHALS

Figure 13A

NC_007359.1

AGCAAAAGCAGGTACTGATCCAAAATGGAAGACTTTGTGCGACAATGCTTCAATCCAATGATTGTCGAGCT
TGCGGAAAAGGCAATGAAAGAATATGGGGAAGATCCGAAAATCGAAACGAACAAATTTGCCGCAATATGCA
CGCACTTAGAAGTCTGTTTCATGTATTCAGATTTCCACTTTATTGATGAACGGGGCGAATCAACAATTATA
GAATCTGGCGATCCCAATGCATTATTGAAACACCGGTTTGAAATAATCGAAGGGAGGGACCGAACAATGGC
CTGGACAGTGGTGAATAGTATCTGCAACACCACAGGAGTTGAGAAGCCTAAATTTCTCCCAGATTTGTATG
ACTACAAGGAGAACCGATTTATTGAAATTGGAGTGACACGGAGGGAAGTTCACACATACTATCTAGAAAAA
GCCAACAAGATAAAATCTGAGAAGACACACATTCACATATTCTCATTCACTGGAGAGGAAATGGCCACCAA
AGCGGACTACACCCTTGATGAAGAAAGCAGGGCCCGAATCAAAACCAGGCTGTTCACTATAAGGCAGGAAA
TGGCCAGTAGGGGTTTATGGGATTCCTTTCGTCAGTCCGAGAGAGGCGAAGAGACAGTTGAAGAAAGATTT
GAAATCACAGGGACTATGTGCAGGCTTGCCGACCAAAGTCTCCCACCTAATTTCTCCAGCCTTGAAAAATT
TAGAGCCTATGTGGATGGATTCGAACCGAACGGCTGCATTGAGGGCAAGCTTTCTCAAATGTCGAAAGAAG
TAAACGCCAGAATTGAGCCATTTCTGAAGACAACACCACGCCCTCTTAGATTACCTGATGGGCCTCCCTGC
TCTCAGCGGTCGAAGTTTTTGCTGATGGATGCCCTTAAATTAAGCATCGAAGACCCGAGTCATGAGGGGGA
GGGGATACCGCTATATGATGCAATCAAATGCATGAAAACATTTTTCGGCTGGAAAGAGCCCAACATTGTAA
AACCACATGAAAAAGGCATAAACCCCAATTACCTCCTGGCTTGGAAGCAGGTGCTGGCAGAGCTCCAAGAT
ATTGAAAACGAGGAGAAAATTCCAAAGACAAAGAACATGAGGAAAACAAGCCAATTGAAGTGGGCACTTGG
TGAGAATATGGCACCAGAGAAAGTAGACTTTGAGGATTGCAAAGATGTTAGCGATCTAAGGCAGTATGACA
GTGATGAACCAAAGCCTAGATCACTAGCAAGCTGGATCCAGAGTGAATTCAACAAGGCATGCGAATTGACA
GATTCAAGTTGGATTGAACTTGATGAAATAGGGGAAGACGTTGCTCCAATTGAGCACATTGCAAGTATGAG
AAGGAACTATTTCACAGCGGAAGTATCCCATTGCAGGGCTACTGAATACATAATGAAGGGAGTGTACATAA
ACACAGCTTTGTTGAATGCATCCTGTGCAGCCATGGATGACTTCCAACTGATCCCAATGATAAGCAAATGC
AGAACCAAAGAAGGAAGACGGAAAACTAACCTGTATGGATTCCTTATAAAAGGAAGATCCCATTTGAGAAA
TGACACCGATGTGGTAAACTTTGTGAGTATGGAATTCTCTCTTACTGATCCGAGGCTGGAGCCACACAGAT
GGGAAAAGTACTGCGTTCTTCGGATAGGAGACATGCTCTTACGGACTGAAATAGGCCAAGTGTCAAGGCCC
ATGTTTCTTTATGTGAGAACCAATGGAACCTCCAAGATCAAGATGAAATGGGGCATGGAAATGAGGCGATG
CCCTTTTCAATCCCTTCAACAGATTGAGAGCATGATTGAGGCCGAGTCTTCTGTCAAAGAAAAAGACATGA
CTAAAGAATTCTTTGAAAACAAATCAGAAACATGGCCAATTGGAGAATCACCCAAGGGAGTGGAGGAAGGC
TCCATCGGGAAGGTGTGCAGAACCTTACTGGCTAAATCTGTTTTCAACAGTCTATATGCATCTCCACAACT
CGAGGGGTTTTCAGCTGAATCAAGAAAATTGCTTCTCATTGTTCAGGCACTTAGGGACAACCTGGAACCTG
GAACCTTCGATCTTGGGGGGCTATATGAAGCAATTGAGGAGTGCCTGATTAATGATCCCTGGGTTTTGCTT
AATGCATCTTGGTTCAACTCCTTCCTCACACATGCACTAAGATAGTTGTGGCAATGCTACTATTTGCTATC
CATACTGTCCAAAAAAGTACCTTGTTTCTACT

Figure 13B

YP_308666.1

MEDFVRQCFNPMIVELAEKAMKEYGEDPKIETNKFAAICTHLEVCFMYSDFHFIDERGESTIIESGDPNAL
LKHRFEIIEGRDRTMAWTVVNSICNTTGVEKPKFLPDLYDYKENRFIEIGVTRREVHTYYLEKANKIKSEK
THIHIFSFTGEEMATKADYTLDEESRARIKTRLFTIRQEMASRGLWDSFRQSERGEETVEERFEITGTMCR
LADQSLPPNFSSLEKFRAYVDGFEPNGCIEGKLSQMSKEVNARIEPFLKTTPRPLRLPDGPPCSQRSKFLL
MDALKLSIEDPSHEGEGIPLYDAIKCMKTFFGWKEPNIVKPHEKGINPNYLLAWKQVLAELQDIENEEKIP
KTKNMRKTSQLKWALGENMAPEKVDFEDCKDVSDLRQYDSDEPKPRSLASWIQSEFNKACELTDSSWIELD
EIGEDVAPIEHIASMRRNYFTAEVSHCRATEYIMKGVYINTALLNASCAAMDDFQLIPMISKCRTKEGRRK
TNLYGFLIKGRSHLRNDTDVVNFVSMEFSLTDPRLEPHRWEKYCVLRIGDMLLRTEIGQVSRPMFLYVRTN
GTSKIKMKWGMEMRRCPFQSLQQIESMIEAESSVKEKDMTKEFFENKSETWPIGESPKGVEEGSIGKVCRT
LLAKSVFNSLYASPQLEGFSAESRKLLLIVQALRDNLEPGTFDLGGLYEAIEECLINDPWVLLNASWFNSF
LTHALR

