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(54) Title: CANINE INFLUENZA VIRUS AND RELATED COMPOSITIONS AND METHODS OF USE

(57) Abstract: The present invention provides an isolated canine influenza virus of subtype H3N8 comprising an HA having SEQ ID NO: 4 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 4, with the proviso that the amino acids at positions 94 and 233 are identical to SEQ ID NO: 4; a composition comprising attenuated or inactivated virus; isolated or purified HA, NM, NP, Ml, NSl, PA, PBl, and PB2 proteins and fragments thereof and compositions comprising same or nucleic acids, optionally as part of a vector, encoding same; and a method of inducing an immune response to canine influenza virus in an animal comprising administering to the animal an aforementioned composition.

CANINE INFLUENZA VIRUS AND RELATED COMPOSITIONS AND METHODS OF USE

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 60/727,808, filed October 18, 2005, and U.S. Utility Patent Application No. 11/539,123, filed October 5, 2006, the contents of which are incorporated herein by reference in their entirety.

TECHNICAL FIELD OF THE INVENTION

[0002] The present invention relates to the fields of virology, molecular biology, and immunology. In particular, the present invention relates to canine influenza virus, as well as related compositions and methods of use in inducing an immune response in animals.

BACKGROUND OF THE INVENTION

[0003] Influenza virus is an RNA virus belonging to the family Orthomyxoviridae. The viral RNA consists of eight independent segments, which easily recombine among influenza viruses to produce new subtypes.

[0004] Nucleoprotein (NP), which is the primary component of the nucleocapsid, is encoded in the fifth segment. The NP and the matrix protein are used to classify the influenza virus into group A, B or C. Since NP is an internal protein, it is not subject to the pressure of selection by a host's immune system. It binds RNA, is part of the transcriptase complex, and is involved in the nuclear-cytoplasmic transport of viral RNA (vRNA).

[0005] Neuraminidase (NM), which splits the α-keto bond that joins a terminal sialic acid and the next sugar residue, thereby allowing the release of viral progeny from infected cells, is encoded by the sixth segment. Nine subtypes (N1-N9) of this enzyme have been identified. All subtypes have two structural regions – a stalk and a head. All N8 proteins have 470 amino acids, the first eight of which are highly conserved. The following region is rich in hydrophobic amino acids and is considered to be the transmembrane domain. The next 51 amino acids make up the stalk region, and the head region begins at Cys91. The last region contains the catalytic site of the enzyme. Cysteine residues in the head and stalk region tend to be highly conserved. There are 6-8 putative N-glycosylation sites.

[0006] Hemagglutinin (HA), which is a membrane glycoprotein responsible for the adsorption of the virus into the host cell, is the main antigen to which neutralizing

antibodies are directed. Its antigenic variation is the major cause of influenza epidemics. It is encoded by the fourth segment. Sixteen different subtypes (H1-H16) have been identified. HA has a signal peptide of 16 amino acids and two polypeptides (HA1 and HA2) joined by disulfide bridges. HA1 has the amino terminal end, whereas HA2 has the carboxyl terminal end. A hydrophobic region in HA2 anchors HA to the viral membrane. Cysteine residues tend to be highly conserved. There are six putative glycosylation sites, which enable the virus to mask its antigenic sites (Skehel et al., PNAS USA 81: 1779 (1984)).

[0007] Other proteins include matrix (M or M1 and M2), nonstructural (NS or NS1 and NS2), PA, PB1, and PB2. The M1 protein is a major component of the virion that binds to the plasma membrane of infected cells by means of two hydrophobic regions at the N-terminus of the protein, whereas M2 is an ion channel and, therefore, an integral membrane protein. The NS1 protein is found in the nucleus and affects cellular RNA transport, splicing, and translation. The NS2 protein is found in the nucleus and cytoplasm and has unknown function. The PA protein is a transcriptase and may have protease activity, whereas the PB1 protein functions in transcription elongation and the PB2 protein functions in transcription cap binding.

[0008] Globally, influenza is the most economically significant respiratory disease in humans, pigs, horses and poultry (Wright et al., Orthomyxoviruses. In: *Fields Virology*. Knipe et al., eds. Lippincott Williams & Wilkins, Philadelphia, 2001. pp. 1533-1579.). Influenza virus is known for its continuous genetic and antigenic changes, which impede effective control of the virus (Wright et al. (2001), *supra*; Webster et al., Microbiol. Rev. 56: 152-179 (1992)). Of particular concern for prevention of epidemics and pandemics is the emergency of a new subtype of the virus by genetic re-assortment or inter-species transmission (Wright et al. (2001), *supra*).

