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(54) Title: INFLUENZA NUCLEIC ACID MOLECULES AND VACCINES MADE THEREFROM

(57) Abstract: Provided herein are nucleic acid sequences that encode novel consensus amino acid sequences of HA hemagglutinin, as well as genetic constructs/vectors and vaccines expressing the sequences. Also provided herein are methods for generating an immune response against one or more Influenza A serotpyes using the vaccines that are provided.

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INFLUENZA NUCLEIC ACID MOLECULES AND VACCINES MADE THEREFROM

FIELD OF THE INVENTION

The present invention relates to improved influenza viral vaccines, improved methods for inducing immune responses against influenza, improved methods for diagnosing vaccinated vs. infected influenza mammalian hosts and for prophylactically and/or therapeutically immunizing individuals against influenza.

BACKGROUND OF THE INVENTION

Influenza, commonly referred to as the flu, is an infectious disease caused by RNA viruses of the family *Orthomyxoviridae*. Influenza or flu viruses infect birds and mammals. Three of the five genera of *Orthomyxoviridae* are influenza viruses: Influenza A, Influenza B and Influenza C. Of these, Influenza A is the most common.

Influenza is typically transmitted through the air in aerosols produced by coughs or sneezes and by direct contact with body fluids containing the virus or contaminated surfaces. Seasonal epidemics of influenza occur worldwide and result in hundreds of thousands of deaths annually. In some years, pandemics occur and cause millions of deaths. In addition, livestock, particularly poultry and swine, are also susceptible to annual epidemics and occasional pandemics which cause large numbers of animal deaths and monetary losses.

Structurally, influenza viruses are similar, having generally spherical or filamentous virus particles of about 80–120 nm made up of similar molecular component. A central core comprising viral proteins and viral RNA is covered by a viral envelope made up of two different glycoproteins and a lipid coat derived from the cell that the viral particle is produced in. Two additional different glycoproteins are anchored within the viral envelope and include portions which project outward on the surface.

The influenza virus RNA genome is typically provided as eight different single stranded, negative sense RNA segments that together make up the genome's eleven viral genes which encode the eleven proteins (HA, NA, NP, M1, M2, NS1, NEP, PA, PB1, PB1-F2, PB2). The eight RNA segments are: 1) HA, which encodes hemagglutinin (about 500 molecules of hemagglutinin are needed to make one virion); 2) NA, which encodes neuraminidase (about 100

molecules of neuraminidase are needed to make one virion); 3) NP, which encodes nucleoprotein; 4) M, which encodes two matrix proteins (the M1 and the M2) by using different reading frames from the same RNA segment (about 3000 matrix protein molecules are needed to make one virion); 5) NS, which encodes two distinct non-structural proteins (NS1 and NEP) by using different reading frames from the same RNA segment; 6) PA, which encodes an RNA polymerase; 7) PB1, which encodes an RNA polymerase and PB1-F2 protein (induces apoptosis) by using different reading frames from the same RNA segment; and 8) PB2, which encodes an RNA polymerase.

Of these eleven proteins, hemagglutinin (HA) and neuraminidase (NA) are two large glycoproteins anchored in the viral envelope and present on the outer surface of the viral particles. These proteins serve as immunogens for immune responses against influenza. HA, which is a lectin that mediates binding of the virus to target cells and entry of the viral genome into the target cell, is expressed as a single gene product, HA0, and later processed by host proteases to produce two subunits, HA1 and HA2, which together form a complex on the surface of influenza viral particles. NA is involved in the release of newly produced mature viral particles produced in infected cells.

There are sixteen known HA serotypes and nine known NA serotypes for Influenza A viruses. The identity of the different serotypes present in a viral particle typically is used to describe a virus. For example, H1N1 is an influenza virus with HA serotype H1 and NA serotype N1; H5N1 is an influenza virus with HA serotype H5 and NA serotype N1. Only H1, H2 and H3 serotypes, and N1 and N2 serotypes usually infect humans.

Influenza strains are generally species or genus specific; i.e. an influenza strain which can infect pigs (a swine influenza virus) typically does not infect humans or birds; an influenza strain which can infect birds (an avian influenza virus) does not infect humans or pigs; and an influenza strain which can infect humans (a human influenza virus) does not infect birds or pigs. Influenza strains, however, can mutate and become infective from one species to another. For example, a strain which only infects pigs, a swine influenza, can mutate or recombine to become a strain that can infect humans only or both pigs and humans. A flu virus commonly referred to as "swine flu" is an influenza virus strain, such as an H1N1 strain, which can infect humans and which was derived from a strain that was previously specific for pigs (i.e. a swine flu virus is a swine origin

human influenza or swine derived human influenza). A flu virus commonly referred to as "bird flu" is an influenza virus strain, such as an H5N1 strain, which can infect humans and which was derived from a strain that was previously specific for birds (i.e. a bird flu virus avian origin human influenza or avian derived human influenza).

Vaccinations against influenza are provided seasonally to many humans in developed countries and sometime to livestock. The vaccines used are limited in their protective results because the immune responses induced by the vaccines are specific for certain subtypes of virus. Different influenza vaccines are developed and administered annually based upon international surveillance and scientists' estimations of which types and strains of viruses will circulate in a given year. The virus changes significantly by mutation, recombination and reassortment of the segments. Thus, vaccines given in one year are not considered protective against the seasonal strains that are widely transmitted the following year.

The "flu shot" commonly promoted U.S. Centers for Disease Control and Prevention usually contains three killed/inactivated influenza viruses: one A (H3N2) virus, one A (H1N1) virus, and one B virus. Thus, it is apparent that vaccinations are limited to predictions of subtypes, and the availability of a specific vaccine to that subtype.

The direct administration of nucleic acid sequences to vaccinate against animal and human diseases has been studied and much effort has focused on effective and efficient means of nucleic acid delivery in order to yield necessary expression of the desired antigens, resulting immunogenic response and ultimately the success of this technique.

DNA vaccines have many conceptual advantages over more traditional vaccination methods, such as live attenuated viruses and recombinant protein-based vaccines. DNA vaccines are safe, stable, easily produced, and well tolerated in humans with preclinical trials indicating little evidence of plasmid integration [Martin, T., et al., Plasmid DNA malaria vaccine: the potential for genomic integration after intramuscular injection. Hum Gene Ther, 1999. 10(5): p. 759-68; Nichols, W.W., et al., Potential DNA vaccine integration into host cell genome. Ann N Y Acad Sci, 1995. 772: p. 30-9]. In addition, DNA vaccines are well suited for repeated administration due to the fact that efficacy of the vaccine is not influenced by pre-existing antibody titers to the vector [Chattergoon, M., J. Boyer, and D.B. Weiner, Genetic immunization: a new era in vaccines and immune therapeutics. FASEB J, 1997. 11(10): p. 753-63]. However,

one major obstacle for the clinical adoption of DNA vaccines has been a decrease in the platform's immunogenicity when moving to larger animals [Liu, M.A. and J.B. Ulmer, Human clinical trials of plasmid DNA vaccines. Adv Genet, 2005. 55: p. 25-40]. Recent technological advances in the engineering of DNA vaccine immunogen, such has codon optimization, RNA optimization and the addition of immunoglobulin leader sequences have improved expression and immunogenicity of DNA vaccines [Andre, S., et al., Increased immune response elicited by DNA vaccination with a synthetic gp120 sequence with optimized codon usage. J Virol, 1998. 72(2): p. 1497-503; Deml, L., et al., Multiple effects of codon usage optimization on expression and immunogenicity of DNA candidate vaccines encoding the human immunodeficiency virus type 1 Gag protein. J Virol, 2001. 75(22): p. 10991-1001; Laddy, D.J., et al., Immunogenicity of novel consensus-based DNA vaccines against avian influenza. Vaccine, 2007. 25(16): p. 2984-9; Frelin, L., et al., Codon optimization and mRNA amplification effectively enhances the immunogenicity of the hepatitis C virus nonstructural 3/4A gene. Gene Ther, 2004. 11(6): p. 522-33], as well as, recently developed technology in plasmid delivery systems such as electroporation [Hirao, L.A., et al., Intradermal/subcutaneous immunization by electroporation improves plasmid vaccine delivery and potency in pigs and rhesus macaques. Vaccine, 2008. 26(3): p. 440-8; Luckay, A., et al., Effect of plasmid DNA vaccine design and in vivo electroporation on the resulting vaccine-specific immune responses in rhesus macaques. J Virol, 2007. 81(10): p. 5257-69; Ahlen, G., et al., In vivo electroporation enhances the immunogenicity of hepatitis C virus nonstructural 3/4A DNA by increased local DNA uptake, protein expression, inflammation, and infiltration of CD3+ T cells. J Immunol, 2007. 179(7): p. 4741-53]. In addition, studies have suggested that the use of consensus immunogens can be able to increase the breadth of the cellular immune response as compared to native antigens alone [Yan, J., et al., Enhanced cellular immune responses elicited by an engineered HIV-1 subtype B consensus-based envelope DNA vaccine. Mol Ther, 2007. 15(2): p. 411-21; Rolland, M., et al., Reconstruction and function of ancestral center-of-tree human immunodeficiency virus type 1 proteins. J Virol, 2007. 81(16): p. 8507-14].

One method for delivering nucleic acid sequences such as plasmid DNA is the electroporation (EP) technique. The technique has been used in human clinical trials to deliver

anti-cancer drugs, such as bleomycin, and in many preclinical studies on a large number of animal species.

There remains a need for an immunogenic influenza consensus hemagglutinin protein, for nucleic acid constructs that encode such a protein and for compositions useful to induce immune responses against multiple strains of influenza. There remains a need for effective vaccines against influenza that are economical and effective across numerous influenza subtypes for treating individuals.

SUMMARY OF THE INVENTION

Provided herein are isolated nucleic acid molecules comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NO:1, a nucleic acid sequence that is 95% homologous to SEQ ID NO:1; a fragment of SEQ ID NO:1; a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:1; SEQ ID NO:3; a nucleic acid sequence that is 95% homologous to SEQ ID NO:3; a fragment of SEQ ID NO:3; a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:3; SEQ ID NO:6; a nucleic acid sequence that is 95% homologous to SEQ ID NO:6; a fragment of SEQ ID NO:6; a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:6; SEQ ID NO:9,a nucleic acid sequence that is 95% homologous to SEQ ID NO:9; a fragment of SEQ ID NO:9; a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:9; SEQ ID NO:11, a nucleic acid sequence that is 95% homologous to SEQ ID NO:11; a fragment of SEQ ID NO:11; a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:11; SEQ ID NO:13; a nucleic acid sequence that is 95% homologous to SEQ ID NO:13; a fragment of SEQ ID NO:13; a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:13;.and SEQ ID NO:15; a nucleic acid sequence that is 95% homologous to SEQ ID NO:15; a fragment of SEQ ID NO:15; a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:15.

Also provided are compositions comprising: a) a first nucleic acid sequence selected from the group consisting of one or more of: SEQ ID NO:1, a nucleic acid sequence that is 95% homologous to SEQ ID NO:1; a fragment of SEQ ID NO:1; a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:1; SEQ ID NO:3; a nucleic acid sequence that is 95% homologous to SEQ ID NO:3; a fragment of SEQ ID NO:3; a nucleic acid sequence that is 95%

homologous to a fragment of SEQ ID NO:3; SEQ ID NO:6; a nucleic acid sequence that is 95% homologous to SEQ ID NO:6; a fragment of SEQ ID NO:6; a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:6; SEQ ID NO:9; a nucleic acid sequence that is 95% homologous to SEQ ID NO:9; a fragment of SEQ ID NO:9; a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:9; SEQ ID NO:11; a nucleic acid sequence that is 95% homologous to SEQ ID NO:11; a fragment of SEQ ID NO:11; and a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:11SEQ ID NO:13; a nucleic acid sequence that is 95% homologous to SEQ ID NO:13; a fragment of SEQ ID NO:13; a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:13; SEQ ID NO:15; a nucleic acid sequence that is 95% homologous to SEQ ID NO:15; a fragment of SEQ ID NO:15; and a nucleic acid sequence that is 95% homologous to a fragment of SEQ ID NO:15; and b) a second nucleic acid sequence that encodes a protein selected from the group consisting of one or more of: influenza A H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin, neuraminidase and fragments thereof.

Some aspects of the invention provide methods of inducing an immune response comprising the step of: administering to an individual such nucleic acid molecules and/or compositions.

Additional aspects of the invention provide methods of protecting an individual against infection. The methods comprise the step of: administering to said individual a prophylactically effective amount of a nucleic acid molecule comprising such nucleic acid sequence or compositions; wherein the nucleic acid sequence is expressed in cells of said individual and a protective immune response is induced against a protein encoded by said nucleic acid sequence. In some embodiment, the immune response is a protective immune response against swine origin human influenza.

In some aspects of the invention, methods are provided for treating an individual who has been infected by Influenza. The methods comprise the step of: administering to said individual a therapeutically effective amount of such nucleic acid molecules and/or composition. In some embodiment, the immune response is a therapeutic immune response against swine origin human influenza.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a map of the 2999 basepair backbone vector plasmid pVAX1 (Invitrogen, Carlsbad CA). The CMV promoter is located at bases 137-724. The T7 promoter/priming site is at bases 664-683. Multiple cloning sites are at bases 696-811. Bovine GH polyadenylation signal is at bases 829-1053. The Kanamycin resistance gene is at bases 1226-2020. The pUC origin is at bases 2320-2993.

Based upon the sequence of pVAX1 available from Invitrogen, the following mutations were found in the sequence of pVAX1 that was used as the backbone for pGX2009:

C>G 241 in CMV promoter

C>T 1942 backbone, downstream of the bovine growth hormone polyadenylation signal (bGHpolyA)

A> - 2876 backbone, downstream of the Kanamycin gene

C>T 3277 in pUC origin of replication (Ori) high copy number mutation (see Nucleic Acid Research 1985)

G>C 3753 in very end of pUC Ori upstream of RNASeH site

Base pairs 2, 3 and 4 are changed from ACT to CTG in backbone, upstream of CMV promoter.

Figure 2 shows two maps of the plasmid pGX2009, which is also referred to as pH1HA09. The nucleic acid sequence of the plasmid pGX2009 (SEQ ID NO:5) includes the coding sequence for the consensus H1 protein construct (amino acid SEQ ID NO:4 encoded by SEQ ID NO:3) which includes the IgE leader(amino acid SEQ ID NO:17) linked to the N terminal of the consensus H1 amino acid sequence(amino acid SEQ ID NO:2 encoded by SEQ ID NO:1) which is linked at its C terminal to the HA Tag (SEQ ID NO:18). The consensus H1 protein (amino acid SEQ ID NO:4 encoded by SEQ ID NO:3) is labeled SwiHum Con HA and H1HA09.

Figure 3 shows a maps of the plasmid pGX2006. The nucleic acid sequence of the plasmid pGX2006 (SEQ ID NO:8) includes the coding sequence for consensus H2 protein (amino acid SEQ ID NO:7 encoded by SEQ ID NO:6) which is labeled H2HA.

Figure 4 shows data from hemagglutination inhibition assays performed with sera from immunized ferrets.

Figure 5 shows results of a challenge of immunized and unimmunized ferrets with a novel H1N1 strain.

DETAILED DESCRIPTION

Consensus amino acid sequences of each of influenza A H1 and H2 (referred to herein as "consensus H1" (SEQ ID NO:2) and "consensus H2" (SEQ ID NO:7), respectively), as well as a novel synthetic hybrid consensus H1 influenza A hemagglutinin amino acid sequence (referred to herein as "consensus U2" (SEQ ID NO:10)) and a consensus amino acid sequence of influenza B hemagglutinin (referred to herein as "consensus BHA" (SEQ ID NO:13)) are provided, which can provide protection of mammals against influenza. In addition, proteins are provided which comprise the consensus H1 amino acid sequence, the consensus H2 amino acid sequence, the consensus U2 amino acid sequence and/or the consensus BHA amino acid sequence. In some aspects, nucleic acid sequences are provided which encode proteins comprising the consensus H1 amino acid sequence (for example (SEQ ID NO:1) or (SEQ ID NO:3)), the consensus H2 amino acid sequence (for example (SEQ ID NO:6)), the consensus U2 amino acid sequence(for example (SEQ ID NO:9) or (SEQ ID NO:11)), and/or the consensus BHA amino acid sequence(for example (SEQ ID NO:13) or (SEQ ID NO:15)).

While not being bound by scientific theory, a vaccine that can be used to elicit an immune response (humoral, cellular, or both) broadly against multiple influenza subtypes may comprise one or more of the following: 1) a nucleic acid sequence that encodes a protein comprising the consensus H1 amino acid sequence; 2) a protein comprising the consensus H1 amino acid sequence; 3) a nucleic acid sequence that encodes a protein comprising the consensus H2 amino acid sequence; 4) a protein comprising the consensus H2 amino acid sequence; 5) a nucleic acid sequence that encodes a protein comprising the consensus U2 amino acid sequence; 6) a protein comprising the consensus U2 amino acid sequence; 7) a nucleic acid sequence that encodes a protein comprising the consensus BHA amino acid sequence; and 8) a protein comprising the consensus BHA amino acid sequence.

Immunization methods can be performed and vaccines can be prepared which use and/or combine two or more of the following components: 1) a nucleic acid sequence that encodes a protein comprising the consensus H1 amino acid sequence; 2) a protein comprising the consensus H1 amino acid sequence; 3) a nucleic acid sequence that encodes a protein comprising the consensus H2 amino acid sequence, 4) a protein comprising the consensus H2 amino acid sequence; 5) a nucleic acid sequence that encodes a protein comprising the consensus U2 amino acid sequence, 6) a protein comprising the consensus U2 amino acid sequence, 7) a nucleic acid sequence that encodes a protein comprising the consensus BHA amino acid sequence, and 8) a protein comprising the consensus BHA amino acid sequence. For more broad based treatments against influenza, immunization methods can be performed and vaccines can be prepared which use and/or combine one or more other influenza proteins such as influenza A H1-H16, influenza A N1-N9, influenza B hemagglutinin, influenza B neuraminidase and/or genes encoding these proteins together with one or more of the following components: 1) a nucleic acid sequence that encodes a protein comprising the consensus H1 amino acid sequence; 2) a protein comprising the consensus H1 amino acid sequence; 3) a nucleic acid sequence that encodes a protein comprising the consensus H2 amino acid sequence, 4) a protein comprising the consensus H2 amino acid sequence; 5) a nucleic acid sequence that encodes a protein comprising the consensus U2 amino acid sequence, 6) a protein comprising the consensus U2 amino acid sequence, 7) a nucleic acid sequence that encodes a protein comprising the consensus BHA amino acid sequence, and 8) a protein comprising the consensus BHA amino acid sequence.