Figure 14A

NC_007376.1

AGCAAAAGCAGGTACTGATTCGAAATGGAAGATTTTGTGCGACAATGCTTCAATCCGATGATTGTCGA
ACTTGCGGAAAAGGCAATGAAAGAGTATGGAGAAGATCTGAAAATCGAAACAAACAAATTTGCAGCAA
TATGCACTCACTTGGAAGTATGCTTCATGTATTCAGATTTTCATTTCATCAATGAGCAAGGCGAGTCA
ATAATGGTAGAGCTTGATGATCCAAATGCACTTTTGAAGCACAGATTTGAAATAATAGAGGGAAGAGA
TCGCACAATGGCCTGGACAGTAGTAAACAGTATTTGCAACACCACAGGAGCTGAGAAACCGAAGTTTC
TGCCAGATTTGTATGATTACAAGGAGAATAGATTCATCGAGATTGGAGTGACAAGGAGAGAAGTCCAC
ATATACTATCTTGAAAAGGCCAATAAAATTAAATCTGAGAATACACACATCCACATTTTCTCATTCAC
TGGGGAAGAAATGGCCACAAAGGCCGACTACACTCTCGATGAGGAAAGCAGGGCTAGGATCAAAACCA
GACTATTCACCATAAGACAAGAAATGGCCAACAGAGGCCTCTGGGATTCCTTTCGTCAGTCCGAAAGA
GGCGAAGAAACAATTGAAGAAAGATTTGAAATCACAGGGACAATGCGCAGGCTTGCCGACCAAAGTCT
CCCGCCGAACTTCTCCTGCCTTGAGAATTTTAGAGCCTATGTGGATGGATTCGAACCGAACGGCTACA
TTGAGGGCAAGCTTTCTCAAATGTCCAAAGAAGTAAATGCAAAAATTGAACCTTTTCTGAAAACAACA
CCAAGACCAATTAGACTTCCGGATGGGCCTCCTTGTTTTCAGCGGTCCAAATTCCTGCTGATGGATGC
TTTAAAATTAAGCATTGAGGACCCAAGTCACGAAGGGGAGGGAATACCACTATATGATGCGATCAAGT
GCATGAGAACATTCTTTGGATGGAAAGAACCCTATATTGTTAAACCACACGAAAAGGGAATAAATCCA
AATTATCTGCTGTCATGGAAGCAAGTACTGGCGGAACTGCAGGACATTGAGAATGAGGAGAAGATTCC
AAGAACTAAAAACATGAAGAAAACGAGTCAGCTAAAGTGGGCACTTGGTGAGAACATGGCACCAGAGA
AGGTAGACTTTGACAACTGTAGAGACATAAGCGATTTGAAGCAATATGATAGTGACGAACCTGAATTA
AGGTCACTTTCAAGCTGGATCCAGAATGAGTTCAACAAGGCATGCGAGCTGACCGATTCAATCTGGAT
AGAGCTCGATGAGATTGGAGAAGACGTGGCTCCAATTGAACACATTGCAAGCATGAGAAGGAATTACT
TCACAGCAGAGGTGTCCCATTGCAGAGCCACAGAATATATAATGAAGGGGGTATACATTAATACTGCC
TTGCTTAATGCATCCTGTGCAGCAATGGACGATTTCCAACTAATTCCCATGATAAGCAAGTGTAGAAC
TAAAGAGGGAAGGCGAAAGACCAATTTATATGGTTTCATCATAAAAGGAAGATCTCACTTAAGGAATG
ACACCGACGTGGTAAACTTTGTGAGCATGGAGTTTTCTCTCACTGACCCGAGACTTGAGCCACACAAA
TGGGAGAAGTACTGTGTCCTTGAGATAGGAGATATGCTACTAAGAAGTGCCATAGGCCAGATGTCAAG
GCCTATGTTCTTGTATGTGAGGACAAATGGAACATCAAAGATTAAAATGAAATGGGGAATGGAGATGA
GGCCTTGCCTCCTTCAGTCACTACAACAAATCGAGAGTATGGTTGAAGCCGAGTCCTCTGTCAAAGAG
AAAGACATGACCAAAGAGTTTTTTGAGAATAAATCAGAAACATGGCCCATTGGGGAGTCCCCCAAAGG
AGTGGAAGAAGGTTCCATTGGGAAGGTCTGCAGGACTTTATTAGCCAAGTCGGTATTCAATAGCCTGT
ATGCATCCCCACAATTAGAAGGATTTTCAGCTGAATCAAGAAAACTGCTTCTTGTCGTTCAGGCTCTT
AGGGACAATCTTGAACCTGGAACCTTTGATCTTGGGGGGCTATATGAAGCAATTGAGGAGTGCCTGAT
TAATGATCCCTGGGTTTTGCTTAATGCGTCTTGGTTCAACTCCTTCCTAACACATGCATTAAGATAGT
TGTGGCAATGCTACTATTTGCTATCCATACTGTCCAAAAAAGTACCTTGTTTCTACT

Figure 14B

YP_308852.1

MEDFVRQCFNPMIVELAEKAMKEYGEDLKIETNKFAAICTHLEVCFMYSDFHFINEQGESIMVELDDP
NALLKHRFEIIEGRDRTMAWTVVNSICNTTGAEKPKFLPDLYDYKENRFIEIGVTRREVHIYYLEKAN
KIKSENTHIHIFSFTGEEMATKADYTLDEESRARIKTRLFTIRQEMANRGLWDSFRQSERGEETIEER
FEITGTMRRLADQSLPPNFSCLENFRAYVDGFEPNGYIEGKLSQMSKEVNAKIEPFLKTTPRPIRLPD
GPPCFQRSKFLLMDALKLSIEDPSHEGEGIPLYDAIKCMRTFFGWKEPYIVKPHEKGINPNYLLSWKQ
VLAELQDIENEEKIPRTKNMKKTSQLKWALGENMAPEKVDFDNCRDISDLKQYDSDEPELRSLSSWIQ
NEFNKACELTDSIWIELDEIGEDVAPIEHIASMRRNYFTAEVSHCRATEYIMKGVYINTALLNASCAA
MDDFQLIPMISKCRTKEGRRKTNLYGFIIKGRSHLRNDTDVVNFVSMEFSLTDPRLEPHKWEKYCVLE
IGDMLLRSAIGQMSRPMFLYVRTNGTSKIKMKWGMEMRPCLLQSLQQIESMVEAESSVKEKDMTKEFF
ENKSETWPIGESPKGVEEGSIGKVCRTLLAKSVFNSLYASPQLEGFSAESRKLLLVVQALRDNLEPGT
FDLGGLYEAIEECLINDPWVLLNASWFNSFLTHALR

Figure 15A

NC_002020.1

AGCAAAAGCAGGGTGACAAAGACATAATGGATCCAAACACTGTGTCAAGCTTTCAGGTA
GATTGCTTTCTTTGGCATGTCCGCAAACGAGTTGCAGACCAAGAACTAGGTGATGCCCC
ATTCCTTGATCGGCTTCGCCGAGATCAGAAATCCCTAAGAGGAAGGGGCAGCACTCTTG
GTCTGGACATCGAGACAGCCACACGTGCTGGAAAGCAGATAGTGGAGCGGATTCTGAAA
GAAGAATCCGATGAGGCACTTAAAATGACCATGGCCTCTGTACCTGCGTCGCGTTACCT
AACCGACATGACTCTTGAGGAAATGTCAAGGGAATGGTCCATGCTCATACCCAAGCAGA
AAGTGGCAGGCCCTCTTTGTATCAGAATGGACCAGGCGATCATGGATAAAAACATCATA
CTGAAAGCGAACTTCAGTGTGATTTTTGACCGGCTGGAGACTCTAATATTGCTAAGGGC
TTTCACCGAAGAGGGAGCAATTGTTGGCGAAATTTCACCATTGCCTTCTCTTCCAGGAC
ATACTGCTGAGGATGTCAAAAATGCAGTTGGAGTCCTCATCGGAGGACTTGAATGGAAT
GATAACACAGTTCGAGTCTCTGAAACTCTACAGAGATTCGCTTGGAGAAGCAGTAATGA
GAATGGGAGACCTCCACTCACTCCAAAACAGAAACGAGAAATGGCGGGAACAATTAGGT
CAGAAGTTTGAAGAAATAAGATGGTTGATTGAAGAAGTGAGACACAAACTGAAGGTAAC
AGAGAATAGTTTTGAGCAAATAACATTTATGCAAGCCTTACATCTATTGCTTGAAGTGG
AGCAAGAGATAAGAACTTTCTCATTTCAGCTTATTTAATAATAAAAAACACCCTTGTTT
CTACT

Figure 15B

NP_040983.1

MDPNTVSSFQDILLRMSKMQLESSSEDLNGMITQFESLKLYRDSLGEAVMRMGDLHSLQ
NRNEKWREQLGQKFEEIRWLIEEVRHKLKVTENSFEQITFMQALHLLLEVEQEIRTFSF
QLI

Figure 16A

NC_004906.1

AGCAAAAGCAGGGTGACAAAGACATAATGGATTCCAACACTGTGTCAAGCTTTCAGGTA
GACTGCTTTCTTTGGCATGTCCGCAAACGATTTGCAGACCAAGAACTGGGTGATGCCCC
ATTCCTTGACCGGCTTCGCCGAGATCAGAAGTCCCTAAGAGGAAGAGGCAGCACTCTTG
GTCTGGACATCAGAACTGCCACTCGTGAAGGAAAGCATATAGTGGAGCGGATTCTGGAG
GAAGAATCTGACGAGGCACTTAAAATGACTATCGCTTCAGTGCCTGCTTCACGCTACCT
AACTGAAATGACTCTTGAGGAAATGTCAAGGGATTGGTTAATGCTCATTCCCAAGCAGA
AAGTGACAGGGCCCCTTTGCATTAGAATGGACCAGGCAGTAATGGGTAAAACCATCATA
TTGAAAGCAAACTTTAGTGTGATTTTTAATCGACTTGAAGCTCTGATACTACTTAGAGC
GTTTACAGATGAAGGAGCAATAGTGGGCGAAATCTCACCATTACCTTCCCTTCCAGGAC
ATACTGACGAGGATGTCAAAAATGCAATTGGGGTCCTCATCGGAGGACTTGAATGGAAT
GATAACACAGTTCGAGTCTCTGAAACTCTACAGAGATTCACTTGGAGAAGCAGTGATGA
GAATGGGAGATCTCCACTCCCTCCAAAACAGAAACGGAAAGTGGAGAGAACAATTGAGC
CAGAAGTTTGAAGAGATAAGATGGTTAATTGAAGAAATGCGACATAGGTTAAGAATTAC
AGAGAATAGCTTTGAGCAAATAACCTTTATGCAAGCCTTACAACTATTGCTTGAAGTGGAG
CAAGAGATAAGAACTTTCTCGTTTCAGCTTATTTAATGATAAAAAACACCCTTGTTTCTACT

Figure 16B

YP_581750.1

MDSNTVSSFQDILTRMSKMQLGSSSEDLNGMITQFESLKLYRDSLGEAVMRMGDLHSLQ
NRNGKWREQLSQKFEEIRWLIEEMRHRLRITENSFEQITFMQALQLLLEVEQEIRTFSF
QLI

Figure 17A

NC_007370.1

AGCAAAAGCAGGGTGACAAAGACATAATGGATTCCAACACTGTGTCAAGTTTCCAGGTA
GATTGCTTTCTTTGGCATATCCGGAAACAAGTTGTAGACCAAGAACTGAGTGATGCCCC
ATTCCTTGATCGGCTTCGCCGAGATCAGAGGTCCCTAAGGGGAAGAGGCAATACTCTCG
GTCTAGACATCAAAGCAGCCACCCATGTTGGAAAGCAAATTGTAGAAAAGATTCTGAAA
GAAGAATCTGATGAGGCACTTAAAATGACCATGGTCTCCACACCTGCTTCGCGATACAT
AACTGACATGACTATTGAGGAATTGTCAAGAAACTGGTTCATGCTAATGCCCAAGCAGA
AAGTGGAAGGACCTCTTTGCATCAGAATGGACCAGGCAATCATGGAGAAAAACATCATG
TTGAAAGCGAATTTCAGTGTGATTTTTGACCGACTAGAGACCATAGTATTACTAAGGGC
TTTCACCGAAGAGGGAGCAATTGTTGGCGAAATCTCACCATTGCCTTCTTTTCCAGGAC
ATACTATTGAGGATGTCAAAAATGCAATTGGGGTCCTCATCGGAGGACTTGAATGGAAT
GATAACACAGTTCGAGTCTCTAAAAATCTACAGAGATTCGCTTGGAGAAGCAGTAATGA
GAATGGGGGACCTCCACTTACTCCAAAACAGAAACGGAAAATGGCGAGAACAGCTAGGT
CAAAAGTTTGAAGAGATAAGATGGCTGATTGAAGAAGTGAGACACAGACTAAAAACAAC
TGAAAATAGCTTTGAACAAATAACATTCATGCAAGCATTACAACTGCTGTTTGAAGTGG
AACAGGAGATAAGAACTTTCTCATTTCAGCTTATTTAATGATAAAAAACACCCTTGTTT
CTACT

Figure 17B

YP_308844.1

MDSNTVSSFQDILLRMSKMQLGSSSEDLNGMITQFESLKIYRDSLGEAVMRMGDLHLLQ
NRNGKWREQLGQKFEEIRWLIEEVRHRLKTTENSFEQITFMQALQLLFEVEQEIRTFSF
QLI

Figure 18A

NC_002016.1

AGCGAAAGCAGGTAGATATTGAAAGATGAGTCTTCTAACCGAGGTCGAAACGTACGTTC
TCTCTATCATCCCGTCAGGCCCCCTCAAAGCCGAGATCGCACAGAGACTTGAAGATGTC
TTTGCAGGGAAGAACACCGATCTTGAGGTTCTCATGGAATGGCTAAAGACAAGACCAAT
CCTGTCACCTCTGACTAAGGGGATTTTAGGATTTGTGTTCACGCTCACCGTGCCCAGTG
AGCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCTTAATGGGAACGGGGATCCA
AATAACATGGACAAAGCAGTTAAACTGTATAGGAAGCTCAAGAGGGAGATAACATTCCA
TGGGGCCAAAGAAATCTCACTCAGTTATTCTGCTGGTGCACTTGCCAGTTGTATGGGCC
TCATATACAACAGGATGGGGGCTGTGACCACTGAAGTGGCATTTGGCCTGGTATGTGCA
ACCTGTGAACAGATTGCTGACTCCCAGCATCGGTCTCATAGGCAAATGGTGACAACAAC
CAACCCACTAATCAGACATGAGAACAGAATGGTTTTAGCCAGCACTACAGCTAAGGCTA
TGGAGCAAATGGCTGGATCGAGTGAGCAAGCAGCAGAGGCCATGGAGGTTGCTAGTCAG
GCTAGGCAAATGGTGCAAGCGATGAGAACCATTGGGACTCATCCTAGCTCCAGTGCTGG
TCTGAAAAATGATCTTCTTGAAAATTTGCAGGCCTATCAGAAACGAATGGGGGTGCAGA
TGCAACGGTTCAAGTGATCCTCTCGCTATTGCCGCAAATATCATTGGGATCTTGCACTT
GATATTGTGGATTCTTGATCGTCTTTTTTTCAAATGCATTTACCGTCGCTTTAAATACG
GACTGAAAGGAGGGCCTTCTACGGAAGGAGTGCCAAAGTCTATGAGGGAAGAATATCGA
AAGGAACAGCAGAGTGCTGTGGATGCTGACGATGGTCATTTTGTCAGCATAGAGCTGGA
GTAAAAAACTACCTTGTTTCTACT

Figure 18B

NP_040978.1

MSLLTEVETYVLSIIPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRPILSPLTKGI
LGFVFTLTVPSERGLQRRRFVQNALNGNGDPNNMDKAVKLYRKLKREITFHGAKEISLS
YSAGALASCMGLIYNRMGAVTTEVAFGLVCATCEQIADSQHRSHRQMVTTTNPLIRHEN
RMVLASTTAKAMEQMAGSSEQAAEAMEVASQARQMVQAMRTIGTHPSSSAGLKNDLLEN
LQAYQKRMGVQMQRFK

Figure 19A

NC_007377.1

AGCAAAAGCAGGTAGATATTGAAAGATGAGCCTTCTAACCGAGGTCGAAACGTACGTTC
TCTCTATCGTCCCGTCAGGCCCCCTCAAAGCCGAGATCGCACAGAGACTTGAAGATGTC
TTTGCTGGGAAGAACACAGATCTTGAGGCTCTCATGGAATGGCTAAAGACAAGACCAAT
CCTGTCACCTCTGACTAAGGGGATTTTGGGATTTGTATTCACGCTCACCGTGCCAAGTG
AGCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCTCAATGGGAATGGGGATCCA
AATAACATGGACAGAGCAGTTAAACTGTATAGAAAGCTTAAGAGGGAGATAACATTCCA
TGGGGCCAAAGAAGTAGCGCTCAGTTATTCTGCTGGTGCACTTGCCAGTTGCATGGGCC
TCATATACAACAGGATGGGGGCTGTGACCACTGAAGTGGCCTTTGCCGTGGTATGTGCA
ACCTGTGAACAGATTGCTGACTCCCAGCATAGGTCTCACAGGCAAATGGTGACAACAAC
CAATCCACTAATAAGACATGAGAACAGAATGGTTCTGGCCAGCACTACAGCTAAGGCTA
TGGAGCAAATGGCTGGATCGAGTGAGCAAGCAGCAGAGGCCATGGAGGTTGCTAGTCAG
GCCAGGCAAATGGTGCAGGCAATGAGAGCCATTGGGACTCCTCCTAGCTCCAGTGCTGG
TCTAAAAGATGATCTTCTTGAAAATTTGCAGGCCTATCAGAAACGAATGGGGGTGCAGA
TGCAACGATTCAAGTGACCCCCTTGTTGTTGCTGCGAGTATCATTGGGATCTTGCACTT
TATATTGTGGATTCTTGATCGTCTTTTTTTCAAATGCATTTATCGCTTCTTTAAACACG
GTCTGAAAAGAGGGCCTTCTACGGAAGGAGTACCTGAGTCTATGAGGGAAGAATATCGA
AAGGAACAGCAGAGTGCTGTGGATGCTGACGATAGTCATTTTGTCAGCATAGAGCTGGA
GTAAAAAACTACCTTGTTTCTACT