1. Definitions.

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

For recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6,9, and 7.0 are explicitly contemplated.

a. Adjuvant

"Adjuvant" as used herein means any molecule added to the DNA plasmid vaccines described herein to enhance the immunogenicity of the antigens encoded by the DNA plasmids and the encoding nucleic acid sequences described hereinafter.

b. Antibody

"Antibody" as used herein means an antibody of classes IgG, IgM, IgA, IgD or IgE, or fragments, fragments or derivatives thereof, including Fab, F(ab')2, Fd, and single chain antibodies, diabodies, bispecific antibodies, bifunctional antibodies and derivatives thereof. The antibody can be an antibody isolated from the serum sample of mammal, a polyclonal antibody, affinity purified antibody, or mixtures thereof which exhibits sufficient binding specificity to a desired epitope or a sequence derived therefrom.

c. Coding Sequence

"Coding sequence" or "encoding nucleic acid" as used herein means the nucleic acids (RNA or DNA molecule) that comprise a nucleotide sequence which encodes a protein. The coding sequence can further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to whom the nucleic acid is administered.

d. Complement

"Complement" or "complementary" as used herein means a nucleic acid can mean Watson-Crick (*e.g.*, A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

e. Consensus or Consensus Sequence

"Consensus" or "consensus sequence" as used herein means a polypeptide sequence based on analysis of an alignment of multiple subtypes of a particular influenza antigen. Nucleic acid sequences that encode a consensus polypeptide sequence may be prepared. Vaccines comprising proteins that comprise consensus sequences and/or nucleic acid molecules that encode such proteins can be used to induce broad immunity against multiple subtypes or serotypes of a particular influenza antigen. Consensus influenza antigens can include influenza A consensus hemagglutinin amino acid sequences, including for example consensus H1, consensus H2, or influenza B consensus hemagglutinin amino acid sequences.

f. Constant Current

"Constant current" as used herein means a current that is received or experienced by a tissue, or cells defining said tissue, over the duration of an electrical pulse delivered to same tissue. The electrical pulse is delivered from the electroporation devices described herein. This current remains at a constant amperage in said tissue over the life of an electrical pulse because the electroporation device provided herein has a feedback element, preferably having instantaneous feedback. The feedback element can measure the resistance of the tissue (or cells) throughout the duration of the pulse and cause the electroporation device to alter its electrical energy output (e.g., increase voltage) so current in same tissue remains constant throughout the electrical pulse (on the order of microseconds), and from pulse to pulse. In some embodiments, the feedback element comprises a controller.

g. Current Feedback or Feedback

"Current feedback" or "feedback" can be used interchangeably and means the active response of the provided electroporation devices, which comprises measuring the current in tissue between electrodes and altering the energy output delivered by the EP device accordingly in order to maintain the current at a constant level. This constant level is preset by a user prior to initiation of a pulse sequence or electrical treatment. The feedback can be accomplished by the electroporation component, e.g., controller, of the electroporation device, as the electrical circuit therein is able to continuously monitor the current in tissue between electrodes and compare that monitored current (or current within tissue) to a preset current and continuously make energy-output adjustments to maintain the monitored current at preset levels. The feedback loop can be instantaneous as it is an analog closed-loop feedback.

h. Decentralized Current

"Decentralized current" as used herein means the pattern of electrical currents delivered from the various needle electrode arrays of the electroporation devices described herein, wherein the patterns minimize, or preferably eliminate, the occurrence of electroporation related heat stress on any area of tissue being electroporated.

i. Electroporation

"Electroporation," "electro-permeabilization," or "electro-kinetic enhancement" ("EP") as used interchangeably herein means the use of a transmembrane electric field pulse to induce

microscopic pathways (pores) in a bio-membrane; their presence allows biomolecules such as plasmids, oligonucleotides, siRNA, drugs, ions, and water to pass from one side of the cellular membrane to the other.

j. Feedback Mechanism

"Feedback mechanism" as used herein means a process performed by either software or hardware (or firmware), which process receives and compares the impedance of the desired tissue (before, during, and/or after the delivery of pulse of energy) with a present value, preferably current, and adjusts the pulse of energy delivered to achieve the preset value. A feedback mechanism can be performed by an analog closed loop circuit.

k. Fragment

"Fragment" as used herein with respect to nucleic acid sequences means a nucleic acid sequence or a portion thereof, that encodes a polypeptide capable of eliciting an immune response in a mammal that cross reacts with a full length wild type strain influenza antigen, including, e.g., an influenza A H1 hemagglutinin, an influenza A H2 hemagglutinin or an influenza B hemagglutinin. The fragments can be DNA fragments selected from at least one of the various nucleotide sequences that encode the consensus amino acid sequences and constructs comprising such sequences, including SEQ ID NOS: 1, 3, 6, 9, 11 13 and 15. DNA fragments can comprise coding sequences for the immunoglobulin leader such as IgE or IgG sequences. The DNA fragments can be 30 or more nucleotides in length, 45 or more, 60 or more, 75 or more, 90 or more, 120 or more, 150 or more, 180 or more, 210 or more, 240 or more, 270 or more, 300 or more, 360 or more, 420 or more, 480 or more, 540 or more, 600 or more, 660 or more, 720 or more, 780 or more, 840 or more, 900 or more, 960 or more, 1020 or more, 1080 or more, 1140 or more, 1200 or more, 1260 or more, 1320 or more, 1380 or more, 1440 or more, 1500 or more, 1560 or more, 1620 or more, 1680 or more, 1740 or more, 1800 or more, 1860 or more, 1820 or more, 1880 or more, 1940 or more, 2000 or more, 2600 or more, 2700 or more, 2800 or more, 2900 or more, 2910 or more, 2920 or more, 2930 or more, 2931 or more, 2932 or more, 2933 or more, 2934 or more, 2935 or more, 2936 or more, 2937 or more, or 2938 or more in length. DNA fragments can be fewer than 10 nucleotides, fewer than 20, fewer than 30, fewer than 40, fewer than 50, fewer than 60, fewer than 75, fewer than 90, fewer than 120, fewer than 150, fewer than 180, fewer than 210, fewer than 240, fewer than 270, fewer than 300, fewer than

360, fewer than 420, fewer than 480, fewer than 540, fewer than 600, fewer than 660, fewer than 720, fewer than 780, fewer than 840, fewer than 900, fewer than 960, fewer than 1020, fewer than 1080, fewer than 1140, fewer than 1200, fewer than 1260, fewer than 1320, fewer than 1380, fewer than 1440, fewer than 1500, fewer than 1560, fewer than 1620, fewer than 1680, or fewer than 1740 nucleotides, fewer than 1800, fewer than 1860, fewer than 1820, fewer than 1880, fewer than 1940, fewer than 2000, fewer than 2600, fewer than 2700, fewer than 2800, fewer than 2900, fewer than 2910, fewer than 2920, fewer than 2930, fewer than 2931, fewer than 2932, fewer than 2933, fewer than 2934, fewer than 2935, fewer than 2936, fewer than 2937, or fewer than 2938.

"Fragment" with respect to polypeptide sequences means a polypeptide capable of eliciting an immune response in a mammal that cross reacts with a full length wild type strain influenza antigen, including, e.g., an influenza A H1 hemagglutinin, an influenza A H2 hemagglutinin or an influenza B hemagglutinin. The fragment can be polypeptide fragment selected from at least one of the various polypeptide sequences of the present invention, including SEQ ID NOS: 2, 4, 7, 10, 12, 14 and 16. Polypeptide fragments can be analyzed to contact at least one antigenic epitope as provided by a publicly available database such as the Los Alamos National Laboratory's HA Sequence Database. Polypeptides HA fragments can further comprise amino acid sequences for the immunoglobulin leader such as IgE or IgG. The polypeptide fragments can be 30 or more amino acids in length, 45 or more, 60 or more, 75 or more, 90 or more, 120 or more, 150 or more, 180 or more, 210 or more, 240 or more, 270 or more, 300 or more, 360 or more, 420 or more, 480 or more, 540 or more, 600 or more, 660 or more, or 710 amino acids or more in length. Polypeptide fragments can be fewer than 10 amino acids, fewer than 20, fewer than 30, fewer than 40, fewer than 50, fewer than 60, fewer than 75, fewer than 90, fewer than 120, fewer than 150, fewer than 180, fewer than 210, fewer than 240, fewer than 270, fewer than 300, fewer than 360, fewer than 420, fewer than 480, fewer than 540, fewer than 600, fewer than 660, fewer than 700, fewer than 701, fewer than 702, fewer than 703, fewer than 704, fewer than 705, fewer than 706, fewer than 707, fewer than 708, fewer than 709, or fewer than 710 amino acids in length.

l. Genetic construct

As used herein, the term “genetic construct" refers to the DNA or RNA molecules that comprise a nucleotide sequence which encodes a protein. The coding sequence includes initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of the individual to whom the nucleic acid molecule is administered. As used herein, the term "expressible form" refers to gene constructs that contain the necessary regulatory elements operable linked to a coding sequence that encodes a protein such that when present in the cell of the individual, the coding sequence will be expressed.

m. Identical

"Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences, means that the sequences have a specified percentage of residues that are the same over a specified region. The percentage can be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) can be considered equivalent. Identity can be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

n. Impedance

“Impedance” can be used when discussing the feedback mechanism and can be converted to a current value according to Ohm’s law, thus enabling comparisons with the preset current.

o. Immune Response

“Immune response” as used herein means the activation of a host’s immune system, e.g., that of a mammal, in response to the introduction of antigen such as an influenza hemagglutinin

consensus antigen. The immune response can be in the form of a cellular or humoral response, or both.

p. Nucleic Acid

"Nucleic acid" or "oligonucleotide" or "polynucleotide" as used herein means at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid can be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that can hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

Nucleic acids can be single stranded or double stranded, or can contain portions of both double stranded and single stranded sequence. The nucleic acid can be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid can contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids can be obtained by chemical synthesis methods or by recombinant methods.

q. Operably Linked

"Operably linked" as used herein means that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter can be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene can be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance can be accommodated without loss of promoter function.

r. Promoter

"Promoter" as used herein means a synthetic or naturally-derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter can comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter can also comprise distal enhancer or repressor elements, which can be located as much as several

thousand base pairs from the start site of transcription. A promoter can be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter can regulate the expression of a gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents. Representative examples of promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, SV40 early promoter or SV40 late promoter and the CMV IE promoter.

s. Stringent Hybridization Conditions

"Stringent hybridization conditions" as used herein means conditions under which a first nucleic acid sequence (e.g., probe) will hybridize to a second nucleic acid sequence (e.g., target), such as in a complex mixture of nucleic acids. Stringent conditions are sequence-dependent and will be different in different circumstances. Stringent conditions can be selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m can be the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions can be those in which the salt concentration is less than about 1.0 M sodium ion, such as about 0.01-1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., about 10-50 nucleotides) and at least about 60°C for long probes (e.g., greater than about 50 nucleotides). Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal can be at least 2 to 10 times background hybridization. Exemplary stringent hybridization conditions include the following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C.

t. Substantially Complementary

"Substantially complementary" as used herein means that a first sequence is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the complement of a

second sequence over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 180, 270, 360, 450, 540, 630, 720, 810, 900, 990, 1080, 1170, 1260, 1350, 1440, 1530, 1620, 1710, 1800, 1890, 1980, 2070 or more nucleotides or amino acids, or that the two sequences hybridize under stringent hybridization conditions.

u. Substantially Identical

"Substantially identical" as used herein means that a first and second sequence are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 180, 270, 360, 450, 540, 630, 720, 810, 900, 990, 1080, 1170, 1260, 1350, 1440, 1530, 1620, 1710, 1800, 1890, 1980, 2070 or more nucleotides or amino acids, or with respect to nucleic acids, if the first sequence is substantially complementary to the complement of the second sequence.

v. Subtype or Serotype

"Subtype" or "serotype": as used herein, interchangeably, and in reference to influenza virus, means genetic variants of an influenza virus such that one subtype is recognized by an immune system apart from a different subtype.

w. Variant

"Variant" used herein with respect to a nucleic acid means (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto.

"Variant" with respect to a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Variant can also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change.

These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte et al., J. Mol. Biol. 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ±2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions can be performed with amino acids having hydrophilicity values within ±2 of each other. Both the hyrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

x. Vector

"Vector" as used herein means a nucleic acid sequence containing an origin of replication. A vector can be a vector, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector can be a DNA or RNA vector. A vector can be a self-replicating extrachromosomal vector, and preferably, is a DNA plasmid.

2. Influenza antigen

Provided herein are antigens capable of eliciting an immune response in a mammal against one or more influenza serotypes. The antigen can be capable of eliciting an immune response in a mammal against one or more influenza serotypes, including against one or more pandemic strains, such as 2009 H1N1 swine originated influenza. The antigen can be capable of eliciting an immune response in a mammal against one or more influenza serotype, including against one or more strains of swine derived human influenza. The antigen can comprise

epitopes that make them particularly effective as immunogens against which anti-influenza immune responses can be induced.

The antigen can comprise the full length translation product HA0, subunit HA1, subunit HA2, a variant thereof, a fragment thereof or a combination thereof. The influenza hemagglutinin antigen can be a consensus sequence derived from multiple strains of influenza A serotype H1, a consensus sequence derived from multiple strains of influenza A serotype H2, a hybrid sequence containing portions of two different consensus sequences derived from different sets of multiple strains of influenza A serotype H1 or a consensus sequence derived from multiple strains of influenza B. The influenza hemagglutinin antigen can be from influenza B. The antigen can contain at least one antigenic epitope that can be effective against particular influenza immunogens against which an immune response can be induced. The antigen may provide an entire repertoire of immunogenic sites and epitopes present in an intact influenza virus. The antigen may be a consensus hemagglutinin antigen sequence that can be derived from hemagglutinin antigen sequences from a plurality of influenza A virus strains of one serotype such as a plurality of influenza A virus strains of serotype H1 or of serotype H2. The antigen may be a hybrid consensus hemagglutinin antigen sequence that can be derived from combining two different consensus hemagglutinin antigen sequences or portions thereof. Each of two different consensus hemagglutinin antigen sequences may be derived from a different set of a plurality of influenza A virus strains of one serotype such as a plurality of influenza A virus strains of serotype H1. The antigen may be a consensus hemagglutinin antigen sequence that can be derived from hemagglutinin antigen sequences from a plurality of influenza B virus strains.

The consensus hemagglutinin antigen may be a protein comprising SEQ ID NO: 2 (the consensus H1 amino acid sequence) wherein amino acids 1-343 correspond to the HA1 subunit of the precursor HA0 consensus H1 amino acid sequence and amino acids 344-566 correspond to the HA2 subunit of the HA0 consensus H1 amino acid sequence. The consensus hemagglutinin antigen may be a protein comprising SEQ ID NO: 7 (the consensus H2 amino acid sequence). The consensus hemagglutinin antigen may be a synthetic hybrid consensus H1 sequences comprising portions of two different consensus H1 sequences which are each derived from a different set of sequences from the other. An example of a consensus HA antigen that is a synthetic hybrid consensus H1 protein is a protein comprising SEQ ID NO: 10 (the U2 amino

acid sequence). The consensus hemagglutinin antigen may be a consensus hemagglutinin protein derived from hemagglutinin sequences from influenza B strains, such as a protein comprising SEQ ID NO: 14 (the consensus BHA amino acid sequence).

The consensus hemagglutinin antigen may further comprise one or more additional amino acid sequence elements. The consensus hemagglutinin antigen may further comprise on its N-terminal an IgE or IgG leader amino acid sequence. The IgE leader amino acid sequence may be SEQ ID NO: 17. The consensus hemagglutinin antigen may further comprise an immunogenic tag which is a unique immunogenic epitope that can be detected by readily available antibodies. An example of such an an immunogenic tag is the 9 amino acid influenza HA Tag which may be linked on the consensus hemagglutinin C terminus. The HA Tag amino acid sequence may be SEQ ID NO:18. In some embodiments, consensus hemagglutinin antigen may further comprise on its N-terminal an IgE or IgG leader amino acid sequence and on its C terminal an HA tag.

The consensus hemagglutinin antigen may be a consensus hemagglutinin protein that consists of consensus influenza amino acid sequences or fragments and variants thereof. The consensus hemagglutinin antigen may be a consensus hemagglutinin protein that comprises non-influenza protein sequences and influenza protein sequences or fragments and variants thereof.

Examples of a consensus H1 protein include those that may consist of the consensus H1 amino acid sequence (SEQ ID NO:2) or those that further comprise additional elements such as an IgE leader sequence, or an HA Tag or both an IgE leader sequence and an HA Tag. An example of the consensus H1 protein that includes both an IgE leader sequence and an HA Tag is SEQ ID NO: 4, which comprises the consensus H1 amino acid coding sequence (SEQ ID NO:2) linked to the IgE leader amino acid sequence (SEQ ID NO: 17) at its N terminal and linked to the HA Tag (SEQ ID NO:18) at its C terminal.

Examples of consensus H2 proteins include those that may consist of the consensus H2 amino acid sequence (SEQ ID NO:7) or those that further comprise an IgE leader sequence, or an HA Tag, or both an IgE leader sequence and an HA Tag.

Examples of hybrid consensus H1 proteins include those that may consist of the consensus U2 amino acid sequence (SEQ ID NO:10) or those that further comprise an IgE leader sequence, or an HA Tag, or both an IgE leader sequence and an HA Tag. An example of the

consensus U2 protein is SEQ ID NO:12, which comprises the consensus U2 amino acid sequence (SEQ ID NO:10) linked to the IgE leader amino acid sequence (SEQ ID NO: 17) at its N terminal and linked to the HA Tag (SEQ ID NO:18) at its C terminal.

Examples of hybrid consensus influenza B hemagglutinin proteins include those that may consist of the consensus BHA amino acid sequence (SEQ ID NO:14) or it may comprise an IgE leader sequence, or a an HA Tag, or both an IgE leader sequence and an HA Tag. An example of the consensus BHA protein is SEQ ID NO:16 which comprises the consensus BHA amino acid sequence (SEQ ID NO:14) linked to the IgE leader amino acid sequence (SEQ ID NO: 17) at its N terminal and linked to the HA Tag (SEQ ID NO:18) at its C terminal.