Figure 19B

YP_308854.1

MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEALMEWLKTRPILSPLTKGI
LGFVFTLTVPSERGLQRRRFVQNALNGNGDPNNMDRAVKLYRKLKREITFHGAKEVALS
YSAGALASCMGLIYNRMGAVTTEVAFAVVCATCEQIADSQHRSHRQMVTTTNPLIRHEN
RMVLASTTAKAMEQMAGSSEQAAEAMEVASQARQMVQAMRAIGTPPSSSAGLKDDLLEN
LQAYQKRMGVQMQRFK

Figure 20A

NC_007363.1

AGCAAAAGCAGGTAGATATTGAAAAATGAGTCTTCTAACCGAGGTCGAAACGTACGTTC
TCTCTATCGTCCCGTCAGGCCCCCTCAAAGCCGAGATCGCGCAGAGACTTGAGGATGTC
TTTGCAGGAAAGAACACCGATCTCGAGGCTCTCATGGAATGGCTAAAGACAAGACCAAT
CCTGTCACCTCTGACTAAAGGGATTTTAGGATTTGTGTTCACGCTCACCGTGCCCAGTG
AGCGAGGACTGCAGCGTAGACGCTTTGTCCAGAATGCCTTAAATGGAAATGGAGATCCA
AACAATATGGATAGGGCAGTTAAGCTATACAAGAAGCTGAAAAGAGAAATAACATTCCA
TGGGGCTAAGGAGGTCGCACTCAGCTACTCAACCGGTGCACTTGCCAGTTGTATGGGTC
TCATATACAACAGGATGGGAACGGTGACCACAGAAGTGGCTTTTGGCCTAGTGTGTGCC
ACTTGTGAGCAGATTGCAGATTCACAGCATCGGTCTCACAGACAGATGGCAACTACCAC
CAACCCACTAATCAGGCATGAGAACAGAATGGTGCTGGCCAGCACTACAGCTAAGGCTA
TGGAGCAGATGGCTGGATCGAGTGAGCAGGCAGCGGAAGCCATGGAGGTTGCTAGTCAG
GCTAGGCAGATGGTGCAGGCAATGAGGACAATTGGGACTCATCCTAGCTCCAGTGCCGG
TCTGAAAGATAATCTTCTTGAAAATTTGCAGGCCTACCAAAAACGAATGGGAGTGCAAA
TGCAGCGATTCAAGTGATCCTCTTGTTGTTGCCGCAAGTATCATTGGGATACTGCACTT
GATATTGTGGATTCTTGATCGTCTTTTCTTCAAATGCATTTATCGTCGCCTTAAATACG
GTTTGAAAAGAGGGCCTTCTACGGAAGGGGTACCTGAGTCTATGAGGGAAGAGTATCGG
CAGGAACAGCAGAGTGCTGTGGATGTTGACGATGGTCATTTTGTCAACATAGAGCTGGA
GTAAAAAACTACCTTGTTTCTACT

Figure 20B

YP_308671.1

MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEALMEWLKTRPILSPLTKGI
LGFVFTLTVPSERGLQRRRFVQNALNGNGDPNNMDRAVKLYKKLKREITFHGAKEVALS
YSTGALASCMGLIYNRMGTVTTEVAFGLVCATCEQIADSQHRSHRQMATTTNPLIRHEN
RMVLASTTAKAMEQMAGSSEQAAEAMEVASQARQMVQAMRTIGTHPSSSAGLKDNLLEN
LQAYQKRMGVQMQRFK

Figure 21A

NC_002020.1

AGCAAAAGCAGGGTGACAAAGACATAATGGATCCAAACACTGTGTCAAGCTTTCAGGTA
GATTGCTTTCTTTGGCATGTCCGCAAACGAGTTGCAGACCAAGAACTAGGTGATGCCCC
ATTCCTTGATCGGCTTCGCCGAGATCAGAAATCCCTAAGAGGAAGGGGCAGCACTCTTG
GTCTGGACATCGAGACAGCCACACGTGCTGGAAAGCAGATAGTGGAGCGGATTCTGAAA
GAAGAATCCGATGAGGCACTTAAAATGACCATGGCCTCTGTACCTGCGTCGCGTTACCT
AACCGACATGACTCTTGAGGAAATGTCAAGGGAATGGTCCATGCTCATACCCAAGCAGA
AAGTGGCAGGCCCTCTTTGTATCAGAATGGACCAGGCGATCATGGATAAAAACATCATA
CTGAAAGCGAACTTCAGTGTGATTTTTGACCGGCTGGAGACTCTAATATTGCTAAGGGC
TTTCACCGAAGAGGGAGCAATTGTTGGCGAAATTTCACCATTGCCTTCTCTTCCAGGAC
ATACTGCTGAGGATGTCAAAAATGCAGTTGGAGTCCTCATCGGAGGACTTGAATGGAAT
GATAACACAGTTCGAGTCTCTGAAACTCTACAGAGATTCGCTTGGAGAAGCAGTAATGA
GAATGGGAGACCTCCACTCACTCCAAAACAGAAACGAGAAATGGCGGGAACAATTAGGT
CAGAAGTTTGAAGAAATAAGATGGTTGATTGAAGAAGTGAGACACAAACTGAAGGTAAC
AGAGAATAGTTTTGAGCAAATAACATTTATGCAAGCCTTACATCTATTGCTTGAAGTGG
AGCAAGAGATAAGAACTTTCTCATTTCAGCTTATTTAATAATAAAAAACACCCTTGTTT
CTACT

Figure 21B

NP_040984.1

MDPNTVSSFQVDCFLWHVRKRVADQELGDAPFLDRLRRDQKSLRGRGSTLGLDIETATR
AGKQIVERILKEESDEALKMTMASVPASRYLTDMTLEEMSREWSMLIPKQKVAGPLCIR
MDQAIMDKNIILKANFSVIFDRLETLILLRAFTEEGAIVGEISPLPSLPGHTAEDVKNA
VGVLIGGLEWNDNTVRVSETLQRFAWRSSNENGRPPLTPKQKREMAGTIRSEV

Figure 22A

NC_007370.1

AGCAAAAGCAGGGTGACAAAGACATAATGGATTCCAACACTGTGTCAAGTTTCCAGGTA
GATTGCTTTCTTTGGCATATCCGGAAACAAGTTGTAGACCAAGAACTGAGTGATGCCCC
ATTCCTTGATCGGCTTCGCCGAGATCAGAGGTCCCTAAGGGGAAGAGGCAATACTCTCG
GTCTAGACATCAAAGCAGCCACCCATGTTGGAAAGCAAATTGTAGAAAAGATTCTGAAA
GAAGAATCTGATGAGGCACTTAAAATGACCATGGTCTCCACACCTGCTTCGCGATACAT
AACTGACATGACTATTGAGGAATTGTCAAGAAACTGGTTCATGCTAATGCCCAAGCAGA
AAGTGGAAGGACCTCTTTGCATCAGAATGGACCAGGCAATCATGGAGAAAAACATCATG
TTGAAAGCGAATTTCAGTGTGATTTTTGACCGACTAGAGACCATAGTATTACTAAGGGC
TTTCACCGAAGAGGGAGCAATTGTTGGCGAAATCTCACCATTGCCTTCTTTTCCAGGAC
ATACTATTGAGGATGTCAAAAATGCAATTGGGGTCCTCATCGGAGGACTTGAATGGAAT
GATAACACAGTTCGAGTCTCTAAAAATCTACAGAGATTCGCTTGGAGAAGCAGTAATGA
GAATGGGGGACCTCCACTTACTCCAAAACAGAAACGGAAAATGGCGAGAACAGCTAGGT
CAAAAGTTTGAAGAGATAAGATGGCTGATTGAAGAAGTGAGACACAGACTAAAAACAAC
TGAAAATAGCTTTGAACAAATAACATTCATGCAAGCATTACAACTGCTGTTTGAAGTGG
AACAGGAGATAAGAACTTTCTCATTTCAGCTTATTTAATGATAAAAAACACCCTTGTTT
CTACT