The consensus hemagglutinin protein can be encoded by a consensus hemagglutinin nucleic acid, a variant thereof or a fragment thereof. Unlike the consensus hemagglutinin protein which may be a consensus sequence derived from a plurality of different hemagglutinin sequences from different strains and variants, the consensus hemagglutinin nucleic acid refers to a nucleic acid sequence that encodes a consensus protein sequence and the coding sequences used may differ from those used to encode the particular amino acid sequences in the plurality of different hemagglutinin sequences from which the consensus hemagglutinin protein sequence is derived. The consensus nucleic acid sequence may be codon optimized and/or RNA optimized. The consensus hemagglutinin nucleic acid sequence may comprise a Kozak's sequence in the 5' untranslated region. The consensus hemagglutinin nucleic acid sequence may comprise nucleic acid sequences that encode a leader sequence. The coding sequence of an N terminal leader sequence is 5' of the hemagglutinin coding sequence. The N-terminal leader can be facilitate secretion. The N-terminal leader can be an IgE leader or an IgG leader. The consensus hemagglutinin nucleic acid sequence can comprise nucleic acid sequences that encode an immunogenic tag. The immunogenic tag can be on the C terminus of the protein and the sequence encoding it is 3' of the HA coding sequence. The immunogenic tag provides a unique epitope for which there are readily available antibodies so that such antibodies can be used in assays to detect and confirm expression of the protein. The immunogenic tag can be an H Tag at the C-terminus of the protein.

Consensus hemagglutinin nucleic acid may have a polynucleotide sequence that encodes a protein that comprises the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:7, SEQ ID

NO:10 or SEQ ID NO:14. A consensus hemagglutinin nucleic acid that encodes SEQ ID NO: 2, SEQ ID NO:7, SEQ ID NO:10 or SEQ ID NO:14 may be SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:9 or SEQ ID NO:13, respectively. The consensus hemagglutinin nucleic acid can further comprise a polynucleotide sequence encoding the IgE leader amino acid sequence, or a polynucleotide sequence encoding an HA Tag amino acid sequence, or both. SEQ ID NO: 17 is an IgE leader polypeptide sequence. SEQ ID NO: 18 is an HA Tag polypeptide sequence. Examples of hemagglutinin consensus nucleic acids that further comprise polynucleotide sequences encoding an IgE leader sequence and an HA Tag include nucleic acid molecules that encode proteins that comprise the amino acid sequence of SEQ ID NO:4, SEQ ID NO:12 or SEQ ID NO:16. A consensus hemagglutinin nucleic acid that encodes SEQ ID NO:4, SEQ ID NO:12 or SEQ ID NO:16 may be SEQ ID NO:3, SEQ ID NO:11 or SEQ ID NO:15, respectively.

3. Genetic Constructs and Plasmids

Provided herein are genetic constructs that can comprise a nucleic acid sequence that encodes the hemagglutinin antigen. The genetic construct can be present in the cell as a functioning extrachromosomal molecule comprising the nucleic acid encoding the hemagglutinin antigen. The genetic construct comprising the nucleic acid encoding the hemagglutinin antigen can be linear minichromosome including centromere, telomers or plasmids or cosmids.

The genetic construct can also be part of a genome of a recombinant viral vector, including recombinant adenovirus, recombinant adenovirus associated virus and recombinant vaccinia. The genetic construct can be part of the genetic material in attenuated live microorganisms or recombinant microbial vectors which live in cells.

The genetic constructs can comprise regulatory elements for gene expression of the hemagglutinin nucleic acid. The regulatory elements can be a promoter, an enhacer an initiation codon, a stop codon, or a polyadenylation signal.

Compositions may comprise a first nucleic acid sequence which encodes the hemagglutinin consensus antigen selected from the group consisting of one or more of: influenza A consensus hemagglutinin H1 antigen, influenza A consensus hemagglutinin H2 antigen, influenza A consensus hemagglutinin U2 antigen, and influenza B consensus hemagglutinin protein BHA, and may further comprise one or more additional nucleic acid sequence(s) that encodes one or more protein(s) selected from the group consisting of: influenza A hemagglutinin

proteins H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, influenza A neuraminidase N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin (BHA) and influenza B neuraminidase (BNA). The first and additional nucleic acid sequences may be present on the same nucleic acid molecule or different nucleic acid molecules. The first and additional nucleic acid sequences can be under the control of regulatory elements that function in a human cell. The additional coding sequence may encode one or more H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, BHA and BNA from one or more strains of influenza, or be a consensus derived from a plurality of strains having the serotype, or be a hybrid which includes sequences from two or more consensus sequences.

The nucleic acid sequences may make up a genetic construct that can be a vector. The vector can be capable of expressing a consensus hemagglutinin antigen in the cell of a mammal in a quantity effective to elicit an immune response in the mammal. The vector can be recombinant. The vector can comprise heterologous nucleic acid encoding the consensus hemagglutinin antigen. The vector can be a plasmid. The vector can be useful for transfecting cells with nucleic acid encoding a consensus hemagglutinin antigen, which the transformed host cell is cultured and maintained under conditions wherein expression of the consensus hemagglutinin antigen takes place.

The vector can comprise heterologous nucleic acid encoding a consensus hemagglutinin antigen and can further comprise an initiation codon, which can be upstream of the consensus hemagglutinin coding sequence, and a stop codon, which can be downstream of the consensus hemagglutinin coding sequence. The initiation and termination codon can be in frame with the consensus hemagglutinin coding sequence. The vector can also comprise a promoter that is operably linked to the consensus hemagglutinin coding sequence. The promoter operably linked to the consensus hemagglutinin coding sequence can be a promoter from simian virus 40 (SV40), a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter, Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. The promoter can also be a

promoter from a human gene such as human actin, human myosin, human hemoglobin, human muscle creatine, or human metalothionein. The promoter can also be a tissue specific promoter, such as a muscle or skin specific promoter, natural or synthetic. Examples of such promoters are described in US patent application publication no.US20040175727, the contents of which are incorporated herein in its entirety.

The vector can also comprise a polyadenylation signal, which can be downstream of the HA coding sequence. The polyadenylation signal can be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β-globin polyadenylation signal. The SV40 polyadenylation signal can be a polyadenylation signal from a pCEP4 vector (Invitrogen, San Diego, CA).

The vector can also comprise an enhancer upstream of the consensus hemagglutinin coding. The enhancer can be necessary for DNA expression. The enhancer can be human actin, human myosin, human hemoglobin, human muscle creatine or a viral enhancer such as one from CMV, HA, RSV or EBV. Polynucleotide function enhances are described in U.S. Patent Nos. 5,593,972, 5,962,428, and WO94/016737, the contents of each are fully incorporated by reference.

The vector can also comprise a mammalian origin of replication in order to maintain the vector extrachromosomally and produce multiple copies of the vector in a cell. The vector can be pVAX1 (Figure 1), pCEP4 or pREP4 from Invitrogen (San Diego, CA), which can comprise the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region, which can produce high copy episomal replication without integration. The vector can be pVAX1 with changes such as those described in the paragraph referring to Figure 1 in the Brief Description of the Figures section above. The backbone of the vector can be pAV0242. The vector can be a replication defective adenovirus type 5 (Ad5) vector.

The vector can also comprise a regulatory sequence, which can be well suited for gene expression in a mammalian or human cell into which the vector is administered. The consensus hemagglutinin coding sequence can comprise a codon, which can allow more efficient transcription of the coding sequence in the host cell.

The vector can be pSE420 (Invitrogen, San Diego, Calif.), which can be used for protein production in *Escherichia coli* (E.coli). The vector can also be pYES2 (Invitrogen, San Diego, Calif.), which can be used for protein production in Saccharomyces cerevisiae strains of yeast. The vector can also be of the MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, Calif.), which can be used for protein production in insect cells. The vector can also be pcDNA I or pcDNA3 (Invitrogen, San Diego, Calif.), which maybe used for protein production in mammalian cells such as Chinese hamster ovary (CHO) cells. The vector can be expression vectors or systems to produce protein by routine techniques and readily available starting materials including Sambrook et al., Molecular Cloning an Laboratory Manual, Second Ed. , Cold Spring Harbor (1989) ,which is incorporated fully by reference.

The vector can be pGX2009 or pGX2006, which can be used for expressing the consensus hemagglutinin antigen. The vector pGX2009 (4739 bp, Figure 2; SEQ ID NO: 5) is a modified pVAX1 plasmid with a nucleic acid sequence that encodes a consensus H1 protein (amino acid SEQ ID NO:4 encoded by SEQ ID NO:3) that comprises an IgE leader sequence (amino acid SEQ ID NO:12 encoded by SEQ ID NO:11) linked to a consensus H1 amino acid sequence (amino acid SEQ ID NO:2 encoded by SEQ ID NO:1). The vector pGX2006 (4628 bp; Figure 3, SEQ ID NO:8) is a pVAX1 plasmid with a nucleic acid sequence that encodes a consensus H2 protein (amino acid SEQ ID NO:7 encoded by SEQ ID NO:6).

The genetic constructs and components disclosed herein which include consensus hemagglutinin coding sequences may be used to express other influenza proteins such as influenza A H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin or neuraminidase protein whereby coding sequences for influenza A proteins H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin or neuraminidase protein are included in place of consensus hemagglutinin coding sequences.

4. Pharmaceutical compositions

Provided herein are pharmaceutical compositions according to the present invention which comprise about 1 nanogram to about 10 mg of DNA. In some embodiments, pharmaceutical compositions according to the present invention comprise from between: 1) at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 nanograms, or at

least 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95,100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, 495, 500, 605, 610, 615, 620, 625, 630, 635, 640, 645, 650, 655, 660, 665, 670, 675, 680, 685, 690, 695, 700, 705, 710, 715, 720, 725, 730, 735, 740, 745, 750, 755, 760, 765, 770, 775, 780, 785, 790, 795, 800, 805, 810, 815, 820, 825, 830, 835, 840, 845, 850, 855, 860, 865, 870, 875, 880, 885, 890, 895. 900, 905, 910, 915, 920, 925, 930, 935, 940, 945, 950, 955, 960, 965, 970, 975, 980, 985, 990, 995 or 1000 micrograms, or at least 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 mg or more; and 2) up to and including 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 nanograms, or up to and including 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95,100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, 495, 500, 605, 610, 615, 620, 625, 630, 635, 640, 645, 650, 655, 660, 665, 670, 675, 680, 685, 690, 695, 700, 705, 710, 715, 720, 725, 730, 735, 740, 745, 750, 755, 760, 765, 770, 775, 780, 785, 790, 795, 800, 805, 810, 815, 820, 825, 830, 835, 840, 845, 850, 855, 860, 865, 870, 875, 880, 885, 890, 895. 900, 905, 910, 915, 920, 925, 930, 935, 940, 945, 950, 955, 960, 965, 970, 975, 980, 985, 990, 995, or 1000 micrograms, or up to and including 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 mg. In some embodiments, pharmaceutical compositions according to the present invention comprise about 5 nanogram to about 10 mg of DNA. In some embodiments, pharmaceutical compositions according to the present invention comprise about 25 nanogram to about 5 mg of DNA. In some embodiments, the pharmaceutical compositions contain about 50 nanograms to about 1 mg of DNA. In some embodiments, the pharmaceutical compositions contain about 0.1 to about 500 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 1 to about 350 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 5 to about 250 micrograms of DNA. In some embodiments, the

pharmaceutical compositions contain about 10 to about 200 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 15 to about 150 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 20 to about 100 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 25 to about 75 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 30 to about 50 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 35 to about 40 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 100 to about 200 microgram DNA. In some embodiments, the pharmaceutical compositions comprise about 10 microgram to about 100 micrograms of DNA. In some embodiments, the pharmaceutical compositions comprise about 20 micrograms to about 80 micrograms of DNA. In some embodiments, the pharmaceutical compositions comprise about 25 micrograms to about 60 micrograms of DNA. In some embodiments, the pharmaceutical compositions comprise about 30 nanograms to about 50 micrograms of DNA. In some embodiments, the pharmaceutical compositions comprise about 35 nanograms to about 45 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 0.1 to about 500 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 1 to about 350 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 25 to about 250 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 100 to about 200 microgram DNA.

The pharmaceutical compositions according to the present invention are formulated according to the mode of administration to be used. In cases where pharmaceutical compositions are injectable pharmaceutical compositions, they are sterile, pyrogen free and particulate free. An isotonic formulation is preferably used. Generally, additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol and lactose. In some cases, isotonic solutions such as phosphate buffered saline are preferred. Stabilizers include gelatin and albumin. In some embodiments, a vasoconstriction agent is added to the formulation.

Preferably the pharmaceutical composition is a vaccine, and more preferably a DNA vaccine.

Provided herein is a vaccine capable of generating in a mammal an immune response against one or more influenza serotypes. The vaccine can comprise the genetic construct as discussed above. The vaccine can comprise a plurality of the vectors each directed to one or more Influenza A serotypes such as H1-H16 Influenza B hemagglutinin or combinations thereof. The vaccine may comprise one or more nucleic acid sequences that encode one or more consensus hemagglutinin antigens. When the vaccine comprises more than one consensus hemagglutinin nucleic acid sequences, all such sequences may be present on a single nucleic acid molecule or each such sequences may be present on a different nucleic acid molecule. Alternatively, vaccines that comprise more than one consensus hemagglutinin nucleic acid sequences may comprise nucleic acid molecules with a single consensus hemagglutinin nucleic acid sequences and nucleic acid molecules with more than one consensus hemagglutinin nucleic acid sequences. In addition, vaccines comprising one or more consensus hemagglutinin nucleic acid sequences may further comprise coding sequences for one or more proteins selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9 and influenza B neuraminise.

In some embodiments, vaccines may comprise proteins. Some vaccines may comprise one or more consensus hemagglutinin antigens such as H1, H2, U2 and BHA. The vaccines may comprise one or more other proteins selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9 and influenza B neuraminidase. The vaccines may comprise one or more consensus hemagglutinin antigens in combination with one or more other proteins selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin and neuraminidase.

The vaccine may be a DNA vaccine. The DNA vaccine may comprise a plurality of the same or different plasmids comprising one or more of consensus hemagglutinin nucleic acid sequences. The DNA vaccine may comprise one or more nucleic acid sequences that encode one or more consensus hemagglutinin antigens. When the DNA vaccine comprises more than one consensus hemagglutinin nucleic acid sequences, all such sequences may be present on a single plasmid, or each such sequences may be present on a different plasmids, or some plasmids may comprise a single consensus hemagglutinin nucleic acid sequences while other plasmids have

more than one consensus hemagglutinin nucleic acid sequences. In addition, DNA vaccines may further comprise one or more consensus coding sequences for one or more proteins selected from the group consisting of influenza A H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin and neuramidase. Such additional coding sequences may be on the same or different plasmids from each other and from the plasmids comprising one or more of consensus hemagglutinin nucleic acid sequences.

In some embodiments, vaccines may comprise nucleic acid sequences that encode influenza antigens in combination with influenza antigens. In some embodiments, the nucleic acid sequences encode one or more consensus hemagglutinin antigens such as H1, H2, U2 and BHA. In some embodiments, the nucleic acid sequences encode one or more one or more other proteins selected from the group consisting of , influenza A H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin and neuramidase. In some embodiments, the vaccines comprise one or more consensus hemagglutinin antigens such as H1, H2, U2 and BHA. In some embodiments, the vaccines comprise one or more one or more other proteins selected from the group consisting of influenza A H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin and neuramidase.

In some embodiments, vaccines comprise a combination of three or more consensus hemagglutinin nucleic acid sequences including those encoding one or more of H1, H2, U2 and BHA. In some embodiments, vaccines comprise a combination of three or more hemagglutinin nucleic acid sequences including those encoding consensus U2, consensus BHA and an H3 hemagglutinin. In some embodiments, vaccines comprise a combination of three or more hemagglutinin nucleic acid sequences including those encoding consensus BHA, an H1 hemagglutinin and an H3 hemagglutinin. In some embodiments, vaccines comprise one or more nucleic acid sequences that encode one or more influenza antigens disclosed in U.S. Serial No. 12/375,518, which is incorporated herein by reference and/or U.S. Serial No. 12/269,824, which is incorporated herein by reference. In some embodiments, vaccines comprise a nucleic acid sequence SEQ ID NO:19 which encodes SEQ ID NO:20 (which is an H1 hemagglutinin disclosed in U.S. Serial No. 12/375,518 as SEQ ID NO:36 and SEQ ID NO:37 respectively

therein) and/or nucleic acid sequence SEQ ID NO:21 which encodes SEQ ID NO:22 (which is an H1 hemagglutinin disclosed in U.S. Serial No. 12/269,824 as SEQ ID NO:9 and SEQ ID NO:10 respectively therein). In some embodiments, vaccines comprise a nucleic acid sequence SEQ ID NO:23 which encodes SEQ ID NO:24 (which is an H3 hemagglutinin disclosed in U.S. Serial No. 12/269,824 as SEQ ID NO:11 and SEQ ID NO:12 respectively therein.

In some embodiments, vaccines comprise a combination of three or more consensus hemagglutinin proteins including one or more of H1, H2, U2 and BHA. In some embodiments, vaccines comprise a combination of three or more hemagglutinin proteins including consensus U2, consensus BHA and an H3 hemagglutinin. In some embodiments, vaccines comprise a combination of three or more hemagglutinin proteins including consensus BHA, an H1 hemagglutinin and an H3 hemagglutinin. In some embodiments, vaccines comprise one or more antigens from U.S. Serial No. 12/375,518 and/or U.S. Serial No. 12/269,824. In some embodiments, vaccines comprise SEQ ID NO:20 and/or SEQ ID NO:22 and/or SEQ ID NO:24.

In some embodiments, vaccines comprise a combination of 1) the consensus hemagglutinin U2 protein and/or a nucleic acid sequences encoding the consensus hemagglutinin U2 protein, 2) the consensus hemagglutinin BHA protein and/or a nucleic acid sequences encoding the consensus hemagglutinin BHA protein, and 3) a hemagglutinin H3 protein disclosed in SEQ ID NO:24.

In some embodiments, vaccines comprise a combination of 1) the consensus hemagglutinin BHA protein and/or a nucleic acid sequences encoding the consensus hemagglutinin BHA protein, 2) a hemagglutinin H1 protein having SEQ ID NO:20 and/or SEQ ID NO:22 nd/or a hemagglutinin H1 protein encoding nucleic acid sequences SEQ ID NO19 and/or SEQ ID NO:21, and 3) a hemagglutinin H3 protein having SEQ ID NO:24 and/or a hemagglutinin H3 protein encoding nucleic acid sequence SEQ ID NO:23 therein).

DNA vaccines are disclosed in US Patent Nos. 5,593,972, 5,739,118, 5,817,637, 5,830,876, 5,962,428, 5,981,505, 5,580,859, 5,703,055, and 5,676,594, which are incorporated herein fully by reference. The DNA vaccine can further comprise elements or reagents that inhibit it from integrating into the chromosome. The vaccine can be an RNA of the hemagglutinin antigen. The RNA vaccine can be introduced into the cell.

The vaccine can be a recombinant vaccine comprising the genetic construct or antigen described above. The vaccine can also comprise one or more consensus hemagglutinin antigen in the form of one or more protein subunits, one or more killed influenza particles comprising one or more consensus hemagglutinin antigens, or one or more attenuated influenza particles comprising one or more consensus hemagglutinin antigens. The attenuated vaccine can be attenuated live vaccines, killed vaccines and vaccines that use recombinant vectors to deliver foreign genes that encode one or more consensus hemagglutinin antigens, and well as subunit and glycoprotein vaccines. Examples of attenuated live vaccines, those using recombinant vectors to deliver foreign antigens, subunit vaccines and glycoprotein vaccines are described in U.S. Patent Nos.: 4,510,245; 4,797,368; 4,722,848; 4,790,987; 4,920,209; 5,017,487; 5,077,044; 5,110,587; 5,112,749; 5,174,993; 5,223,424; 5,225,336; 5,240,703; 5,242,829; 5,294,441; 5,294,548; 5,310,668; 5,387,744; 5,389,368; 5,424,065; 5,451,499; 5,453,3 64; 5,462,734; 5,470,734; 5,474,935; 5,482,713; 5,591,439; 5,643,579; 5,650,309; 5,698,202; 5,955,088; 6,034,298; 6,042,836; 6,156,319 and 6,589,529, which are each incorporated herein by reference.

The vaccine can comprise vectors and/or proteins directed to Influenza A serotypes from particular regions in the world, for example, Asia. The vaccine can also be directed against Influenza A serotypes of swine origin that now infect humans. The vaccine can comprise vectors and/or proteins directed to Influenza B from particular regions in the world. The vaccine can also be directed against Influenza B that infect humans. The vaccine can comprise one or more vectors and/or one or more proteins directed to one or more strains of Influenza A and/or B.

The vaccine provided may be used to induce immune responses including therapeutic or prophylactic immune responses. Antibodies and/or killer T cells may be generated which are directed to the consensus hemagglutinin antigen, and also broadly across multiple subtypes of influenza viruses. Such antibodies and cells may be isolated.

The vaccine can further comprise a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient can be functional molecules as vehicles, adjuvants, carriers, or diluents. The pharmaceutically acceptable excipient can be a transfection facilitating agent, which can include surface active agents, such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A,

muramyl peptides, quinone analogs, vesicles such as squalene and squalene, hyaluronic acid, lipids, liposomes, calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents.

The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. The transfection facilitating agent is poly-L-glutamate, and more preferably, the poly-L-glutamate is present in the vaccine at a concentration less than 6 mg/ml. The transfection facilitating agent can also include surface active agents such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic acid can also be used administered in conjunction with the genetic construct. In some embodiments, the DNA vector vaccines can also include a transfection facilitating agent such as lipids, liposomes, including lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture (see for example W09324640), calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents. Preferably, the transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. Concentration of the transfection agent in the vaccine is less than 4 mg/ml, less than 2 mg/ml, less than 1 mg/ml, less than 0.750 mg/ml, less than 0.500 mg/ml, less than 0.250 mg/ml, less than 0.100 mg/ml, less than 0.050 mg/ml, or less than 0.010 mg/ml.

The pharmaceutically acceptable excipient may be an adjuvant. The adjuvant may be other genes that are expressed in alternative plasmid or are delivered as proteins in combination with the plasmid above in the vaccine. The adjuvant may be selected from the group consisting of: α-interferon(IFN- α), β-interferon (IFN-β), γ-interferon, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15, MHC, CD80,CD86 including IL-15 having the signal sequence deleted and optionally including the signal peptide from IgE. The adjuvant may be IL-12, IL-15, IL-28, CTACK, TECK, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, or a combination thereof.

Other genes which may be useful adjuvants include those encoding: MCP-1, MIP-la, MIP-1p, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, pl50.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP1, TAP2 and functional fragments thereof.

The vaccine can further comprise a genetic vaccine facilitator agent as described in U.S. Serial No. 021,579 filed April 1, 1994, which is fully incorporated by reference.

5. Methods of Delivery

Provided herein is a method for delivering the pharmaceutical formulations, preferably vaccines, for providing genetic constructs and proteins of the hemagglutinin antigen which comprise epitopes that make them particular effective immunogens against which an immune response to influenza viral infections can be induced. The method of delivering the vaccine, or vaccination, can be provided to induce a therapeutic and/or prophylactic immune response. The vaccination process can generate in the mammal an immune response against a plurality of influenza subtypes, including a H1N1 serotype, such as the 2009 swine originated H1N1, or other seasonal and/or pandemic varieties. The vaccine can be delivered to an individual to modulate the activity of the mammal's immune system and enhance the immune response. The delivery of the vaccine can be the transfection of the HA antigen as a nucleic acid molecule that is expressed in the cell and delivered to the surface of the cell upon which the immune system recognized and induces a cellular, humoral, or cellular and humoral response. The delivery of the vaccine can be use to induce or elicit and immune response in mammals against a plurality of influenza viruses by administering to the mammals the vaccine as discussed herein.

Upon delivery of the vaccine to the mammal, and thereupon the vector into the cells of the mammal, the transfected cells will express and secrete the corresponding influenza protein,

including at least one of the consensus antigens, and preferably H1, H2, U2, and BHA. These secreted proteins, or synthetic antigens, will be recognized as foreign by the immune system, which will mount an immune response that can include: antibodies made against the antigens, and T-cell response specifically against the antigen. In some examples, a mammal vaccinated with the vaccines discussed herein will have a primed immune system and when challenged with an influenza viral strain, the primed immune system will allow for rapid clearing of subsequent influenza viruses, whether through the humoral, cellular, or both.. The vaccine can be delivered to an individual to modulate the activity of the individual's immune system thereby enhancing the immune response.

The vaccine can be delivered in the form of a DNA vaccine and methods of delivering a DNA vaccines are described in U.S. Patent Nos. 4,945,050 and 5,036,006, which are both incorporated fully by reference.

The vaccine can be administered to a mammal to elicit an immune response in a mammal. The mammal can be human, non-human primate, cow, pig, sheep, goat, antelope, bison, water buffalo, bovids, deer, hedgehogs, elephants, llama, alpaca, mice, rats, or chicken, and preferably human, cow, pig, or chicken.

a. Combination Treatments

The pharmaceutical compositions, preferably vaccines, can be administered in combination with one or more other influenza proteins or genes encoding influenza A H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin and neuramidase. The vaccine can be administered in combination with proteins or genes encoding adjuvants, which can include: α-interferon(IFN- α), β-interferon (IFN-β), γ-interferon, IL-12, IL-15, IL-28, CTACK, TECK, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, MCP-1, MIP-la, MIP-1p, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, pl50.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1,

Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP1, or TAP2, or functional fragments thereof.

b. Routes of Administration

The vaccine can be administered by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intranasal intrathecal, and intraarticular or combinations thereof. For veterinary use, the composition can be administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can readily determine the dosing regimen and route of administration that is most appropriate for a particular animal.. The vaccine can be administered by traditional syringes, needleless injection devices, "microprojectile bombardment gone guns", or other physical methods such as electroporation ("EP"), "hydrodynamic method", or ultrasound.

The vector of the vaccine can be delivered to the mammal by several well known technologies including DNA injection (also referred to as DNA vaccination) with and without in vivo electroporation, liposome mediated, nanoparticle facilitated, recombinant vectors such as recombinant adenovirus, recombinant adenovirus associated virus and recombinant vaccinia. The HA antigen can be delivered via DNA injection and along with in vivo electroporation.

c. Electroporation

Administration of the vaccine via electroporation of the plasmids of the vaccine may be accomplished using electroporation devices that can be configured to deliver to a desired tissue of a mammal a pulse of energy effective to cause reversible pores to form in cell membranes, and preferable the pulse of energy is a constant current similar to a preset current input by a user. The electroporation device may comprise an electroporation component and an electrode assembly or handle assembly. The electroporation component may include and incorporate one or more of the various elements of the electroporation devices, including: controller, current waveform generator, impedance tester, waveform logger, input element, status reporting element, communication port, memory component, power source, and power switch. The

electroporation may be accomplished using an in vivo electroporation device, for example CELLECTRA® EP system (VGX Pharmaceuticals, Blue Bell, PA) or Elgen electroporator (Genetronics, San Diego, CA) to facilitate transfection of cells by the plasmid.

The electroporation component may function as one element of the electroporation devices, and the other elements are separate elements (or components) in communication with the electroporation component. The electroporation component may function as more than one element of the electroporation devices, which may be in communication with still other elements of the electroporation devices separate from the electroporation component. The elements of the electroporation devices existing as parts of one electromechanical or mechanical device may not limited as the elements can function as one device or as separate elements in communication with one another. The electroporation component may be capable of delivering the pulse of energy that produces the constant current in the desired tissue, and includes a feedback mechanism. The electrode assembly may include an electrode array having a plurality of electrodes in a spatial arrangement, wherein the electrode assembly receives the pulse of energy from the electroporation component and delivers same to the desired tissue through the electrodes. At least one of the plurality of electrodes is neutral during delivery of the pulse of energy and measures impedance in the desired tissue and communicates the impedance to the electroporation component. The feedback mechanism may receive the measured impedance and can adjust the pulse of energy delivered by the electroporation component to maintain the constant current.

A plurality of electrodes may deliver the pulse of energy in a decentralized pattern. The plurality of electrodes may deliver the pulse of energy in the decentralized pattern through the control of the electrodes under a programmed sequence, and the programmed sequence is input by a user to the electroporation component. The programmed sequence may comprise a plurality of pulses delivered in sequence, wherein each pulse of the plurality of pulses is delivered by at least two active electrodes with one neutral electrode that measures impedance, and wherein a subsequent pulse of the plurality of pulses is delivered by a different one of at least two active electrodes with one neutral electrode that measures impedance.

The feedback mechanism may be performed by either hardware or software. The feedback mechanism may be performed by an analog closed-loop circuit. The feedback occurs

every 50 μs, 20 μs, 10 μs or 1 μs, but is preferably a real-time feedback or instantaneous (i.e., substantially instantaneous as determined by available techniques for determining response time). The neutral electrode may measure the impedance in the desired tissue and communicates the impedance to the feedback mechanism, and the feedback mechanism responds to the impedance and adjusts the pulse of energy to maintain the constant current at a value similar to the preset current. The feedback mechanism may maintain the constant current continuously and instantaneously during the delivery of the pulse of energy.

Examples of electroporation devices and electroporation methods that may facilitate delivery of the DNA vaccines of the present invention, include those described in U.S. Patent No. 7,245,963 by Draghia-Akli, et al., U.S. Patent Pub. 2005/0052630 submitted by Smith, et al., the contents of which are hereby incorporated by reference in their entirety. Other electroporation devices and electroporation methods that may be used for facilitating delivery of the DNA vaccines include those provided in co-pending and co-owned U.S. Patent Application, Serial No. 11/874072, filed October 17, 2007, which claims the benefit under 35 USC 119(e) to U.S. Provisional Applications Ser. Nos. 60/852,149, filed October 17, 2006, and 60/978,982, filed October 10, 2007, all of which are hereby incorporated in their entirety.

U.S. Patent No. 7,245,963 by Draghia-Akli, et al. describes modular electrode systems and their use for facilitating the introduction of a biomolecule into cells of a selected tissue in a body or plant. The modular electrode systems may comprise a plurality of needle electrodes; a hypodermic needle; an electrical connector that provides a conductive link from a programmable constant-current pulse controller to the plurality of needle electrodes; and a power source. An operator can grasp the plurality of needle electrodes that are mounted on a support structure and firmly insert them into the selected tissue in a body or plant. The biomolecules are then delivered via the hypodermic needle into the selected tissue. The programmable constant-current pulse controller is activated and constant-current electrical pulse is applied to the plurality of needle electrodes. The applied constant-current electrical pulse facilitates the introduction of the biomolecule into the cell between the plurality of electrodes. The entire content of U.S. Patent No. 7,245,963 is hereby incorporated by reference.

U.S. Patent Pub. 2005/0052630 submitted by Smith, et al. describes an electroporation device which may be used to effectively facilitate the introduction of a biomolecule into cells of

a selected tissue in a body or plant. The electroporation device comprises an electro-kinetic device ("EKD device") whose operation is specified by software or firmware. The EKD device produces a series of programmable constant-current pulse patterns between electrodes in an array based on user control and input of the pulse parameters, and allows the storage and acquisition of current waveform data. The electroporation device also comprises a replaceable electrode disk having an array of needle electrodes, a central injection channel for an injection needle, and a removable guide disk. The entire content of U.S. Patent Pub. 2005/0052630 is hereby incorporated by reference.

The electrode arrays and methods described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/0052630 may be adapted for deep penetration into not only tissues such as muscle, but also other tissues or organs. Because of the configuration of the electrode array, the injection needle (to deliver the biomolecule of choice) is also inserted completely into the target organ, and the injection is administered perpendicular to the target issue, in the area that is pre-delineated by the electrodes The electrodes described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/005263 are preferably 20 mm long and 21 gauge.

Additionally, contemplated in some embodiments that incorporate electroporation devices and uses thereof, there are electroporation devices that are those described in the following patents: US Patent 5,273,525 issued December 28, 1993, US Patents 6,110,161 issued August 29, 2000, 6,261,281 issued July 17, 2001, and 6,958,060 issued October 25, 2005, and US patent 6,939,862 issued September 6, 2005. Furthermore, patents covering subject matter provided in US patent 6,697,669 issued February 24, 2004, which concerns delivery of DNA using any of a variety of devices, and US patent 7,328,064 issued February 5, 2008, drawn to method of injecting DNA are contemplated herein. The above-patents are incorporated by reference in their entirety.

d. Method of Preparing Vaccine

Provided herein is methods for preparing the DNA plasmids that comprise the DNA vaccines discussed herein. The DNA plasmids, after the final subcloning step into the mammalian expression plasmid, can be used to inoculate a cell culture in a large scale fermentation tank, using known methods in the art.

The DNA plasmids for use with the EP devices of the present invention can be formulated or manufactured using a combination of known devices and techniques, but preferably they are manufactured using an optimized plasmid manufacturing technique that is described in a licensed, co-pending U.S. provisional application U.S. Serial No. 60/939,792, which was filed on May 23, 2007. In some examples, the DNA plasmids used in these studies can be formulated at concentrations greater than or equal to 10 mg/mL. The manufacturing techniques also include or incorporate various devices and protocols that are commonly known to those of ordinary skill in the art, in addition to those described in U.S. Serial No. 60/939792, including those described in a licensed patent, US Patent No. 7,238,522, which issued on July 3, 2007. The above-referenced application and patent, US Serial No. 60/939,792 and US Patent No. 7,238,522, respectively, are hereby incorporated in their entirety.

EXAMPLES

The present invention is further illustrated in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

Example 1

pGX2009 (pH1HA09) – Plasmid Encoding 2009 H1N1 Influenza (Swine Flu) Hemagglutinin Antigen

The backbone of pGX2009 (H1HA09) is the modified expression vector pVAX1 (Invitrogen, Carlsbad, CA) under the control of the cytomegalovirus immediate-early (CMV) promoter. The original pVAX1 was purchased from Invitrogen (Catalog number V260-20) and maintained at -20^{0}C. As noted above, sequence analysis revealed differences between the sequence of pVAX1 used as the backbone of pGX2009 and the pVAX1 sequence available from Invitrogen. The differences are set forth above.

Plasmid pGX2009, also referred to as pH1HA09, comprises a nucleic acid sequence that encodes a consensus 2009 H1N1 influenza (swine flu) hemagglutinin molecule. The 79 primary sequences used to generate the consensus sequence were selected from The Influenza Sequence Database.

The accession numbers for nucleotide sequences encoding the amino acid sequence for the various influenza A hemagglutinin H1 proteins as well as the amino acid sequences encoded by the nucleotide sequences are in the GenBank database corresponding to the following accession numbers. The accession numbers not in parentheses disclose nucleotide sequences and additional list amino acid sequences encoded by them. The accession numbers in parentheses are for entries of the corresponding amino acid sequence in GenBank's protein database. The accession numbers are as follows: GQ323579.1 (ACS72657.1), GQ323564.1 (ACS72654.1), GQ323551.1 (ACS72652.1), GQ323530.1 (ACS72651.1), GQ323520.1 (ACS72650.1), GQ323495.1 (ACS72648.1), GQ323489.1 (ACS72647.1), GQ323486.1 (ACS72646.1), GQ323483.1 (ACS72645.1), GQ323455.1 (ACS72641.1), GQ323451.1 (ACS72640.1), GQ323443.1 (ACS72638.1), GQ293077.1 (ACS68822.1), GQ288372.1 (ACS54301.1), GQ287625.1 (ACS54262.1), GQ287627.1 (ACS54263.1), GQ287623.1 (ACS54261.1), GQ287621.1 (ACS54260.1), GQ286175.1 (ACS54258.1), GQ283488.1 (ACS50088.1), GQ280797.1 (ACS45035.1), GQ280624.1 (ACS45017.1), GQ280121.1 (ACS45189.1), GQ261277.1 (ACS34968.1), GQ253498.1 (ACS27787.1), GQ323470.1 (ACS72643.1), GQ253492.1 (ACS27780.1), FJ981613.1 (ACQ55359.1), FJ971076.1 (ACP52565.1), FJ969540.1 (ACP44189.1), FJ969511.1 (ACP44150.1), FJ969509.1 (ACP44147.1), GQ255900.1 (ACS27774.1),GQ255901.1 (ACS27775.1), FJ966974.1 (ACP41953.1), GQ261275.1 (ACS34967.1), FJ966960.1 (ACP41935.1),FJ966952.1 (ACP41926.1), FJ966082.1 (ACP41105.1),GQ255897.1 (ACS27770.1), CY041645.1 (ACS27249.1), CY041637.1 (ACS27239.1),CY041629 (ACS27229.1), GQ323446.1 (ACS72639.1),CY041597.1 (ACS27189.1), CY041581.1 (ACS14726.1),CY040653.1 (ACS14666.1), CY041573.1 (ACS14716.1),CY041565.1 (ACS14706.1), CY041541.1 (ACS14676.1),GQ258462.1 (ACS34667.1), CY041557.1 (ACS14696.1), CY041549.1 (ACS14686.1), GQ283484.1 (ACS50084.1), GQ283493.1 (ACS50095.1), GQ303340.1 (ACS71656.1), GQ287619.1 (ACS54259.1), GQ267839.1 (ACS36632.1), GQ268003.1

(ACS36645.1), CY041621.1 (ACS27219.1), CY041613.1 (ACS27209.1), CY041605.1 (ACS27199.1), FJ966959.1 (ACP41934.1), FJ966982.1 (ACP41963.1), CY039527.2 (ACQ45338.1), FJ981612.1 (ACQ55358.1), FJ981615.1 (ACQ55361.1), FJ982430.1 (ACQ59195.1), FJ998208.1 (ACQ73386.1), GQ259909.1 (ACS34705.1), GQ261272.1 (ACS34966.1), GQ287621.1 (ACS54260.1), GQ290059.1 (ACS66821.1), GQ323464.1 (ACS72642.1), GQ323473.1 (ACS72644.1), GQ323509.1 (ACS72649.1), GQ323560.1 (ACS72653.1), GQ323574.1 (ACS72655.1), and GQ323576.1 (ACS72656.1). The amino acid sequences were downloaded from the NCBI Sequence Database, and an alignment and consensus sequence generated using Clustal X. A highly efficient leader sequence, the IgE leader, was fused in frame upstream of the start codon to facilitate the expression. In order to have a higher level of expression, the codon usage of this fusion gene was adapted to the codon bias of Homo Sapiens genes. In addition, RNA optimization was also performed: regions of very high (>80%) or very low (<30%) GC content and the cis-acting sequence motifs such as internal TATA boxes, chi-sites and ribosomal entry sites were avoided. The entire sequence was synthetically produced at Geneart (Regensburg, Germany). The synthetic engineered H1HA09 gene was 1818 bp in length (SEQ ID NO:1) and was cloned into pVAX1 at BamHI and XhoI sites by Geneart (Figure 2).