Figure 22B

YP_308845.1

MDSNTVSSFQVDCFLWHIRKQVVDQELSDAPFLDRLRRDQRSLRGRGNTLGLDIKAATH
VGKQIVEKILKEESDEALKMTMVSTPASRYITDMTIEELSRNWFMLMPKQKVEGPLCIR
MDQAIMEKNIMLKANFSVIFDRLETIVLLRAFTEEGAIVGEISPLPSFPGHTIEDVKNA
IGVLIGGLEWNDNTVRVSKNLQRFAWRSSNENGGPPLTPKQKRKMARTARSKV

Figure 23A

NC_004906.1

AGCAAAAGCAGGGTGACAAAGACATAATGGATTCCAACACTGTGTCAAGCTTTCAGGTA
GACTGCTTTCTTTGGCATGTCCGCAAACGATTTGCAGACCAAGAACTGGGTGATGCCCC
ATTCCTTGACCGGCTTCGCCGAGATCAGAAGTCCCTAAGAGGAAGAGGCAGCACTCTTG
GTCTGGACATCAGAACTGCCACTCGTGAAGGAAAGCATATAGTGGAGCGGATTCTGGAG
GAAGAATCTGACGAGGCACTTAAAATGACTATCGCTTCAGTGCCTGCTTCACGCTACCT
AACTGAAATGACTCTTGAGGAAATGTCAAGGGATTGGTTAATGCTCATTCCCAAGCAGA
AAGTGACAGGGCCCCTTTGCATTAGAATGGACCAGGCAGTAATGGGTAAAACCATCATA
TTGAAAGCAAACTTTAGTGTGATTTTTAATCGACTTGAAGCTCTGATACTACTTAGAGC
GTTTACAGATGAAGGAGCAATAGTGGGCGAAATCTCACCATTACCTTCCCTTCCAGGAC
ATACTGACGAGGATGTCAAAAATGCAATTGGGGTCCTCATCGGAGGACTTGAATGGAAT
GATAACACAGTTCGAGTCTCTGAAACTCTACAGAGATTCACTTGGAGAAGCAGTGATGA
GAATGGGAGATCTCCACTCCCTCCAAAACAGAAACGGAAAGTGGAGAGAACAATTGAGC
CAGAAGTTTGAAGAGATAAGATGGTTAATTGAAGAAATGCGACATAGGTTAAGAATTAC
AGAGAATAGCTTTGAGCAAATAACCTTTATGCAAGCCTTACAACTATTGCTTGAAGTGG
AGCAAGAGATAAGAACTTTCTCGTTTCAGCTTATTTAATGATAAAAAACACCCTTGTTT
CTACT

Figure 23B

NP_859034.1

MDSNTVSSFQVDCFLWHVRKRFADQELGDAPFLDRLRRDQKSLRGRGSTLGLDIRTATR
EGKHIVERILEEESDEALKMTIASVPASRYLTEMTLEEMSRDWLMLIPKQKVTGPLCIR
MDQAVMGKTIILKANFSVIFNRLEALILLRAFTDEGAIVGEISPLPSLPGHTDEDVKNA
IGVLIGGLEWNDNTVRVSETLQRFTWRSSDENGRSPLPPKQKRKVERTIEPEV

Figure 24

CY080633.1 Influenza A virus (A/Boston/1/2009(H1N1)) Segment 1

AAAAGCAGGTCAATTATATTCAATATGGAAAGAATAAAAGAGCTAAGGAATTTGATGTC
ACAATCTCGCACTCGCGAGATACTTACCAAAACTACTGTAGACCACATGGCCATAATCA
AGAAATACACATCAGGAAGACAGGAGAAAAACCCATCACTTAGGATGAAATGGATGATG
GCAATGAAATACCCAATTACAGCTGATAAAAGGATAACGGAAATGATTCCTGAAAGAAA
TGAGCATGGACAGACATTATGGAGTAAGGTGAATGATGCCGGATCAGACCGAGTGATGG
TATCACCCCTGGCTGTGACATGGTGGAACAGAAATGGACCAGTGGCAAGTACTATTCAC
TATCCAAAAATCTACAAAACTTACTTTGAAAAGGTTGAAAGGTTAAAACAAGGAACCTT
TGGCCCTGTACACTTTAGAAACCAAGTCAAAATACGCCGAAGAGTCGACATAAATCCTG
GTCATGCAGACCTCAGCGCCAAGGAGGCACAGGATGTAATTATGGAAGTTGTTTTCCCT
AATGAAGTAGGAGCCAGAATACTAACATCAGAATCGCAATTAACGATAACCAAGGAGAA
AAAAGAAGAACTCCAGAATTGCAAAATTTCCCCTTTGATGGTTGCATACATGTTAGAGA
GGGAACTTGTCCGCAAAACAAGATTTCTCCCGGTTGCAGGTGGAACAAGCAGTGTGTAC
ATTGAAGTTTTGCATTTAACACAGGGGACATGCTGGGAGCAGATGTACACTCCAGGTGG
GGAGGTGAGGAATGATGATGTTGATCAAAGCCTAATTATTGCTGCTAGGAACATAGTGA
GAAGAGCTGCAGTATCAGCAGATCCACTAGCATCCTTATTAGAAATGTGCCATAGCACA
CAGATTGGTGGAACAAGGATGGTGGATATTCTCAGGCAAAATCCAACAGAAGAACAAGC
TGTGGACATATGCAAAGCAGCAATGGGGCTGAGAATCAGCTCATCCTTCAGTTTTGGCG
GATTCACATTTAAGAGAACAAGTGGATCATCAGTCAAAAGGGAGGAAGAAGTTCTCACG
GGCAATCTGCAAACATTGAAACTAACCGTGCATGAGGGATATGAAGAGTTCACAATGGT
TGGGAAAAGGGCAACAGCTATACTCAGAAAAGCAACCAGGAGATTGATTCAACTAATAG
TGAGTGGAAGAGACGAACAATCAATAGTCGAAGCAATAGTTGTAGCAATGGTATTCTCA
CAAGAAGATTGCATGGTAAAAGCAGTTAGAGGTGATCTGAATTTCGTTAATAGAGCGAA
TCAGCGGTTGAATCCCATGCATCAACTATTGAGACATTTTCAGAAGGATGCTAAAGTAC
TTTTCTTAAATTGGGGAGTTGAACCTATTGACAATGTGATGGGAATGATTGGGATATTA
CCTGATATGACTCCAAGTACCGAGATGTCAATGAGAGGAGTGAGAGTCAGCAAAATGGG
TGTAGATGAATACTCCAATGCTGAAAGGGTAGTGGTAAGCATTGACCGTTTTTTGAGAG
TCCGGGACCAAAGAGGAAATGTACTACTGTCTCCAGAGGAAGTAAGTGAAACACAAGGG
ACAGAGAAACTGACAATAACTTACTCTTCATCAATGATGTGGGAGATTAATGGCCCTGA
GTCAGTCTTGATCAATACCTATCAGTGGATCATCAGAAACTGGGAGACTGTTAAAATTC
AGTGGTCTCAGAATCCTACGATGCTGTACAATAAAATGGAATTTGAACCATTTCAGTCT
CTAGTCCCCAAGGCCATTAGAGGCCAATACAGTGGGTTTGTTAGAACTCTATTCCAACA
AATGAGGGATGTGCTTGGGACTTTTGACACAACTCAGATAATAAAACTTCTTCCCTTTG
CAGCCGCTCCTCCAAAGCAAAGCAGAATGCAATTCTCGTCATTAACTGTGAATGTGAGG
GGATCAGGAATGAGAATACTTGTGAGGGGTAATTCTCCAGTATTCAACTACAACAAGAC
TACCAAGAGACTCACAGTCCTCGGAAAGGATGCTGGCACTTTAACTGAAGACCCAGATG
AAGGCACAGCTGGAGTGGAATCTGCGGTTCTAAGGGGATTCCTCATTTTAGGCAAAGAA
GATAGAAGATATGGGCCAGCATTAAGCATCAATGAATTGAGCAATCTTGCGAAAGGAGA
AAAAGCTAATGTGCTAATTGGGCAAGGGGATGTAGTGTTGGTAATGAAACGAAAACGGG
ACTCTAGCATACTTACTGACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAAT
TAATTTCGAATAATTTAAAAACGACCTT