Example 2

Challenge of Influenza pGX2009 immunized Ferrets with A/Mexico/InDRE4487/2009

Challenge experiments were carried out using ferrets, a preferred model for influenza. The ferrets were immunized using plasmid pGX2009.

Animals: 4 groups x 5 animals/group, plus one control group with 4 animals = 24 ferrets total (male)

Duration: 18 weeks (including challenge)

Dose: .2mg plasmid

Protocol Summary: Ferrets were allocated randomly into DNA vaccine groups. Animals were immunized at Study Day 0, Day 28, and Day 56. Animals were anesthetized with ketamine/midazolam cocktail, isoflurane or equivalent according to approved anesthesia protocols and vaccinated IM with influenza DNA vaccine combinations. Groups 1 and 2 were immediately electroporated using CELLECTRA® adaptive constant current electroporation (EP)

device at 0.5 Amp, 52 millisecond pulses, 0.2 sec between pulses, 4 sec firing delay, 3 total pulses. Control animals were naïve controls (no plasmid, no EP). Ferrets were allowed to recover from anesthesia in their cages and were closely monitored for 24 hours to ensure full recovery.

Food and water was available ad libitum for the length of the study. On Day 84, animals were challenged by intranasal infection with 1 ml of MX10 (A/Mexico/InDRE4487/2009; 5 x 105 PFU/ml). Animals were monitored daily for clinical signs (weight, temperature, etc.), using an established and approved scoring sheet. On 1, 3, 6, 9 and 15 dpi nasal washes and rectal swabs were collected. Lungs were collected at day 15. Samples were stored in RNAlater for virus load by real-time PCR, medium for infectious virus (TCDI50) and formalin for histology when appropriated.

Figure 4 shows a Hemagglutination Inhibition assay performed with sera from immunized ferrets (3 immunizations). A titer of >1:40 is considered "protective". A dotted line indicates the 1:40 mark. All animals were above the 1:40 mark after 3 immunizations. Figure 5 shows results of a challenge of immunized and unimmunized ferrets with a novel H1N1 strain MX10 (A/Mexico/InDRE4487/2009).. All immunized ferrets survived, while 75% of the naive ferrets died within the 15 day period.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding an amino acid sequence that is at least 99% homologues to SEQ ID NO: 14, and has at least 360 continuous amino acids of SEQ ID NO:14.

2. The isolated nucleic acid molecule of claim 1 comprising the nucleic acid sequence of SEQ ID NO:13.

3. The isolated nucleic acid molecule of claim 1 further comprising a nucleic acid sequence that encodes an IgE leader sequence.

4. The isolated nucleic acid molecule of claim 3 comprising SEQ ID NO: 15.

5. An expression vector comprising the nucleic acid sequence of claim 1 operably linked to regulatory elements capable of directing the expression of the nucleic acid sequence in a cell.

6. The expression vector of claim 5, wherein the regulatory elements are functional in a human cell.

7. The expression vector of claim 6, wherein said expression vector is a plasmid.

8. The expression vector of claim 7, wherein said plasmid is pGX2009.

9. A composition comprising the isolated nucleic acid molecule or the expression vector of any one of claims 1, 2, 3, 5, 6, 7 or 4, further comprising

one or more additional nucleic acid sequences that encode one or more proteins selected from the group consisting of an influenza A hemaggultinin H1, an influenza A hemaggultinin H2, an influenza A hemaggultinin H3, an influenza A H4 an influenza A hemaggultinin H5, an influenza A hemaggultinin H3, an influenza A hemaggultinin H5, an influenza A N1, an

influenza A hemaggultinin H6, an influenza A hemaggultinin H7, an influenza A hemaggultinin H5, an influenza A hemaggultinin H6, an influenza A hemaggultinin H7, an influenza A hemaggultinin H8, an influenza A hemaggultinin H9, an influenza A hemaggultinin H10, an influenza A hemaggultinin H11, an influenza A hemaggultinin H12, an influenza A hemaggultinin H13 ,an influenza A hemaggultinin H14, an influenza A hemaggultinin H15, an influenza A hemaggultinin H16, an influenza A neuraminidase N1, an influenza A neuraminidase N2, an influenza A neuraminidase N3, an influenza A neuraminidase N4, an influenza A neuraminidase N5, an influenza A neuraminidase N6, an influenza A neuraminidase N7, an influenza A neuraminidase N8, an influenza A neuraminidase N9, an influenza B hemaggultinin and an influenza B neuraminidase.

10. A method of inducing an immune response in an individual comprising the step of administering to an individual of the isolated nucleic acid molecule or the expression vector of any one of claims 1, 2, 3, 5, 6, 7 or 4.

11. A method of inducing an immune response comprising the step of administering to an individual the composition of claim 9.

12. A method of protecting an individual against infection by an influenza A strain comprising the step of: administering to said individual a prophylactically effective amount of the composition of claim 9.

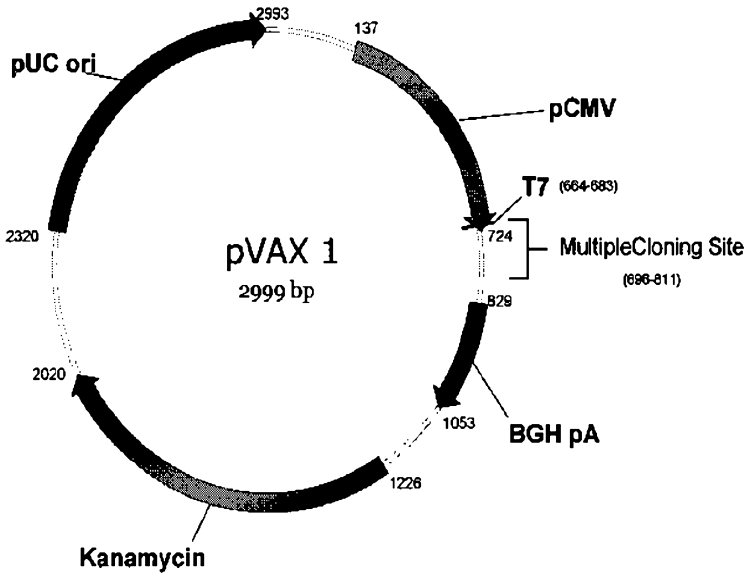
DATED this SIXTEENTH day of APRIL 2013

The Trustees of the University of Pennsylvania

By patent attorneys for the applicant:

FB Rice

FIGURE 1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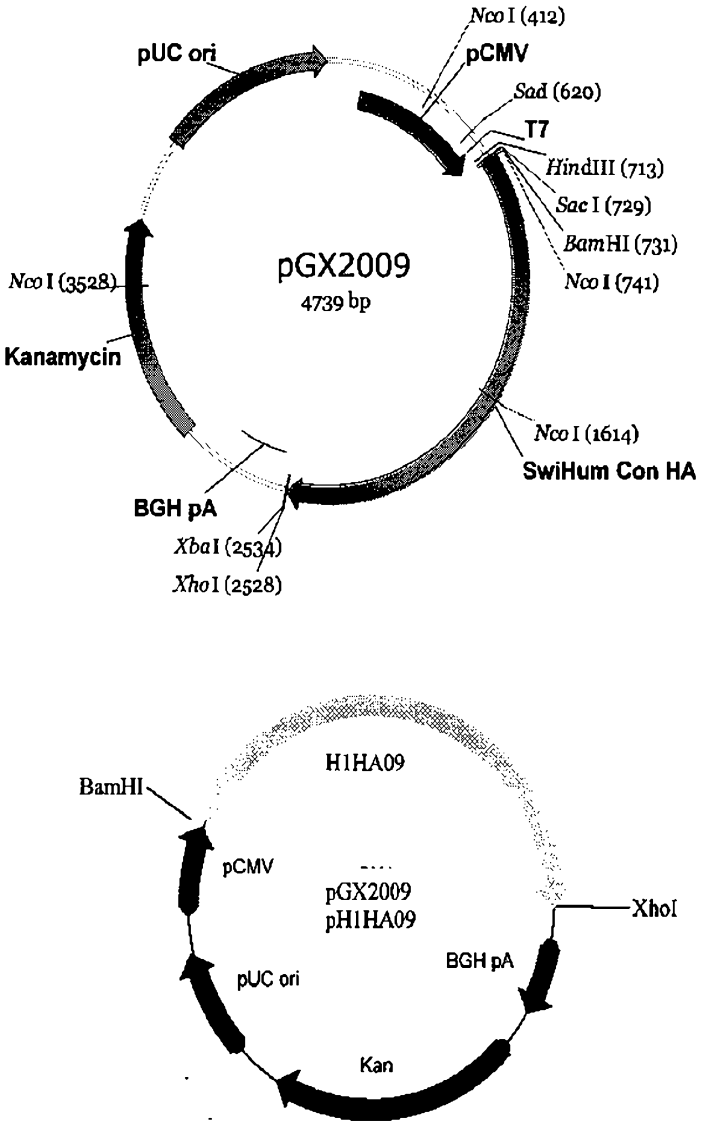
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NcoI (3528)
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NcoI (1614)
SwiHum Con HA
BGH pA
XbaI (2534)
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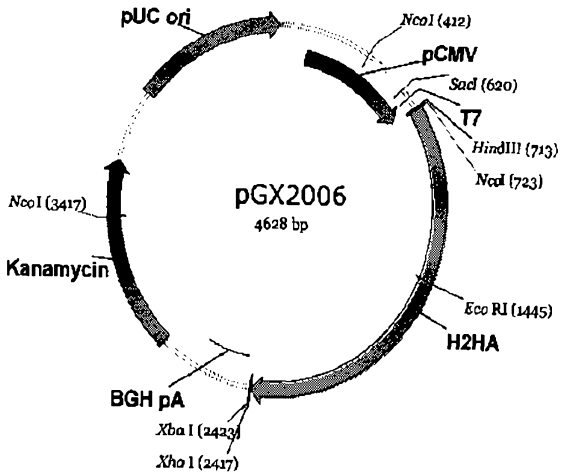
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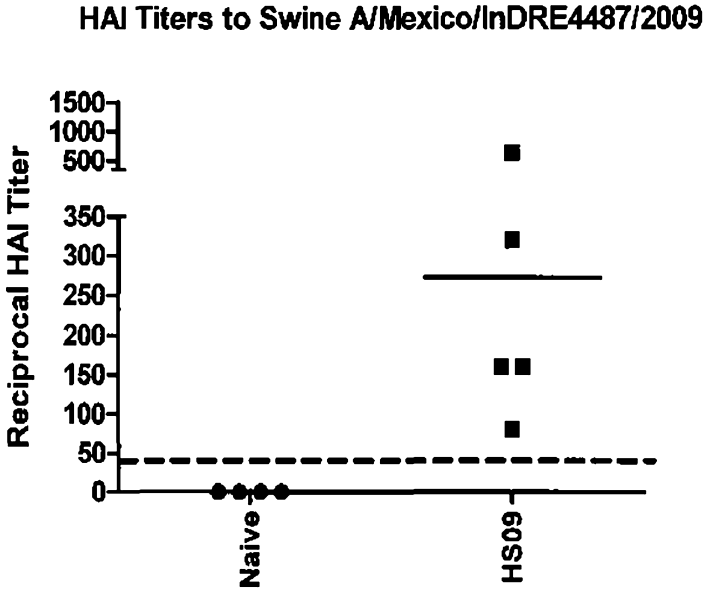
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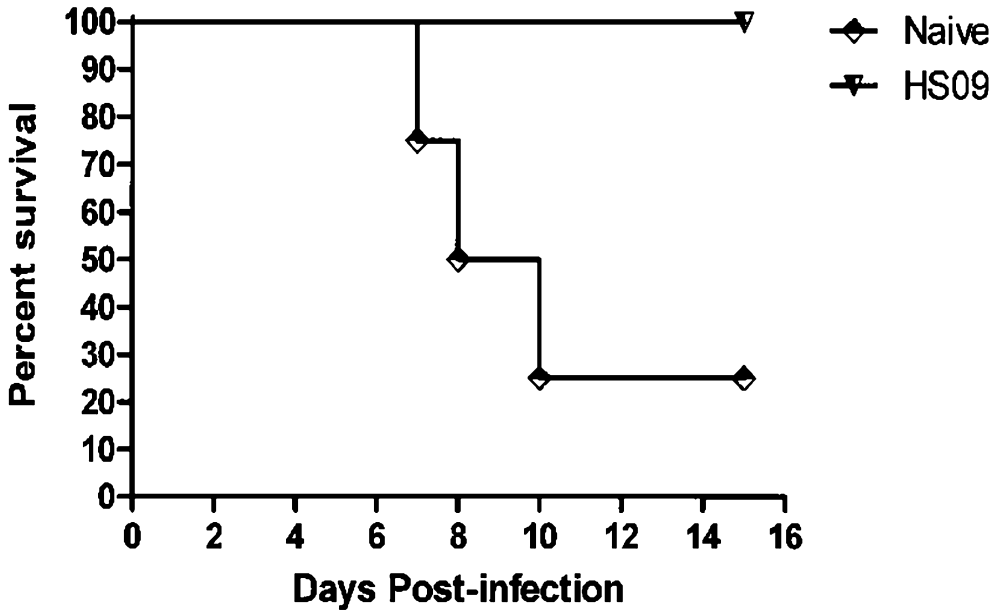
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Days Post-infection
Naive
HS09

SEQUENCE LISTING

<110> The Trustees of the University of Pennsylvania
David, Weiner B.
Yan, Jian
Morrow, Matthew P.

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His Ser Met Lys Ala Ile Leu Val Val Leu Leu Tyr Thr Phe Ala Thr
20 25 30

Ala Asn Ala Asp Thr Leu Cys Ile Gly Tyr His Ala Asn Asn Ser Thr
35 40 45

Asp Thr Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser
50 55 60

Val Asn Leu Leu Glu Asp Lys His Asn Gly Lys Leu Cys Lys Leu Arg
65 70 75 80

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Gly Val Ala Pro Leu His Leu Gly Lys Cys Asn Ile Ala Gly Trp Ile
                85                  90                  95

Leu Gly Asn Pro Glu Cys Glu Ser Leu Ser Thr Ala Ser Ser Trp Ser
            100                 105                 110

Tyr Ile Val Glu Thr Ser Ser Ser Asp Asn Gly Thr Cys Tyr Pro Gly
        115                 120                 125

Asp Phe Ile Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser
    130                 135                 140

Ser Phe Glu Arg Phe Glu Ile Phe Pro Lys Thr Ser Ser Trp Pro Asn
145                 150                 155                 160

His Asp Ser Asn Lys Gly Val Thr Ala Ala Cys Pro His Ala Gly Ala
                165                 170                 175

Lys Ser Phe Tyr Lys Asn Leu Ile Trp Leu Val Lys Lys Gly Asn Ser
            180                 185                 190

Tyr Pro Lys Leu Ser Lys Ser Tyr Ile Asn Asp Lys Gly Lys Glu Val
        195                 200                 205

Leu Val Leu Trp Gly Ile His His Pro Ser Thr Ser Ala Asp Gln Gln
    210                 215                 220

Ser Leu Tyr Gln Asn Ala Asp Ala Tyr Val Phe Val Gly Ser Ser Arg
225                 230                 235                 240

Tyr Ser Lys Lys Phe Lys Pro Glu Ile Ala Ile Arg Pro Lys Val Arg
                245                 250                 255

Asp Gln Glu Gly Arg Met Asn Tyr Tyr Trp Thr Leu Val Glu Pro Gly
            260                 265                 270

Asp Lys Ile Thr Phe Glu Ala Thr Gly Asn Leu Val Val Pro Arg Tyr
        275                 280                 285

Ala Phe Ala Met Glu Arg Asn Ala Gly Ser Gly Ile Ile Ile Ser Asp
    290                 295                 300

Thr Pro Val His Asp Cys Asn Thr Thr Cys Gln Thr Pro Lys Gly Ala
305                 310                 315                 320

Ile Asn Thr Ser Leu Pro Phe Gln Asn Ile His Pro Ile Thr Ile Gly
                325                 330                 335

Lys Cys Pro Lys Tyr Val Lys Ser Thr Lys Leu Arg Leu Ala Thr Gly
            340                 345                 350
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Leu Arg Asn Val Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile
        355                 360                 365

Ala Gly Phe Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr
    370                 375                 380

Gly Tyr His His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Leu
385                 390                 395                 400

Lys Ser Thr Gln Asn Ala Ile Asp Glu Ile Thr Asn Lys Val Asn Ser
                405                 410                 415

Val Ile Glu Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe
            420                 425                 430

Asn His Leu Glu Lys Arg Ile Glu Asn Leu Asn Lys Lys Val Asp Asp
        435                 440                 445

Gly Phe Leu Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu
    450                 455                 460

Glu Asn Glu Arg Thr Leu Asp Tyr His Asp Ser Asn Val Lys Asn Leu
465                 470                 475                 480

Tyr Glu Lys Val Arg Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly
                485                 490                 495

Asn Gly Cys Phe Glu Phe Tyr His Lys Cys Asp Asn Thr Cys Met Glu
            500                 505                 510

Ser Val Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ala
        515                 520                 525

Lys Leu Asn Arg Glu Glu Ile Asp Gly Val Lys Leu Glu Ser Thr Arg
    530                 535                 540

Ile Tyr Gln Ile Leu Ala Ile Tyr Ser Thr Val Ala Ser Ser Leu Val
545                 550                 555                 560

Leu Val Val Ser Leu Gly Ala Ile Ser Phe Trp Met Cys Ser Asn Gly
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Ser Leu Gln Cys Arg Ile Cys Ile Tyr Pro Tyr Asp Val Pro Asp Tyr
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<212> DNA
<213> Artificial Sequence

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<223> pGX2009

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aatgacgtat gttcccatag taacgccaat agggactttc cattgacgtc aatgggtgga 240

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ccctattgac gtcaatgacg gtaaatggcc cgcctggcat tatgcccagt acatgacctt 360

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ggtctatata agcagagctc tctggctaac tagagaaccc actgcttact ggcttatcga 660

aattaatacg actcactata gggagaccca agctggctag cgtttaaact taagcttggt 720

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Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Lys Val Asp
20 25 30

Thr Ile Leu Glu Arg Asn Val Thr Val Thr His Ala Lys Asp Ile Leu
35 40 45

Glu Lys Thr His Asn Gly Lys Leu Cys Lys Leu Asn Gly Ile Pro Pro
50 55 60

Leu Glu Leu Gly Asp Cys Ser Ile Ala Gly Trp Leu Leu Gly Asn Pro
65 70 75 80

Glu Cys Asp Arg Leu Leu Ser Val Pro Glu Trp Ser Tyr Ile Met Glu
85 90 95

Lys Glu Asn Pro Arg Asp Gly Leu Cys Tyr Pro Gly Ser Phe Asn Asp
100 105 110

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Tyr Glu Glu Leu Lys His Leu Leu Ser Ser Val Lys His Phe Glu Lys
        115                 120                 125

Val Lys Ile Leu Pro Lys Asp Arg Trp Thr Gln His Thr Thr Thr Gly
    130                 135                 140

Gly Ser Arg Ala Cys Ala Val Ser Gly Asn Pro Ser Phe Phe Arg Asn
145                 150                 155                 160

Met Val Trp Leu Thr Lys Lys Gly Ser Asn Tyr Pro Val Ala Lys Gly
                165                 170                 175

Ser Tyr Asn Asn Thr Ser Gly Glu Gln Met Leu Ile Ile Trp Gly Val
            180                 185                 190

His His Pro Asn Asp Glu Thr Glu Gln Arg Thr Leu Tyr Gln Asn Val
        195                 200                 205

Gly Thr Tyr Val Ser Val Gly Thr Ser Thr Leu Asn Lys Arg Ser Thr
    210                 215                 220

Pro Glu Ile Ala Thr Arg Pro Lys Val Asn Gly Leu Gly Ser Arg Met
225                 230                 235                 240

Glu Phe Ser Trp Thr Leu Leu Asp Met Trp Asp Thr Ile Asn Phe Glu
                245                 250                 255

Ser Thr Gly Asn Leu Ile Ala Pro Glu Tyr Gly Phe Lys Ile Ser Lys
            260                 265                 270

Arg Gly Ser Ser Gly Ile Met Lys Thr Glu Gly Thr Leu Glu Asn Cys
        275                 280                 285

Glu Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Thr Thr Leu Pro
    290                 295                 300

Phe His Asn Val His Pro Leu Thr Ile Gly Glu Cys Pro Lys Tyr Val
305                 310                 315                 320

Lys Ser Glu Lys Leu Val Leu Ala Thr Gly Leu Arg Asn Val Pro Gln
                325                 330                 335

Ile Glu Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly
            340                 345                 350

Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His Ser Asn
        355                 360                 365

Asp Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala
    370                 375                 380
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Phe Asp Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu Lys Met Asn
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Thr Gln Phe Glu Ala Val Gly Lys Glu Phe Ser Asn Leu Glu Arg Arg
405 410 415

Leu Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp Val Trp
420 425 430

Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg Thr Leu
435 440 445

Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val Arg Met
450 455 460

Gln Leu Arg Asp Asn Val Lys Glu Leu Gly Asn Gly Cys Phe Glu Phe
465 470 475 480

Tyr His Lys Cys Asp Asp Glu Cys Met Asn Ser Val Lys Asn Gly Thr
485 490 495

Tyr Asp Tyr Pro Lys Tyr Glu Glu Glu Ser Lys Leu Asn Arg Asn Glu
500 505 510

Ile Lys Gly Val Lys Leu Ser Ser Met Gly Val Tyr Gln Ile Leu Ala
515 520 525

Ile Tyr Ala Thr Val Ala Gly Ser Leu Ser Leu Ala Ile Met Met Ala
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Gly Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile
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Cys Ile

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gaagacaata gcaggcatgc tggggatgcg gtgggctcta tggcttctac tgggcggttt 2700

tatggacagc aagcgaaccg gaattgccag ctggggcgcc ctctggtaag gttgggaagc 2760

cctgcaaagt aaactggatg gctttcttgc cgccaaggat ctgatggcgc aggggatcaa 2820

gctctgatca agagacagga tgaggatcgt ttcgcatgat tgaacaagat ggattgcacg 2880

caggttctcc ggccgcttgg gtggagaggc tattcggcta tgactgggca caacagacaa 2940

tcggctgctc tgatgccgcc gtgttccggc tgtcagcgca ggggcgcccg gttctttttg 3000

tcaagaccga cctgtccggt gccctgaatg aactgcaaga cgaggcagcg cggctatcgt 3060

ggctggccac gacgggcgtt ccttgcgcag ctgtgctcga cgttgtcact gaagcgggaa 3120

gggactggct gctattgggc gaagtgccgg ggcaggatct cctgtcatct caccttgctc 3180

ctgccgagaa agtatccatc atggctgatg caatgcggcg gctgcatacg cttgatccgg 3240

ctacctgccc attcgaccac caagcgaaac atcgcatcga gcgagcacgt actcggatgg 3300

aagccggtct tgtcgatcag gatgatctgg acgaagagca tcaggggctc gcgccagccg 3360

aactgttcgc caggctcaag gcgagcatgc ccgacggcga ggatctcgtc gtgacccatg 3420

gcgatgcctg cttgccgaat atcatggtgg aaaatggccg cttttctgga ttcatcgact 3480

gtggccggct gggtgtggcg gaccgctatc aggacatagc gttggctacc cgtgatattg 3540

ctgaagagct tggcggcgaa tgggctgacc gcttcctcgt gctttacggt atcgccgctc 3600

ccgattcgca gcgcatcgcc ttctatcgcc ttcttgacga gttcttctga attattaacg 3660

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atgaccaaaa tcccttaacg tgagttttcg ttccactgag cgtcagaccc cgtagaaaag 3960

atcaaaggat cttcttgaga tcctttttt ctgcgcgtaa tctgctgctt gcaaacaaaa 4020

aaaccaccgc taccagcggt ggtttgtttg ccggatcaag agctaccaac tctttttccg 4080

aaggtaactg gcttcagcag agcgcagata ccaaatactg ttcttctagt gtagccgtag 4140

ttaggccacc acttcaagaa ctctgtagca ccgcctacat acctcgctct gctaatcctg 4200

ttaccagtgg ctgctgccag tggcgataag tcgtgtctta ccgggttgga ctcaagacga 4260

tagttaccgg ataaggcgca gcggtcgggc tgaacggggg gttcgtgcac acagcccagc 4320

ttggagcgaa cgacctacac cgaactgaga tacctacagc gtgagctatg agaaagcgcc 4380

acgcttcccg aagggagaaa ggcggacagg tatccggtaa gcggcagggt cggaacagga 4440

gagcgcacga gggagcttcc agggggaaac gcctggtatc tttatagtcc tgtcgggttt 4500

cgccacctct gacttgagcg tcgatttttg tgatgctcgt caggggggcg gagcctatgg 4560

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atgttctt 4628

<210> 9
<211> 1695
<212> DNA
<213> Artificial Sequence

<220>
<223> Influenza U2 DNA sequence

<400> 9
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accgtgaccc acagcgtgaa cctgctggaa gataagcaca acggcaagct gtgcaagctg 180

aagggaatcg ccccctgca gctgggcaag tgcaatatcg ccggctggat tctgggcaac 240

cccgagtgcg agagcctgag cagcaagagc agctggtcct acatcgtgga aacccccaac 300

agcgagaacg gcacctgtta ccccggcgac ttcgccgact acgaggaact gcgcgagcag 360

ctgagcagcg tgtccagctt cgagagattc gagatcttcc ccaagaccag cagctggccc 420

aaccacgacg tgaccaaggg cgtgaccgct agctgtagcc acgcaggcgc cagcagcttc 480

tacaagaacc tgctgtggct gaccaagaag aacggcagct accccaagct gagcaagagc 540

tacatcaaca acaaagaaaa agaggtgctg gtcctctggg gcgtccacca ccccagcaca 600

atcgccgacc agcagagcct gtaccagaac gagaacgcct acgtgtccgt gggcagcagc 660

cactacagcc ggaagttcac ccccgagatc gccaagcggc ccaaagtgcg ggaccaggaa 720

ggccggatca actactactg gaccctgctg gaacccggcg acaccatcat cttcgaggcc 780

aacggcaacc tgatcgcccc cagatacgcc ttcgccctga gcagaggctt cggcagcggc 840

atcatcatca gcaacgcccc catgcacgac tgcgacacca agtgccagac ccctcagggc 900

gccatcaaca gcagcctgcc cttccagaac atccaccccg tgaccatcgg cgagtgcccc 960

aaatacgtgc ggagcaccaa gctgcggatg gccaccggcc tgcggaacat ccccagcatc 1020

cagagcagag gcctgttcgg cgccattgcc ggcttcatcg agggcggctg gaccggaatg 1080

gtggacgggt ggtacggcta ccaccaccag aatgagcagg gcagcggcta cgccgccgac 1140

cagaagtcca cccagaacgc catcgacggc atcaccaaca aagtgaacag cgtgatcgag 1200

aagatgaaca cccagttcac cgccgtgggc aaagagttca acaagctgga aaagcggatg 1260

gaaaacctga acaagaaggt ggacgacggc ttcctggaca tctggaccta caacgccgaa 1320

ctgctcgtgc tgctggaaaa cgagcggacc ctggacttcc acgacagcaa cgtgaagaac 1380

ctgtacgaga aagtgaagtc ccagctgaag aacaacgcca aagagatcgg caacggctgc 1440

ttcgagttct accacaagtg caacaacgag tgcatggaaa gcgtgaagaa cggaacctac 1500

gactacccca agtacagcga ggaaagcaag ctgaaccggg aagagatcga cggcgtgaag 1560

ctggaatcca tgggcgtgta ccagatcctg gccatctaca gcaccgtggc tagcagcctg 1620

gtgctgctgg tgtccctggg cgccatctcc ttttggatgt gctccaacgg cagcctgcag 1680

tgccggatct gcatc 1695

<210> 10
<211> 565
<212> PRT
<213> Artificial Sequence

<220>
<223> Influenza U2 amino acid sequence

<400> 10

Lys Ala Lys Leu Leu Val Leu Leu Cys Thr Phe Ala Ala Thr Asn Ala
1 5 10 15

Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr Val
20 25 30

Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn Leu
35 40 45

Leu Glu Asp Lys His Asn Gly Lys Leu Cys Lys Leu Lys Gly Ile Ala
50 55 60

Pro Leu Gln Leu Gly Lys Cys Asn Ile Ala Gly Trp Ile Leu Gly Asn
65 70 75 80

Pro Glu Cys Glu Ser Leu Ser Ser Lys Ser Ser Trp Ser Tyr Ile Val
85 90 95

Glu Thr Pro Asn Ser Glu Asn Gly Thr Cys Tyr Pro Gly Asp Phe Ala
100 105 110

Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe Glu
115 120 125

Arg Phe Glu Ile Phe Pro Lys Thr Ser Ser Trp Pro Asn His Asp Val
130 135 140

Thr Lys Gly Val Thr Ala Ser Cys Ser His Ala Gly Ala Ser Ser Phe
145 150 155 160

Tyr Lys Asn Leu Leu Trp Leu Thr Lys Lys Asn Gly Ser Tyr Pro Lys
165 170 175

Leu Ser Lys Ser Tyr Ile Asn Asn Lys Glu Lys Glu Val Leu Val Leu
180 185 190

```
Trp Gly Val His His Pro Ser Thr Ile Ala Asp Gln Gln Ser Leu Tyr
        195                 200                 205

Gln Asn Glu Asn Ala Tyr Val Ser Val Gly Ser Ser His Tyr Ser Arg
    210                 215                 220

Lys Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln Glu
225                 230                 235                 240

Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr Ile
                245                 250                 255

Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Arg Tyr Ala Phe Ala
            260                 265                 270

Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Ile Ser Asn Ala Pro Met
        275                 280                 285

His Asp Cys Asp Thr Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn Ser
    290                 295                 300

Ser Leu Pro Phe Gln Asn Ile His Pro Val Thr Ile Gly Glu Cys Pro
305                 310                 315                 320

Lys Tyr Val Arg Ser Thr Lys Leu Arg Met Ala Thr Gly Leu Arg Asn
                325                 330                 335

Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe
            340                 345                 350

Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr His
        355                 360                 365

His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr
    370                 375                 380

Gln Asn Ala Ile Asp Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu
385                 390                 395                 400

Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu
                405                 410                 415

Glu Lys Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu
            420                 425                 430

Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu
        435                 440                 445

Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys
    450                 455                 460
```

Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly Cys
465 470 475 480

Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val Lys
485 490 495

Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu Asn
500 505 510

Arg Glu Glu Ile Asp Gly Val Lys Leu Glu Ser Met Gly Val Tyr Gln
515 520 525

Ile Leu Ala Ile Tyr Ser Thr Val Ala Ser Ser Leu Val Leu Leu Val
530 535 540

Ser Leu Gly Ala Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln
545 550 555 560

Cys Arg Ile Cys Ile
565

<210> 11
<211> 1809
<212> DNA
<213> Artificial Sequence

<220>
<223> IgE-U2-HATAntigen DNA Sequence

<400> 11
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cgggtgcact ctaaggccaa gctgctggtg ctgctgtgca ccttcgccgc caccaacgcc 120