Figure 25

CY080632.1 Influenza A virus (A/Boston/1/2009(H1N1)) Segment 2

AAAGCAGGCAAACCATTTGAATGGATGTCAATCCGACATTACTTTTCTTAAAAGTGCCA
GCACAAAATGCTATAAGCACAACTTTTCCTTATACTGGTGACCCTCCTTACAGCCATGG
GACAGGAACAGGGTACACCATGGATACAGTCAACAGGACACACCAGTACTCAGAAAGAG
GAAGATGGACAAAAAATACCGAAACGGGAGCACCGCAACTTAACCCAATTGATGGTCCC
TTACCGGAAGACAATGAACCAAGTGGCTATGCCCAAACAGATTGTGTATTAGAAGCAAT
GGCTTTCCTTGAAGAATCCCATCCCGGTATCTTTGAAAACTCTTGTATTGAAACAATGG
AGGTTGTTCAACAAACAAGGGTGGACAAACTGACACAAGGCAGACAGACCTATGACTGG
ACTCTAAATAGGAACCAGCCTGCTGCCACAGCATTGGCAAACACTATAGAAGTATTCAG
ATCAAACGGCCTCATAGCAAATGAATCTGGAAGGCTAATAGACTTCCTTAAAGATGTAA
TGGAGTCGATGGACAGAGGCGAAGTAGAGGTCACAACTCATTTTCAAAGAAAGAGGAGA
GTGAGAGACAATGTAACTAAAAAAATGGTGACCCAAAGAACAATAGGCAAAAAGAAACA
TAAATTAGACAAAAGAAGTTACCTAATTAGGGCATTAACCCTGAACACAATGACCAAAG
ATGCTGAGAGGGGGAAACTAAAACGCAGAGCAATTGCAACCCCAGGAATGCAAATAAGG
GGGTTTGTATACTTTGTTGAGACACTGGCAAGAAGCATATGTGAAAAGCTTGAACAATC
AGGATTGCCAGTTGGAGGAAATGAGAAGAAAGCAAAGTTAGCAAATGTTGTAAGGAAGA
TGATGACCAACTCCCAGGACACTGAAATTTCTTTCACCATAACCGGAGACAACACAAAA
TGGAACGAAAATCAAAACCCTAGAATGTTCTTGGCCATGATCACATATATAACCAAAAA
TCAGCCTGAATGGTTCAGAAATATTCTAAGTATTGCTCCAATAATGTTTTCAAACAAGA
TGGCGAGACTAGGTAAGGGGTACATGTTTGAAAGCAAGAGTATGAAACTGAGAACTCAA
ATACCTGCAGAGATGCTAGCCAACATAGACTTGAAATATTTCAATGATTCAACTAAAAA
GAAAATTGAAAAAATCCGACCATTATTAATAGATGGAACTGCATCATTGAGTCCTGGAA
TGATGATGGGCATGTTCAATATGTTGAGCACCGTATTGGGCGTCTCCATTCTGAATCTT
GGGCAAAAGAGATACACCAAGACTACTTACTGGTGGGATGGTCTTCAATCGTCTGATGA
TTTTGCTTTAATTGTGAACGCACCCAACTATGCAGGAATTCAAGCTGGAGTTGACAGGT
TTTATCGAACCTGTAAGCTGCTCGGAATTAATATGAGCAAAAAGAAGTCTTACATAAAC
AGAACAGGTACCTTTGAATTCACGAGCTTTTTCTATCGTTATGGGTTTGTTGCCAATTT
CAGCATGGAGCTTCCTAGTTTTGGGGTGTCTGGGGTCAATGAATCTGCAGACATGAGTA
TTGGAGTCACTGTCATCAAAAACAATATGATAAACAATGACCTTGGCCCAGCAACTGCT
CAAATGGCCCTTCAGTTATTTATAAAAGATTACAGGTACACTTATCGATGCCACCGAGG
TGACACACAAATACAAACCCGAAGATCATTTGAGATAAAGAAACTATGGGACCAAACCC
GCTCAAAAGCTGGACTGTTGGTCTCTGATGGAGGCCCCAATTTGTATAACATTAGAAAT
CTCCATATTCCTGAAGTTTGCTTGAAATGGGAGTTGATGGATGAGGATTACCAGGGGCG
TTTATGCAACCCATTAAACCCGTTTGTCAGCCATAAAGAGATTGAATCAGTGAACAGTG
CAGTGATAATGCCGGCACATGGTCCAGCCAAAAATATGGAGTATGACGCTGTTGCAACA
ACACACTCCTGGGTCCCCAAAAGAAATCGATCCATTTTGAACACGAGCCAAAGGGGGAT
ACTTGAAGATGAGCAAATGTATCAGAGGTGCTGCAATTTATTTGAAAAATTCTTCCCAA
GTAGCTCATACAGAAGACCAGTTGGAATATCCAGTATGGTAGAGGCTATGGTCTCAAGA
GCCCGAATTGATGCACGGATTGATTTCGAATCTGGAAGGATAAAGAAAGAGGAATTTGC

TGAGATCATGAAGATCTGTTCCACCATTGAAGACCTCAGACGGCAAAAATGAGGAATTT
GGCTTGTCCTTCATGAAAAAATGCCTTGTTTCTACT

Figure 26

CY080631.1 Influenza A virus (A/Boston/1/2009(H1N1)) Segment 3

TACTGATTCAAAATGGAAGATTTTGTACGACAATGCTTCAATCCGATGATTGTCGAGCT
TGCAGAAAAGGCAATGAAAGAGTATGGAGAGGACCTGAAAATCGAAACAAACAAATTTG
CAGCAATATGCACCCACTTGGAAGTGTGCTTCATGTATTCAGATTTTCATTTCATAAAT
GAGCAAGGCGAATCAATAATAGTAGAGCCTGAGGACCCAAATGCACTTTTAAAGCACAG
ATTTGAGATAATAGAGGGACGAGATCGTACAATGGCATGGACAGTTGTAAACAGTATTT
GCAACACCACAGGAGCTGAGAAACCAAAGTTTCTGCCAGATCTGTATGATTACAAAGAG
AATAGATTCATCGAGATTGGAGTGACAAGGAGGGAAGTTCACATATACTATCTGGAAAA
GGCCAACAAAATTAAATCTGAGAAGACACACATTCACATTTTCTCATTCACCGGCGAAG
AAATGGCCACAAAGGCTGATTACACTCTCGATGAAGAAAGCAGAGCTAGGATTAAAACC
AGATTGTTCACCATAAGACAAGAAATGGCAAGCAGAGGTCTTTGGGACTCCTTTCGTCA
GTCCGAAAGAGGCGAAGAAACAATTGAAGAAAGATTTGAAATCACAGGGACAATGCGCA
GGCTCGCTGACCAAAGCCTTCCGCCGAACTTCTCCTGCATTGAGAATTTTAGAGCCTAT
GTGGATGGATTTGAACCGAACGGCTACATTGAGGGCAAGCTTTCTCAAATGTCCAAAGA
AGTAAATGCTAGAATTGAGCCTTTTTTGAAAACAACACCTCGACCAATTAGACTTCCGA
ATGGGCCTCCTTGTTTTCAGCGGTCAAAATTCCTGCTGATGGATTCTTTAAAATTAAGC
ATTGAGGATCCAAATCATGAAGGTGAGGGGATACCACTATATGATGCAATCAAGTGTAT
GAGGACATTCTTTGGATGGAAAGAACCCACTGTTGTCAAGCCACACGAGAAGGGAATAA
ATCCAAATTATCTGTTGTCGTGGAAGCAAGTATTGGAAGAGCTGCAGGACATTGAGAGT
GAGGAGAAGATTCCAAGAACAAAAAACATGAAAAAAACTAGTCAGCTAAAGTGGGCACT
TGGTGAGAACATGGCACCAGAGAAGGTGGATTTTGATGACTGTAAAGATATAAGCGATT
TGAAGCAATATGACAGTGACGAACCTGAATTAAGATCATTTTCAAGTTGGATCCAGAAT
GAGTTCAACAAGGCATGCGAGCTGACCGATTCAATCTGGATAGAGCTTGATGAGATTGG
AGAAGATGTGGCTCCGATTGAACACATTGCAAGCATGAGAAGGAATTACTTCACAGCTG
AGGTGTCCCACTGCAGAGCCACAGAATATATAATGAAGGGGGTATACATTAATACTGCT
TTGCTCAATGCATCCTGTGCAGCAATGGATGATTTCCAACTAATTCCCATGATAAGCAA
ATGTAGAACTAAAGAGGGAAGGAGAAAGACCAATTTGTACGGCTTCATCGTAAAAGGAA
GATCTCACTTAAGGAATGACACCGATGTGGTAAACTTTGTGAGCATGGAGTTTTCCCTC
ACTGACCCAAGACTTGAGCCACACAAATGGGAGAAGTACTGCGTTCTTGAGATAGGAGA
TATGCTTCTAAGGAGTGCAATAGGCCAAGTGTCAAGGCCCATGTTCTTGTATGTAAGGA
CAAATGGAACCTCAAAAATTAAAATGAAATGGGGAATGGAGATGAGGCGTTGCCTCCTC
CAATCCCTCCAACAAATAGAGAGCATGATTGAAGCTGAGTCCTCTGTCAAAGAGAAAGA
CATGACAAAAGAGTTTTTTGAGAATAAATCAGAAACATGGCCCATTGGAGAGTCACCAA
AAGGAGTGGAAGAAGGTTCCATTGGGAAAGTATGCAGGACACTGTTGGCTAAGTCAGTA
TTCAATAGCCTGTATGCATCTCCACAATTAGAAGGATTTTCAGCTGAGTCAAGAAAGTT
GCTCCTCATTGTTCAGGCTCTTAGGGACAATCTGGAACCTGGGACCTTTGATCTTGGGG
GGCTATATGAAGCAATTGAGGAGTGCCTGATTAATGATCCCTGGGTTTTGCTTAATGCT
TCTTGGTTCAACTCCTTCCTAACACATGCATTGAGATAGCTGGGGCAATGCTACTATTT
GCTATCCATACTGTCCAAAAAAGTACCTTGTTTC