gacaccatct gcatcggcta ccacgccaac aacagcaccg acaccgtgga taccgtgctg 180

gaaaagaacg tgaccgtgac ccacagcgtg aacctgctgg aagataagca caacggcaag 240

ctgtgcaagc tgaagggaat cgccccctg cagctgggca agtgcaatat cgccggctgg 300

attctgggca accccgagtg cgagagcctg agcagcaaga gcagctggtc ctacatcgtg 360

gaaaccccca acagcgagaa cggcacctgt taccccggcg acttcgccga ctacgaggaa 420

ctgcgcgagc agctgagcag cgtgtccagc ttcgagagat tcgagatctt ccccaagacc 480

agcagctggc ccaaccacga cgtgaccaag ggcgtgaccg ctagctgtag ccacgcaggc 540

gccagcagct tctacaagaa cctgctgtgg ctgaccaaga agaacggcag ctaccccaag 600

ctgagcaaga gctacatcaa caacaaagaa aaagaggtgc tggtcctctg gggcgtccac 660

caccccagca caatcgccga ccagcagagc ctgtaccaga acgagaacgc ctacgtgtcc 720

gtgggcagca gccactacag ccggaagttc acccccgaga tcgccaagcg gcccaaagtg 780

cgggaccagg aaggccggat caactactac tggaccctgc tggaacccgg cgacaccatc 840

atcttcgagg ccaacggcaa cctgatcgcc cccagatacg ccttcgccct gagcagaggc 900

ttcggcagcg gcatcatcat cagcaacgcc cccatgcacg actgcgacac caagtgccag 960

acccctcagg gcgccatcaa cagcagcctg cccttccaga acatccaccc cgtgaccatc 1020

ggcgagtgcc ccaaatacgt gcggagcacc aagctgcgga tggccaccgg cctgcggaac 1080

atccccagca tccagagcag aggcctgttc ggcgccattg ccggcttcat cgagggcggc 1140

tggaccggaa tggtggacgg gtggtacggc taccaccacc agaatgagca gggcagcggc 1200

tacgccgccg accagaagtc cacccagaac gccatcgacg gcatcaccaa caaagtgaac 1260

agcgtgatcg agaagatgaa cacccagttc accgccgtgg gcaaagagtt caacaagctg 1320

gaaaagcgga tggaaaacct gaacaagaag gtggacgacg gcttcctgga catctggacc 1380

tacaacgccg aactgctcgt gctgctggaa aacgagcgga ccctggactt ccacgacagc 1440

aacgtgaaga acctgtacga gaaagtgaag tcccagctga agaacaacgc caaagagatc 1500

ggcaacggct gcttcgagtt ctaccacaag tgcaacaacg agtgcatgga aagcgtgaag 1560

aacggaacct acgactaccc caagtacagc gaggaaagca agctgaaccg ggaagagatc 1620

gacggcgtga agctggaatc catgggcgtg taccagatcc tggccatcta cagcaccgtg 1680

gctagcagcc tggtgctgct ggtgtccctg ggcgccatct ccttttggat gtgctccaac 1740

ggcagcctgc agtgccggat ctgcatctac ccctacgacg tgcccgacta cgcctgatga 1800

ctcgagctc 1809

<210> 12
<211> 592
<212> PRT
<213> Artificial Sequence

<220>
<223> IgE-U2-HATantigen amino acid Sequence

<400> 12

Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val
1 5 10 15

His Ser Lys Ala Lys Leu Leu Val Leu Leu Cys Thr Phe Ala Ala Thr
20 25 30

Asn Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp
35 40 45

Thr Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val
50 55 60

Asn Leu Leu Glu Asp Lys His Asn Gly Lys Leu Cys Lys Leu Lys Gly
65 70 75 80

Ile Ala Pro Leu Gln Leu Gly Lys Cys Asn Ile Ala Gly Trp Ile Leu
85 90 95

Gly Asn Pro Glu Cys Glu Ser Leu Ser Ser Lys Ser Ser Trp Ser Tyr
100 105 110

```
Ile Val Glu Thr Pro Asn Ser Glu Asn Gly Thr Cys Tyr Pro Gly Asp
        115                 120                 125

Phe Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser
    130                 135                 140

Phe Glu Arg Phe Glu Ile Phe Pro Lys Thr Ser Ser Trp Pro Asn His
145                 150                 155                 160

Asp Val Thr Lys Gly Val Thr Ala Ser Cys Ser His Ala Gly Ala Ser
                165                 170                 175

Ser Phe Tyr Lys Asn Leu Leu Trp Leu Thr Lys Lys Asn Gly Ser Tyr
            180                 185                 190

Pro Lys Leu Ser Lys Ser Tyr Ile Asn Asn Lys Glu Lys Glu Val Leu
        195                 200                 205

Val Leu Trp Gly Val His His Pro Ser Thr Ile Ala Asp Gln Gln Ser
    210                 215                 220

Leu Tyr Gln Asn Glu Asn Ala Tyr Val Ser Val Gly Ser Ser His Tyr
225                 230                 235                 240

Ser Arg Lys Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp
                245                 250                 255

Gln Glu Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp
            260                 265                 270

Thr Ile Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Arg Tyr Ala
        275                 280                 285

Phe Ala Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Ile Ser Asn Ala
    290                 295                 300

Pro Met His Asp Cys Asp Thr Lys Cys Gln Thr Pro Gln Gly Ala Ile
305                 310                 315                 320

Asn Ser Ser Leu Pro Phe Gln Asn Ile His Pro Val Thr Ile Gly Glu
                325                 330                 335

Cys Pro Lys Tyr Val Arg Ser Thr Lys Leu Arg Met Ala Thr Gly Leu
            340                 345                 350

Arg Asn Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala
        355                 360                 365

Gly Phe Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly
    370                 375                 380
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Tyr His His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys
385 390 395 400

Ser Thr Gln Asn Ala Ile Asp Gly Ile Thr Asn Lys Val Asn Ser Val
405 410 415

Ile Glu Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn
420 425 430

Lys Leu Glu Lys Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly
435 440 445

Phe Leu Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu
450 455 460

Asn Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr
465 470 475 480

Glu Lys Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn
485 490 495

Gly Cys Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser
500 505 510

Val Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys
515 520 525

Leu Asn Arg Glu Glu Ile Asp Gly Val Lys Leu Glu Ser Met Gly Val
530 535 540

Tyr Gln Ile Leu Ala Ile Tyr Ser Thr Val Ala Ser Ser Leu Val Leu
545 550 555 560

Leu Val Ser Leu Gly Ala Ile Ser Phe Trp Met Cys Ser Asn Gly Ser
565 570 575

Leu Gln Cys Arg Ile Cys Ile Tyr Pro Tyr Asp Val Pro Asp Tyr Ala
580 585 590

<210> 13
<211> 1749
<212> DNA
<213> Artificial Sequence

<220>
<223> BHA DNA Sequence

<400> 13
aaggccatca tcgtgctgct gatggtggtc acaagcaacg ccgaccggat ctgcaccggc 60

atcaccagca gcaacagccc ccacgtggtc aaaaccgcca cccagggcga agtgaacgtg 120

accggcgtga tccccctgac caccaccccc accaagagcc acttcgccaa cctgaagggc 180

accaagaccc ggggaaagct gtgccccaag tgcctgaact gcaccgacct ggacgtggcc 240

ctgggcagac ctatgtgcgt gggcaccacc cctagcgcca aggccagcat cctgcacgaa 300

gtgcggcccg tgaccagcgg ctgcttcccc atcatgcacg accggaccaa gatccggcag 360

ctccccaacc tgctgcgggg ctacgagaac atccggctga gcacccagaa cgtgatcaac 420

gccgagaagg cccctggcgg cccttacaga ctgggcacaa gcggctcttg ccccaacgcc 480

accagcaaga gcggcttttt cgccacaatg gcctgggccg tgcccaagga caacaacaag 540

accgccacca acccccctgac cgtggaagtg ccctacatct gcaccgaggg cgaggaccag 600

atcaccgtgt ggggcttcca cagcgataac aagacccaga tgaagaacct gtacggcgac 660

agcaaccccc agaagttcac cagctccgcc aacggcgtga ccacccacta cgtgtcccag 720

atcggcggct tccccgacca gacagaggat ggcggcctgc cccagagcgg cagaatcgtg 780

gtggactaca tggtgcagaa gcccggcaag accggcacca tcgtgtacca gcggggcatc 840

ctgctgcccc agaaagtgtg gtgcgccagc ggccggtcca aagtgatcaa gggcagcctg 900

cctctgatcg gcgaggccga ttgcctgcac gagaagtacg gcggcctgaa caagagcaag 960

ccctactaca ccggcgagca cgccaaagcc atcggcaact gccccatctg ggtcaaaacc 1020

cccctgaagc tggccaacgg caccaagtac cggcctcccg ccaagctgct gaaagagcgg 1080

ggcttcttcg gcgctatcgc cggctttctg gaaggcggct gggagggcat gatcgccggc 1140

tggcacggct acacatctca cggcgctcat ggcgtggccg tggccgctga tctgaagtcc 1200

acccaggaag ccatcaacaa gatcaccaag aacctgaaca gcctgagcga gctggaagtg 1260

aagaatctgc agcggctgag cggcgccatg gacgagctgc acaacgagat cctggaactg 1320

gacgagaagg tggacgacct gcgggccgac accatctcca gccagatcga gctggccgtg 1380

ctgctgtcca acgagggcat catcaacagc gaggacgagc atctgctggc cctggaacgg 1440

aagctgaaga agatgctggg ccctagcgcc gtggacatcg gcaacggctg cttcgagaca 1500

aagcacaagt gcaaccagac ctgcctggac cggatcgctg ccggcacctt caacgccggc 1560

gagttcagcc tgcccacctt cgacagcctg aacatcaccg ccgccagcct gaacgacgac 1620

ggcctggaca accacaccat cctgctgtac tacagcaccg cagcctccag cctggccgtg 1680

accctgatga tcgccatctt catcgtgtac atggtgtctc gggacaacgt gtcctgcagc 1740

atctgcctg 1749

<210> 14
<211> 583
<212> PRT
<213> Artificial Sequence

<220>
<223> BHA Amino Acid Sequence

<400> 14

Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp Arg
1 5 10 15

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Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys Thr
            20                  25                  30

Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu Thr Thr
        35                  40                  45

Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Lys Thr Arg
    50                  55                  60

Gly Lys Leu Cys Pro Lys Cys Leu Asn Cys Thr Asp Leu Asp Val Ala
65                  70                  75                  80

Leu Gly Arg Pro Met Cys Val Gly Thr Thr Pro Ser Ala Lys Ala Ser
                85                  90                  95

Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro Ile Met
            100                 105                 110

His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg Gly Tyr
        115                 120                 125

Glu Asn Ile Arg Leu Ser Thr Gln Asn Val Ile Asn Ala Glu Lys Ala
    130                 135                 140

Pro Gly Gly Pro Tyr Arg Leu Gly Thr Ser Gly Ser Cys Pro Asn Ala
145                 150                 155                 160

Thr Ser Lys Ser Gly Phe Phe Ala Thr Met Ala Trp Ala Val Pro Lys
                165                 170                 175

Asp Asn Asn Lys Thr Ala Thr Asn Pro Leu Thr Val Glu Val Pro Tyr
            180                 185                 190

Ile Cys Thr Glu Gly Glu Asp Gln Ile Thr Val Trp Gly Phe His Ser
        195                 200                 205

Asp Asn Lys Thr Gln Met Lys Asn Leu Tyr Gly Asp Ser Asn Pro Gln
    210                 215                 220

Lys Phe Thr Ser Ser Ala Asn Gly Val Thr Thr His Tyr Val Ser Gln
225                 230                 235                 240

Ile Gly Gly Phe Pro Asp Gln Thr Glu Asp Gly Gly Leu Pro Gln Ser
                245                 250                 255

Gly Arg Ile Val Val Asp Tyr Met Val Gln Lys Pro Gly Lys Thr Gly
            260                 265                 270

Thr Ile Val Tyr Gln Arg Gly Ile Leu Leu Pro Gln Lys Val Trp Cys
        275                 280                 285
```

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Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro Leu Ile Gly
    290                 295                 300

Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn Lys Ser Lys
305                 310                 315                 320

Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn Cys Pro Ile
                325                 330                 335

Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro
            340                 345                 350

Pro Ala Lys Leu Leu Lys Glu Arg Gly Phe Phe Gly Ala Ile Ala Gly
        355                 360                 365

Phe Leu Glu Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His Gly Tyr
    370                 375                 380

Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu Lys Ser
385                 390                 395                 400

Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu Ser
                405                 410                 415

Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala Met Asp Glu
            420                 425                 430

Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp Leu Arg
        435                 440                 445

Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu Ser Asn
    450                 455                 460

Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu Glu Arg
465                 470                 475                 480

Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Asp Ile Gly Asn Gly
                485                 490                 495

Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp Arg Ile
            500                 505                 510

Ala Ala Gly Thr Phe Asn Ala Gly Glu Phe Ser Leu Pro Thr Phe Asp
        515                 520                 525

Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu Asp Asn
    530                 535                 540

His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu Ala Val
545                 550                 555                 560
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Thr Leu Met Ile Ala Ile Phe Ile Val Tyr Met Val Ser Arg Asp Asn
565 570 575

Val Ser Cys Ser Ile Cys Leu
580

<210> 15
<211> 1865
<212> DNA
<213> Artificial Sequence

<220>
<223> IgE-BHA-HATantigen DNA Sequence

<400> 15
ggtaccggat ccgccaccat ggactggacc tggattctgt tcctggtggc cgctgccaca 60

cgggtgcaca gcaaggccat catcgtgctg ctgatggtgg tcacaagcaa cgccgaccgg 120

atctgcaccg gcatcaccag cagcaacagc ccccacgtgg tcaaaaccgc cacccagggc 180

gaagtgaacg tgaccggcgt gatccccctg accaccaccc ccaccaagag ccacttcgcc 240

aacctgaagg gcaccaagac ccggggaaag ctgtgcccca agtgcctgaa ctgcaccgac 300

ctggacgtgg ccctgggcag acctatgtgc gtgggcacca cccctagcgc caaggccagc 360

atcctgcacg aagtgcggcc cgtgaccagc ggctgcttcc ccatcatgca cgaccggacc 420

aagatccggc agctccccaa cctgctgcgg ggctacgaga acatccggct gagcacccag 480

aacgtgatca acgccgagaa ggcccctggc ggcccttaca gactgggcac aagcggctct 540

tgccccaacg ccaccagcaa gagcggcttt ttcgccacaa tggcctgggc cgtgcccaag 600

gacaacaaca agaccgccac caacccccctg accgtggaag tgccctacat ctgcaccgag 660

ggcgaggacc agatcaccgt gtggggcttc cacagcgata acaagaccca gatgaagaac 720

ctgtacggcg acagcaaccc ccagaagttc accagctccg ccaacggcgt gaccacccac 780

tacgtgtccc agatcggcgg cttccccgac cagacagagg atggcggcct gccccagagc 840

ggcagaatcg tggtggacta catggtgcag aagcccggca agaccggcac catcgtgtac 900

cagcggggca tcctgctgcc ccagaaagtg tggtgcgcca gcggccggtc caaagtgatc 960

aagggcagcc tgcctctgat cggcgaggcc gattgcctgc acgagaagta cggcggcctg 1020

aacaagagca agccctacta caccggcgag cacgccaaag ccatcggcaa ctgccccatc 1080

tgggtcaaaa ccccctgaa gctggccaac ggcaccaagt accggcctcc cgccaagctg 1140

ctgaaagagc ggggcttctt cggcgctatc gccggctttc tggaaggcgg ctgggagggc 1200

atgatcgccg gctggcacgg ctacacatct cacggcgctc atggcgtggc cgtggccgct 1260

gatctgaagt ccacccagga agccatcaac aagatcacca agaacctgaa cagcctgagc 1320

gagctggaag tgaagaatct gcagcggctg agcggcgcca tggacgagct gcacaacgag 1380

atcctggaac tggacgagaa ggtggacgac ctgcgggccg acaccatctc cagccagatc 1440

gagctggccg tgctgctgtc caacgagggc atcatcaaca gcgaggacga gcatctgctg 1500

gccctggaac ggaagctgaa gaagatgctg ggccctagcg ccgtggacat cggcaacggc 1560

tgcttcgaga caaagcacaa gtgcaaccag acctgcctgg accggatcgc tgccggcacc 1620

ttcaacgccg gcgagttcag cctgcccacc ttcgacagcc tgaacatcac cgccgccagc 1680

ctgaacgacg acggcctgga caaccacacc atcctgctgt actacagcac cgcagcctcc 1740

agcctggccg tgaccctgat gatcgccatc ttcatcgtgt acatggtgtc tcgggacaac 1800

gtgtcctgca gcatctgcct gtaccctac gacgtgcccg actacgctga tgactcgagc 1860

tcctc 1865

<210> 16
<211> 610
<212> PRT
<213> Artificial Sequence

<220>
<223> IgE-BHA-HATantigen Amino Acid Sequence

<400> 16

Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val
1 5 10 15

His Ser Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala
20 25 30

Asp Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val
35 40 45

Lys Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu
50 55 60

Thr Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Lys
65 70 75 80

Thr Arg Gly Lys Leu Cys Pro Lys Cys Leu Asn Cys Thr Asp Leu Asp
85 90 95

Val Ala Leu Gly Arg Pro Met Cys Val Gly Thr Thr Pro Ser Ala Lys
100 105 110

Ala Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro
115 120 125

Ile Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg
130 135 140

Gly Tyr Glu Asn Ile Arg Leu Ser Thr Gln Asn Val Ile Asn Ala Glu
145 150 155 160

Lys Ala Pro Gly Gly Pro Tyr Arg Leu Gly Thr Ser Gly Ser Cys Pro
165 170 175

```
Asn Ala Thr Ser Lys Ser Gly Phe Phe Ala Thr Met Ala Trp Ala Val
            180                 185                 190

Pro Lys Asp Asn Asn Lys Thr Ala Thr Asn Pro Leu Thr Val Glu Val
        195                 200                 205

Pro Tyr Ile Cys Thr Glu Gly Glu Asp Gln Ile Thr Val Trp Gly Phe
    210                 215                 220

His Ser Asp Asn Lys Thr Gln Met Lys Asn Leu Tyr Gly Asp Ser Asn
225                 230                 235                 240

Pro Gln Lys Phe Thr Ser Ser Ala Asn Gly Val Thr Thr His Tyr Val
                245                 250                 255

Ser Gln Ile Gly Gly Phe Pro Asp Gln Thr Glu Asp Gly Gly Leu Pro
            260                 265                 270

Gln Ser Gly Arg Ile Val Val Asp Tyr Met Val Gln Lys Pro Gly Lys
        275                 280                 285

Thr Gly Thr Ile Val Tyr Gln Arg Gly Ile Leu Leu Pro Gln Lys Val
    290                 295                 300

Trp Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro Leu
305                 310                 315                 320

Ile Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn Lys
                325                 330                 335

Ser Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn Cys
            340                 345                 350

Pro Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr
        355                 360                 365

Arg Pro Pro Ala Lys Leu Leu Lys Glu Arg Gly Phe Phe Gly Ala Ile
    370                 375                 380

Ala Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His
385                 390                 395                 400

Gly Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu
                405                 410                 415

Lys Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser
            420                 425                 430

Leu Ser Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala Met
        435                 440                 445
```

```
Asp Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp
    450                 455                 460


Leu Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu
465                 470                 475                 480


Ser Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu
                485                 490                 495


Glu Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Asp Ile Gly
            500                 505                 510


Asn Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp
        515                 520                 525


Arg Ile Ala Ala Gly Thr Phe Asn Ala Gly Glu Phe Ser Leu Pro Thr
    530                 535                 540


Phe Asp Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu
545                 550                 555                 560


Asp Asn His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu
                565                 570                 575


Ala Val Thr Leu Met Ile Ala Ile Phe Ile Val Tyr Met Val Ser Arg
            580                 585                 590


Asp Asn Val Ser Cys Ser Ile Cys Leu Tyr Pro Tyr Asp Val Pro Asp
        595                 600                 605


Tyr Ala
    610


<210>  17
<211>  18
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  IgE Leader Amino Acid Sequence

<400>  17

Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val
1               5                   10                  15


His Ser



<210>  18
<211>  9
<212>  PRT
<213>  Artificial Sequence
```