Figure 27

CY080626.1 Influenza A virus (A/Boston/1/2009(H1N1)) Segment 4

GCAAAAGCAGGGGAAAATAAAACAACCAGAATGAAAGTAAAACTACTGGTCCTGTTATG
CACATTTACAGCTACATATGCAGACACAATATGTATAGGCTACCATGCTAACAACTCGA
CCGACACTGTTGACACAGTACTTGAAAAGAATGTGACAGTGACACACTCTGTCAACCTG
CTTGAGAACAGTCACAATGGGAAACTATGTTCATTAAAAGGAATAGCCCCACTACAATT
GGGTAACTGCAGCGTTGCCGGGTGGATCTTAGGAAACCCAGAATGCGAATTACTGATTT
CCAAGGAGTCATGGTCCTACATTGTAGAAAAACCAAATCCTGAGAATGGAACATGTTAC
CCAGGGCATTTCGCTGACTATGAGGAACTGAGGGAGCAATTGAGTTCAGTATCTTCATT
TGAGAGGTTCGAAATATTCCCCAAAGAAAGCTCATGGCCCAACCACACCGTAACCGGAG
TGTCAGCATCATGCTCCCATAATGGGGAAAGCAGTTTTTACAGAAATTTGCTATGGCTG
ACGGTGAAGAATGGTTTGTACCCAAACTTGAGCAAGTCCTATGCAAACAACAAAGAAAA
AGAAGTTCTTGTACTATGGGGTGTTCATCACCCGCCAAACATAGTTGACCAAAAGACCC
TCTATCGTACAGAAAATGCTTATGTTTCTGTAGTGTCTTCACATTATAGCAGAAAATTC
ACCCCAGAAATAGCCAAAAGACCCAAAGTAAGAGATCAAGAAGGAAGAATCAACTACTA
CTGGACTCTGCTTGAACCCGGGGATACAATAATATTTGAGGCAAATGGAAATCTAATAG
CGCCAAGATATGCTTTCGCACTGAGTAGAGGCTTTGGATCAGGAATCATCAACTCAAAT
GCACCAATGGATAAATGTGATGCGAAGTGCCAAACACCTCGGGGAGCTATAAACAGCAG
TCTTCCTTTCCAGAACGTACACCCAGTCACAATAGGAGAGTGTCCAAAGTATGTCAGGA
GTGCAAAATTAAGGATGGTTACAGGACTAAGGAACATCCCATCCATTCAATCCAGAGGT
TTGTTTGGAGCCATTGCCGGTTTCATTGAAGGGGGGTGGACTGGAATGGTAGATGGTTG
GTATGGTTATCATCATCAGAATGAGCAAGGATCTGGCTATGCTGCAGATCAAAAAAGCA
CACAAAATGCCATTAATGGGATTACAAACAAGGTGAATTCTGTAATTGAGAAAATGAAC
ACTCAATTCACAGCAGTGGGCAAAGAATTCAACAAATTGGAAAGAAGGATGGAAAACTT
GAATAAAAAAGTTGATGATGGGTTTATAGACATTTGGACATATAATGCAGAACTGTTGG
TTCTACTGGAAAATGAAAGGACTTTGGATTTCCATGACTCCAATGTGAAGAATCTGTAT
GAGAAAGTAAAAAGCCAGTTAAAGAATAATGCTAAAGAAATAGGAAATGGGTGTTTTGA
ATTCTATCACAAGTGTAACGATGAATGCATGGAGAGTGTAAAGAATGGAACCTATGACT
ATCCAAAATATTCCGAAGAATCAAAGTTAAACAGGGAGAAAATTGATGGAGTGAAATTG
GAATCAATGGGAGTCTATCAGATTCTGGCGATCTACTCAACAGTCGCCAGTTCTCTGGT
TCTTTTGGTCTCCCTGGGGGCAATCAGCTTCTGGATGTGTTCCAATGGGTCTTTGCAGT
GTAGAATATGCATCTAAGACCAGAATTTCAGAAATATAAGGAAAAACACCCTTGTTTCT
A

Figure 28

CY080629.1 Influenza A virus (A/Boston/1/2009(H1N1)) Segment 5

AGCAAAAGCAGGGTAGATAATCACTCACTGAGTGACATTAAAGTCATGGCGTCCCAAGG
CACCAAACGGTCTTACGAACAGATGGAGACTGATGGGGAACGCCAGAATGCAACTGAAA
TCAGAGCATCCGTCGGAAGAATGATTGGTGGAATTGGGCGATTCTACATCCAAATGTGC
ACCGAGCTTAAGCTCAATGATTATGAGGGACGGCTGATCCAGAACAGCTTAACAATAGA
GAGAATGGTGCTCTCTGCTTTTGATGAGAGGAGAAATAAATATCTAGAGGAACATCCCA
GCGCGGGGAAAGATCCTAAGAAAACTGGAGGACCCATATACAAAAGAGTAGATGGAAAG
TGGGTGAGGGAACTCGTCCTTTATGACAAAGAAGAAATAAGGCGGATTTGGCGCCAAGC
CAACAATGGTGATGATGCAACGGCTGGTTTGACTCACATTATGATCTGGCATTCTAATT
TGAATGATACAACTTATCAGAGGACAAGAGCTCTTGTCCGCACCGGAATGGATCCCAGG
ATGTGCTCTTTGATGCAAGGTTCGACTCTCCCTAGAAGATCTGGAGCAGCAGGCGCCGC
AGTCAAAGGAGTTGGGACAATGGTATTGGAATTAATCAGGATGATCAAACGTGGGATCA
ATGACCGAAACTTCTGGAGGGGTGAGAATGGAAGAAAAACAAGGATTGCTTATGAGAGA
ATGTGCAACATTCTAAAAGGAAAATTTCAAACAGCTGCACAAAAAGCAATGATGGACCA
AGTGAGAGAAAGCCGGAACCCAGGAAATGCTGAGATCGAAGATCTCACTTTTCTGGCAC
GGTCTGCACTCATATTGAGAGGATCAGTTGCTCACAAGTCTTGCCTGCCTGCCTGTGTG
TATGGACCAGCCGTAGCCAGTGGGTATGACTTCGAAAAAGAGGGTTACTCTTTGGTAGG
AGTAGACCCTTTCAAACTGCTTCAAACCAGTCAGGTATACAGTCTAATTAGACCAAACG
AGAATCCCGCACACAAGAGCCAGTTGGTGTGGATGGCATGCAATTCTGCTGCATTTGAA
GATCTAAGAGTGTCTAGCTTCATCAGAGGAACAAGAGTACTTCCAAGGGGGAAGCTCTC
CACTAGAGGAGTACAAATTGCTTCAAATGAAAACATGGATGCTATTGTGTCAAGTACTC
TTGAACTGAGAAGCAGATACTGGGCCATAAGAACCAGAAGTGGAGGGAACACTAATCAA
CAAAGGGCCTCTGCGGGCCAAATCAGCACACAACCTACGTTTTCTGTGCAGAGAAACCT
CCCATTTGACAAAGCAACCATCATGGCAGCATTCTCTGGGAATACAGAGGGAAGAACAT
CAGACATGAGGGCAGAAATCATAAAGATGATGGAAAGTGCAAGACCAGAAGAAGTGTCC
TTCCAGGGGCGGGGAGTCTTTGAGCTCTCGGACGAAAGGGCAACGAACCCAATCGTGCC
CTCCTTTGACATGAGTAATGAAGGATCTTATTTCTTCGGAGACAATGCAGAGGAGTACG
ACAATTAATGAAAAATACCC

Figure 29

CY080628.1 Influenza A virus (A/Boston/1/2009(H1N1)) Segment 6

AAAGCAGGAGTTTAAAATGAACCCAAATCAAAAGATAATAACCATTGGATCAATCAGTA
TAGCAATCGGAATAATTAGTCTAATGTTGCAAATAGGAAATATTATTTCAATATGGGCT
AGTCACTCAATCCAAACTGGAAGTCAAAACAACACTGGAATATGCAACCAAAGAATCAT
CACATATGAAAACAGCACCTGGGTGAATCACACATATGTTAATATTAACAACACTAATG
TTGTTGCTGGAGAGGACAAAACGTCAGTGACATTGGCCGGCAATTCATCTCTTTGTTCT
ATCAGTGGATGGGCTATATACACAAAAGACAACAGCATAAGAATTGGCTCCAAAGGAGA
TGTTTTTGTCATAAGAGAACCTTTCATATCATGTTCTCACTTGGAATGCAGAACCTTTT
TTCTGACCCAAGGCGCTCTATTAAATGACAAACATTCAAATGGGACCGTAAAGGACAGA
AGTCCTTATAGGGCCTTAATGAGCTGTCCTCTAGGTGAAGCTCCGTCCCCATACAATTC
AAAGTTCGAATCAGTTGCATGGTCAGCAAGCGCATGCCATGATGGCATGGGCTGGTTGA
CAATCGGAATTTCTGGTCCAGACAATGGAGCTGTGGCTGTACTAAAATACAACGGAATA
ATAACTGGAACCATAAAAAGTTGGAAAAAGCAAATATTAAGAACACAAGAGTCTGAATG
TGTCTGTATGAACGGGTCATGTTTCACCATAATGACCGATGGCCCGAGTAATAAAGCCG
CCTCGTACAAAATTTTCAAGATCGAAAAGGGGAAGGTTACTAAATCAATAGAGTTGAAT
GCACCCAATTTTTATTACGAGGAATGCTCCTGTTACCCAGACACTGGCATAGTGATGTG
TGTATGCAGGGACAACTGGCATGGTTCAAATCGACCTTGGGTGTCTTTTAATCAAAATT
TGGATTATCAAATAGGATACATCTGCAGTGGAGTGTTTGGTGACAATCCGCGTCCCGAA
GATGGAGAGGGCAGCTGCAATCCAGTGACTGTTGATGGAGCAGACGGAGTAAAAGGGTT
TTCATACAAATATGGTAATGGTGTTTGGATAGGAAGGACCAAAAGTAACAGACTTAGAA
AGGGGTTTGAGATGATTTGGGATCCTAATGGATGGACAAATACCGACAGTGATTTCTCA
GTGAAACAGGATGTTGTAGCAATAACTGATTGGTCAGGGTACAGCGGAAGTTTCGTCCA
ACATCCTGAGTTGACAGGATTGGACTGTATAAGACCTTGCTTCTGGGTTGAGTTAGTCA
GAGGGCTGCCTAGAGAAAATACAACAATCTGGACTAGTGGGAGCAGCATTTCTTTTTGT
GGCGTTAATAGTGATACTGCAAACTGGTCTTGGCCAGACGGTGCTGAGTTGCCGTTCAC
CATTGACAAGTAGTTCGTTGAA

Figure 30

CY080627.1| Influenza A virus (A/Boston/1/2009(H1N1)) Segment 7

GCAAAAGCAGGTAGATATTGAAAGATGAGTCTTCTAACCGAGGTCGAAACGTACGTTCT
CTCTATCATCCCGTCAGGCCCCCTCAAAGCCGAGATCGCACAGAGACTTGAAGATGTAT
TTGCTGGAAAGAATACCGATCTTGAGGCTCTCATGGAGTGGCTAAAGACAAGACCAATC
CTGTCACCTCTGACTAAGGGGATTTTAGGATTTGTGTTCACGCTCACCGTGCCCAGTGA
GCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCTTAATGGGAATGGGGATCCAA
ATAATATGGACAGAGCAGTCAAACTTTATCGAAAGCTTAAGAGGGAGATAACATTCCAT
GGAGCCAAAGAAATAGCACTCAGTTATTCTGCTGGTGCACTTGCCAGTTGTATGGGACT
CATATACAACAGGATGGGGGCTGTGACCACCGAATCAGCATTTGGCCTTATATGTGCAA
CCTGTGAACAGATTGCCGACTCCCAGCATAAGTCTCACAGGCAAATGGTAACAACAACC
AATCCATTAATAAGACATGAGAACAGAATGGTTCTGGCCAGCACTACAGCTAAGGCTAT
GGAGCAAATGGCTGGATCGAGCGAACAAGCAGCTGAGGCCATGGAGGTTGCTAGTCAGG
CCAGGCAGATGGTGCAGGCAATGAGAGCCATTGGGACTCATCCTAGCTCTAGCACTGGT
CTGAAAAATGATCTCCTTGAAAATTTACAGGCCTATCAGAAACGAATGGGGGTGCAGAT
GCAACGATTCAAGTGATCCTCTTGTTGTTGCCGCAAGTATAATTGGGATTGTGCACTTG
ATATTGTGGATTATTGATCGCCTTTTTTCCAAAAGCATTTATCGTATCTTTAAACACGG
TTTAAAAAGAGGGCCTTCCACGGAAGGAGTACCAGAGTCTATGAGGGAAGAATATCGAG
AGGAACAGCAGAATGCTGTGGATGCTGACGATGATCATTTTGTCAGCATAGAGCTGGAG
TAAAAAACTACCT

Figure 31

CY080630.1 Influenza A virus (A/Boston/1/2009(H1N1)) Segment 8

AGCGAAAGCAGGGTGGCAAAGACATAATGGATTCCCACACTGTGTCAAGCTTTCAGGTA
GATTGCTTCCTTTGGCATGTCCGCAAACAAGTTGCAGACCAAGATCTAGGCGATGCCCC
CTTCCTTGATCGGCTTCGCCGAGATCAGAAGTCTCTAAAGGGAAGAGGCAGCACTCTCG
GTCTGAACATCGAAACAGCTACTTGTGTTGGAAAGCAAATAGTAGAGAGGATTCTGAAA
GAAGAATCCGATGAGGCACTTAAAATGACCATGGCCTCCGCACTTGCTTCGCGGTACCT
AACTGACATGACTGTTGAAGAAATGTCAAGGGACTGGTTCATGCTCATGCCCAAGCAAA
AAATGGCTGGCCCTCTTTGTGTCAGAATGGACCAGGCAATAATGGATAAGAACATCATA
CTGAAAGCGAATTTCAGTGTGATTTTTGACCGGTTGGAGAATCTGGCATTATTAAGGGC
TTTTACCGAAGAGGGAGCAATTGTTGGCGAAATTTCACCATTGCCTTCTTTTCCAGGAC
ATACTAATGAGGATGTCAAAAATGCAATTGGGGTCCTCATCGGGGGACTTGAATGGAAT
GATAACACAGTTCGAGTCTCTGAAACTCTACAGAGATTCGCTTGGAGAAGCAGTAATGA
GACTGGGGGACCTCCATTCACTACAACACAGAAACGGAAAATGGCGGGAACAACTAGGT
CAGAAGTTTGAAGAAGTAAGATGGCTGATTGAAGAAGTGAGGCATAAATTGAAGACGAC
AGAGAATAGTTTTGAGCAAATAACATTTATGCAAGCATTACAGCTATTATTTGAAGTGG
AACAAGAGATTAGAACGTTTTCGTTTCAGCTTATTTAGTGATAAAAAACACCCT