<220>
<223> HA tag amino acid sequence

<400> 18

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala
1 5

<210> 19
<211> 1707
<212> DNA
<213> Artificial Sequence

<220>
<223> Influenza H1 DNA sequence

<400> 19
atggaaaaga tcgtgctgct gttcgccatc gtgagcctgg tgaagagcga ccagatctgc 60

atcggctacc acgccaacaa cagcaccgag caggtggaca ccatcatgga aaaaaacgtg 120

accgtgaccc acgcccagga catcctggaa aagacccaca acggcaagct gtgcgacctg 180

gacggcgtga agcccctgat cctgcgggac tgcagcgtgg ccggctggct gctgggcaac 240

cccatgtgcg acgagttcat caacgtgccc gagtggagct acatcgtgga gaaggccaac 300

cccgtgaacg acctgtgcta ccccggcgac ttcaacgact acgaggaact gaagcacctg 360

ctgtcccgga tcaaccactt cgagaagatc cagatcatcc ccaagagcag ctggtccagc 420

cacgaggcca gcctgggcgt gagcagcgcc tgcccatacc agggcaagtc cagcttcttc 480

cggaacgtgg tgtggctgat caagaagaac agcacctacc ccaccatcaa gcggagctac 540

aacaacacca accaggaaga tctgctggtc ctgtggggca tccaccaccc caacgacgcc 600

gccgagcaga ccaagctgta ccagaacccc accacctaca tcagcgtggg caccagcacc 660

ctgaaccagc ggctggtgcc ccggatcgcc acccggtcca aggtgaacgg ccagagcggc 720

cggatggaat tcttctggac catcctgaag cccaacgatg ccatcaactt cgagagcaac 780

ggcaacttca tcgcccccga gtacgcctac aagatcgtga agaagggcga cagcaccatc 840

atgaagagcg agctggaata cggcaactgc aacaccaagt gccagacccc catgggcgcc 900

atcaacagca gcatgccctt ccacaacatc cacccccctga ccatcggcga gtgccccaag 960

tacgtgaaga gcaacaggct ggtgctggcc accggcctgc ggaacagccc ccagcgggag 1020

cggcgggccg ccgcccgggg cctgttcggc gccatcgccg gcttcatcga gggcggctgg 1080

cagggcatgg tggacgggtg gtacggctac caccacagca atgagcaggg cagcggctac 1140

gccgccgaca aagagagcac ccagaaggcc atcgacggcg tcaccaacaa ggtgaacagc 1200

atcatcgaca agatgaacac ccagttcgag gccgtgggcc gggagttcaa caacctggaa 1260

cggcggatcg agaacctgaa caagaaaatg gaagatggct tcctggacgt gtggacctac 1320

aacgccgagc tgctggtgct gatggaaaac gagcggaccc tggacttcca cgacagcaac 1380

gtgaagaacc tgtacgacaa agtgcggctg cagctgcggg acaacgccaa agagctgggc 1440

aacggctgct tcgagttcta ccacaagtgc gacaacgagt gcatggaaag cgtgcggaac 1500

ggcacctacg actaccccca gtacagcgag gaagcccggc tgaagcggga ggaaatcagc 1560

ggcgtgaaac tggaaagcat cggcatctac cagatcctga gcatctacag caccgtggcc 1620

agcagcctgg ccctggccat catggtggcc ggcctgagcc tgtggatgtg cagcaacggc 1680

agcctgcagt gccggatctg catctag 1707

<210> 20
<211> 568
<212> PRT
<213> Artificial Sequence

<220>
<223> Influenza Protein H1 Sequence

<400> 20

Met Glu Lys Ile Val Leu Leu Phe Ala Ile Val Ser Leu Val Lys Ser
1 5 10 15

Asp Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Gln Val
20 25 30

Asp Thr Ile Met Glu Lys Asn Val Thr Val Thr His Ala Gln Asp Ile
35 40 45

Leu Glu Lys Thr His Asn Gly Lys Leu Cys Asp Leu Asp Gly Val Lys
50 55 60

Pro Leu Ile Leu Arg Asp Cys Ser Val Ala Gly Trp Leu Leu Gly Asn
65 70 75 80

Pro Met Cys Asp Glu Phe Ile Asn Val Pro Glu Trp Ser Tyr Ile Val
85 90 95

Glu Lys Ala Asn Pro Val Asn Asp Leu Cys Tyr Pro Gly Asp Phe Asn
100 105 110

Asp Tyr Glu Glu Leu Lys His Leu Leu Ser Arg Ile Asn His Phe Glu
115 120 125

Lys Ile Gln Ile Ile Pro Lys Ser Ser Trp Ser Ser His Glu Ala Ser
130 135 140

Leu Gly Val Ser Ser Ala Cys Pro Tyr Gln Gly Lys Ser Ser Phe Phe
145 150 155 160

Arg Asn Val Val Trp Leu Ile Lys Lys Asn Ser Thr Tyr Pro Thr Ile
165 170 175

Lys Arg Ser Tyr Asn Asn Thr Asn Gln Glu Asp Leu Leu Val Leu Trp
180 185 190

Gly Ile His His Pro Asn Asp Ala Ala Glu Gln Thr Lys Leu Tyr Gln

195 200 205

Asn Pro Thr Thr Tyr Ile Ser Val Gly Thr Ser Thr Leu Asn Gln Arg
210 215 220

Leu Val Pro Arg Ile Ala Thr Arg Ser Lys Val Asn Gly Gln Ser Gly
225 230 235 240

Arg Met Glu Phe Phe Trp Thr Ile Leu Lys Pro Asn Asp Ala Ile Asn
245 250 255

Phe Glu Ser Asn Gly Asn Phe Ile Ala Pro Glu Tyr Ala Tyr Lys Ile
260 265 270

Val Lys Lys Gly Asp Ser Thr Ile Met Lys Ser Glu Leu Glu Tyr Gly
275 280 285

Asn Cys Asn Thr Lys Cys Gln Thr Pro Met Gly Ala Ile Asn Ser Ser
290 295 300

Met Pro Phe His Asn Ile His Pro Leu Thr Ile Gly Glu Cys Pro Lys
305 310 315 320

Tyr Val Lys Ser Asn Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Ser
325 330 335

Pro Gln Arg Glu Arg Arg Ala Ala Ala Arg Gly Leu Phe Gly Ala Ile
340 345 350

Ala Gly Phe Ile Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr
355 360 365

Gly Tyr His His Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Lys
370 375 380

Glu Ser Thr Gln Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser
385 390 395 400

Ile Ile Asp Lys Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe
405 410 415

Asn Asn Leu Glu Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp
420 425 430

Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met
435 440 445

Glu Asn Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu
450 455 460

Tyr Asp Lys Val Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly

465 470 475 480

Asn Gly Cys Phe Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu
485 490 495

Ser Val Arg Asn Gly Thr Tyr Asp Tyr Pro Gln Tyr Ser Glu Glu Ala
500 505 510

Arg Leu Lys Arg Glu Glu Ile Ser Gly Val Lys Leu Glu Ser Ile Gly
515 520 525

Ile Tyr Gln Ile Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala
530 535 540

Leu Ala Ile Met Val Ala Gly Leu Ser Leu Trp Met Cys Ser Asn Gly
545 550 555 560

Ser Leu Gln Cys Arg Ile Cys Ile
565

<210> 21
<211> 1728
<212> DNA
<213> Artificial Sequence

<220>
<223> Influenza DNA H1 Sequence

<400> 21
ggtaccaagc ttgccaccat gaaggtgaaa ctgctggtgc tgctgtgcac cttcaccgcc 60

acctacgccg acaccatctg catcggctac cacgccaaca acagcaccga caccgtggat 120

accgtgctgg aaaagaacgt gaccgtgacc cacagcgtga acctgctgga agatagccac 180

aacggcaagc tgtgcctgct gaaaggcatc gccccctgc agctgggcaa ctgcagcgtg 240

gccggctgga tcctgggcaa ccccgagtgc gagctgctga tttccaaaga aagctggtcc 300

tacatcgtgg agacccccaa ccccgagaac ggcacctgct accccggcta cttcgccgac 360

tacgaggaac tgcgggagca gctgtccagc gtgagcagct tcgagcggtt cgagatcttc 420

cccaaagaga gcagctggcc caaccacacc gtgaccggcg tgagcgccag ctgctcccac 480

aatggcaaga gcagcttcta ccggaacctg ctgtggctga ccggcaagaa cggcctgtac 540

cccaacctga gcaagagcta cgccaataac aaagaaaagg aagtgctggt gctgtggggc 600

gtgcaccacc cccccaacat cggcgaccag cgggccctgt accacaccga gaacgcctac 660

gtgagcgtgg tgtccagcca ctacagccgg cggttcaccc ccgagatcgc caagcggccc 720

aaagtgcggg accaggaagg ccggatcaac tactactgga ccctgctgga acccggcgac 780

accatcatct tcgaggccaa cggcaacctg atcgccccca gatacgcctt cgccctgagc 840

cggggcttcg gcagcggcat catcaccagc aacgccccca tggacgagtg cgacgccaag 900

tgccagaccc ctcagggagc tattaacagc agcctgccct tccagaacgt gcaccccgtg 960

accatcggcg agtgccccaa gtacgtgcgg agcgccaagc tgcggatggt gaccggcctg 1020

cggaacatcc ccagcatcca gagcaggggc ctgttcggcg ccatcgccgg cttcatcgag 1080

ggcggctgga ccggcatggt ggacgggtgg tacggctacc accaccagaa cgagcagggc 1140

agcggctacg ccgccgacca gaagagcacc cagaacgcca tcaacggcat caccaacaag 1200

gtgaacagcg tgatcgagaa gatgaacacc cagttcaccg ccgtgggcaa agagttcaac 1260

aagctggaac ggcggatgga aaacctgaac aagaaggtgg acgacggctt cctggacatc 1320

tggacctaca acgccgagct gctggtgctg ctggaaaacg agcggaccct ggacttccac 1380

gacagcaacg tgaagaacct gtacgagaag gtgaaaagcc agctgaagaa caacgccaaa 1440

gagatcggca acggctgctt cgagttctac cacaagtgca acgacgagtg catggaaagc 1500

gtgaagaatg gcacctacga ctaccccaag tacagcgagg aaagcaagct gaaccgggag 1560

aagatcgacg gcgtgaagct ggaaagcatg ggcgtgtacc agatcctggc catctacagc 1620

accgtcgctt ccagcctcgt cctgctcgtg tccctgggcg ccatctcctt ttggatgtgc 1680

agcaacggca gcctgcagtg ccggatctgc atctgatgac tcgagctc 1728

<210> 22
<211> 565
<212> PRT
<213> Artificial Sequence

<220>
<223> Influenza Protein H1 Sequence

<400> 22

Met Lys Val Lys Leu Leu Val Leu Leu Cys Thr Phe Thr Ala Thr Tyr
1 5 10 15

Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr
20 25 30

Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn
35 40 45

Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Leu Leu Lys Gly Ile
50 55 60

Ala Pro Leu Gln Leu Gly Asn Cys Ser Val Ala Gly Trp Ile Leu Gly
65 70 75 80

Asn Pro Glu Cys Glu Leu Leu Ile Ser Lys Glu Ser Trp Ser Tyr Ile
85 90 95

Val Glu Thr Pro Asn Pro Glu Asn Gly Thr Cys Tyr Pro Gly Tyr Phe
100 105 110

Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe
115 120 125

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Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Thr
    130                 135                 140

Val Thr Gly Val Ser Ala Ser Cys Ser His Asn Gly Lys Ser Ser Phe
145                 150                 155                 160

Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro Asn
                165                 170                 175

Leu Ser Lys Ser Tyr Ala Asn Asn Lys Glu Lys Glu Val Leu Val Leu
            180                 185                 190

Trp Gly Val His His Pro Pro Asn Ile Gly Asp Gln Arg Ala Leu Tyr
        195                 200                 205

His Thr Glu Asn Ala Tyr Val Ser Val Val Ser Ser His Tyr Ser Arg
    210                 215                 220

Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln Glu
225                 230                 235                 240

Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr Ile
                245                 250                 255

Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Arg Tyr Ala Phe Ala
            260                 265                 270

Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Thr Ser Asn Ala Pro Met
        275                 280                 285

Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn Ser
    290                 295                 300

Ser Leu Pro Phe Gln Asn Val His Pro Val Thr Ile Gly Glu Cys Pro
305                 310                 315                 320

Lys Tyr Val Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn
                325                 330                 335

Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe
            340                 345                 350

Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr His
        355                 360                 365

His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr
    370                 375                 380

Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu
385                 390                 395                 400
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Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu
                405                 410                 415

Glu Arg Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu
            420                 425                 430

Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu
        435                 440                 445

Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys
    450                 455                 460

Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly Cys
465                 470                 475                 480

Phe Glu Phe Tyr His Lys Cys Asn Asp Glu Cys Met Glu Ser Val Lys
                485                 490                 495

Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu Asn
            500                 505                 510

Arg Glu Lys Ile Asp Gly Val Lys Leu Glu Ser Met Gly Val Tyr Gln
        515                 520                 525

Ile Leu Ala Ile Tyr Ser Thr Val Ala Ser Ser Leu Val Leu Leu Val
    530                 535                 540

Ser Leu Gly Ala Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln
545                 550                 555                 560

Cys Arg Ile Cys Ile
                565
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<210> 23
<211> 1731
<212> DNA
<213> Artificial Sequence

<220>
<223> Influenza DNA H3 Sequence

<400> 23

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ggtaccaagc ttgccaccat gaaaaccatc atcgccctga gctacatcct gtgcctggtg        60

ttcgcccaga agctgcccgg caacgacaac agcaccgcca ccctgtgtct gggccaccac       120

gccgtgccca acggcaccat cgtgaaaaca atcaccaacg accagatcga ggtgaccaac       180

gccaccgagc tggtgcagag cagcagcacc ggcggcatct gcgacagccc ccaccagatc       240

ctggacggcg agaactgcac cctgatcgac gccctgctgg gcgaccctca gtgcgacggc       300

ttccagaaca aaaagtggga cctgttcgtg gagcggagca aggcctacag caactgctac       360

ccctacgacg tgcccgacta cgccagcctg cggagcctgg tggccagcag cggcaccctg       420
```

gaattcaaca acgagagctt caactggacc ggcgtgaccc agaacggcac cagcagcgcc 480

tgcaagcggc ggagcaacaa cagcttcttt tccagactga actggctgac ccacctgaag 540

ttcaagtacc ccgccctgaa cgtgaccatg cccaacaatg agaagttcga caagctgtac 600

atctggggcg tgcaccaccc cggcaccgac aatgaccaga tcagcctgta cgcccaggcc 660

agcggccgga tcaccgtgag caccaagcgg agccagcaga ccgtgatccc caacatcggc 720

agccggccca gagtgagaga catccccagc cggatcagca tctactggac aatcgtgaag 780

cccggcgaca tcctgctgat caactccacc ggcaacctga tcgcccccag gggctacttc 840

aagatcagaa gcggcaagag cagcatcatg cggagcgacg cccccatcgg caagtgcaac 900

agcgagtgca tcacccccaa tggcagcatc cccaacgaca agcccttcca gaacgtgaac 960

cggatcacct acggcgcctg ccccagatac gtgaagcaga acaccctgaa gctggccacc 1020

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gagggcatcg gccaggccgc cgacctgaag agcacccagg ccgccatcaa ccagatcaac 1200

ggcaagctga accggctgat cggcaagacc aacgagaagt tccaccagat cgaaaaagaa 1260

ttcagcgagg tggagggccg gatccaggac ctggaaaagt acgtggagga caccaagatc 1320

gacctgtgga gctacaacgc cgagctgctg gtcgccctgg aaaaccagca caccatcgac 1380

ctgaccgaca gcgagatgaa caagctgttc gagcggacca agaagcagct gcgggagaac 1440

gccgaggaca tgggcaacgg ctgctttaag atctaccaca agtgcgacaa cgcctgcatc 1500

ggcagcatcc ggaacggcac ctacgaccac gacgtgtacc gggacgaggc cctgaacaac 1560

cggttccaga tcaagggcgt ggagctgaag agcggctaca aggactggat cctgtggatc 1620

agcttcgcca tcagctgctt tctgctgtgc gtggccctgc tgggattcat catgtgggcc 1680

tgccagaagg gcaacatccg ctgcaacatc tgcatctgat gactcgagct c 1731

<210> 24
<211> 566
<212> PRT
<213> Artificial Sequence

<220>
<223> Influenza Protein H3 Sequence

<400> 24

Met Lys Thr Ile Ile Ala Leu Ser Tyr Ile Leu Cys Leu Val Phe Ala
1 5 10 15

Gln Lys Leu Pro Gly Asn Asp Asn Ser Thr Ala Thr Leu Cys Leu Gly
20 25 30

His His Ala Val Pro Asn Gly Thr Ile Val Lys Thr Ile Thr Asn Asp
35 40 45

Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ser Ser Thr

```
    50                  55                  60

Gly Gly Ile Cys Asp Ser Pro His Gln Ile Leu Asp Gly Glu Asn Cys
65                  70                  75                  80

Thr Leu Ile Asp Ala Leu Leu Gly Asp Pro Gln Cys Asp Gly Phe Gln
                85                  90                  95

Asn Lys Lys Trp Asp Leu Phe Val Glu Arg Ser Lys Ala Tyr Ser Asn
            100                 105                 110

Cys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser Leu Arg Ser Leu Val
        115                 120                 125

Ala Ser Ser Gly Thr Leu Glu Phe Asn Asn Glu Ser Phe Asn Trp Thr
    130                 135                 140

Gly Val Thr Gln Asn Gly Thr Ser Ser Ala Cys Lys Arg Arg Ser Asn
145                 150                 155                 160

Asn Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr His Leu Lys Phe Lys
                165                 170                 175

Tyr Pro Ala Leu Asn Val Thr Met Pro Asn Asn Glu Lys Phe Asp Lys
            180                 185                 190

Leu Tyr Ile Trp Gly Val His His Pro Gly Thr Asp Asn Asp Gln Ile
        195                 200                 205

Ser Leu Tyr Ala Gln Ala Ser Gly Arg Ile Thr Val Ser Thr Lys Arg
    210                 215                 220

Ser Gln Gln Thr Val Ile Pro Asn Ile Gly Ser Arg Pro Arg Val Arg
225                 230                 235                 240

Asp Ile Pro Ser Arg Ile Ser Ile Tyr Trp Thr Ile Val Lys Pro Gly
                245                 250                 255

Asp Ile Leu Leu Ile Asn Ser Thr Gly Asn Leu Ile Ala Pro Arg Gly
            260                 265                 270

Tyr Phe Lys Ile Arg Ser Gly Lys Ser Ser Ile Met Arg Ser Asp Ala
        275                 280                 285

Pro Ile Gly Lys Cys Asn Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile
    290                 295                 300

Pro Asn Asp Lys Pro Phe Gln Asn Val Asn Arg Ile Thr Tyr Gly Ala
305                 310                 315                 320

Cys Pro Arg Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr Gly Met
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325 330 335

Arg Asn Val Pro Glu Lys Gln Thr Arg Gly Ile Phe Gly Ala Ile Ala
340 345 350

Gly Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly
355 360 365

Phe Arg His Gln Asn Ser Glu Gly Ile Gly Gln Ala Ala Asp Leu Lys
370 375 380

Ser Thr Gln Ala Ala Ile Asn Gln Ile Asn Gly Lys Leu Asn Arg Leu
385 390 395 400

Ile Gly Lys Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe Ser
405 410 415

Glu Val Glu Gly Arg Ile Gln Asp Leu Glu Lys Tyr Val Glu Asp Thr
420 425 430

Lys Ile Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu Glu
435 440 445

Asn Gln His Thr Ile Asp Leu Thr Asp Ser Glu Met Asn Lys Leu Phe
450 455 460

Glu Arg Thr Lys Lys Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Asn
465 470 475 480

Gly Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Gly Ser
485 490 495

Ile Arg Asn Gly Thr Tyr Asp His Asp Val Tyr Arg Asp Glu Ala Leu
500 505 510

Asn Asn Arg Phe Gln Ile Lys Gly Val Glu Leu Lys Ser Gly Tyr Lys
515 520 525

Asp Trp Ile Leu Trp Ile Ser Phe Ala Ile Ser Cys Phe Leu Leu Cys
530 535 540

Val Ala Leu Leu Gly Phe Ile Met Trp Ala Cys Gln Lys Gly Asn Ile
545 550 555 560

Arg Cys Asn Ile Cys Ile
565