[0009] Recently, influenza outbreaks have occurred in species, e.g., feline and canine, which historically do not carry influenza virus (Keawcharoen et al., Emerg. Infect. Dis. 10: 2189-2191 (2004); Crawford et al., Science 310: 398-485 (October 21, 2005; published online September 29, 2005); Dubovi et al., Isolation of equine influenza virus from racing greyhounds with fatal hemorrhagic pneumonia. In: *Proceedings of the 47th Annual Meeting of American Association of Veterinary Laboratory Diagnosticians*, Greensboro, NC, October 2005. p. 158; and Yoon et al., Emerg. Infect. Dis. 11(12): 1974-1976 (December 2005)). Therefore, the host range of influenza virus is expanding.

[0010] Outbreaks of respiratory disease in racing greyhounds caused by infection with influenza virus have occurred in Florida in 2004, in eastern and western Iowa in April 2005, and in Texas in 2005. The disease was characterized by rapid onset of fever and cough, rapid respiration, and hemorrhagic nasal discharge. The morbidity was almost 100% in both race track compounds in Iowa, although the mortality was less than 5%. While a large percentage of affected dogs recovered, many succumbed to hemorrhagic pneumonia. Therapeutic administration of broad-spectrum antibiotics reduced the severity of the disease but could not control it.

[0011] In view of the above, it is an object of the present invention to provide the influenza virus that infects canines. It is another object of the present invention to provide materials and methods for inducing an immune response to the influenza virus in canines. These and other objects and advantages, as well as additional inventive features, will become apparent from the detailed description provided herein.

BRIEF SUMMARY OF THE INVENTION

[0012] The present invention provides an isolated canine influenza virus of subtype H3N8 comprising an HA having SEQ ID NO: 4 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 4, with the proviso that the amino acids at positions 94 and 233 are identical to SEQ ID NO: 4. In particular, the present invention provides an isolated canine influenza virus of subtype H3N8 deposited with the American Type Culture Collection (Manassas, VA) on June 29, 2006, as Patent Deposit No. PTA-7694. Accordingly, the present invention also provides a composition comprising attenuated virus as well as a composition comprising inactivated virus.

[0013] The present invention also provides isolated or purified proteins. In one embodiment, the present invention provides an isolated or purified HA, which (i) has the amino acid sequence of SEQ ID NO: 4 or (ii) is derived from an influenza virus and which has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 4, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 4 at amino acid positions 94 and 233, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 94 or 233 of SEQ ID NO: 4.

[0014] In another embodiment, the present invention provides an isolated or purified NM, which (i) comprises the amino acid sequence of SEQ ID NO: 2 or (ii) is derived from an influenza virus and which comprises an amino acid sequence that is greater than 99%

identical to SEQ ID NO: 2, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 2 at amino acid positions 68 and 134, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 68 or 134 of SEQ ID NO: 2.

[0015] In yet another embodiment, the present invention provides an isolated or purified NP, which (i) has the amino acid sequence of SEQ ID NO: 6 or (ii) is derived from an influenza virus and which has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 6, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 6 at amino acid position 402, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 402 of SEQ ID NO: 6.

[0016] In still yet another embodiment, the present invention provides an isolated or purified M1, which (i) has the amino acid sequence of SEQ ID NO: 8 or (ii) is derived from an influenza virus and which has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 8, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 8 at amino acid position 111, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 111 of SEQ ID NO: 8.

[0017] Also provided is an isolated or purified NS1, which has the amino acid sequence of SEQ ID NO: 10.

[0018] Further provided is an isolated or purified PA protein, which (i) has the amino acid sequence of SEQ ID NO: 12 or (ii) is derived from an influenza virus and which has an amino acid sequence that is greater than 98% (or 99%) identical to SEQ ID NO: 12, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 12 at amino acid positions 233, 256, 327, and 561, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 233, 256, 327, and 561, of SEQ ID NO: 12.

[0019] Still further provided is an isolated or purified PB1, which (i) has the amino acid sequence of SEQ ID NO: 14 or (ii) is derived from an influenza virus and which has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 14, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 14 at amino acid positions 200 and 213, or a fragment of (i) or (ii), wherein the fragment comprises at least nine

contiguous amino acids, at least one of which is identical to the amino acid at position 200 or 213 of SEQ ID NO: 14.

[0020] Even still further provided is an isolated or purified PB2, which (i) has the amino acid sequence of SEQ ID NO: 16 or (ii) is derived from an influenza virus and which has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 16, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 16 at amino acid positions 107, 221, 292, and 661, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 107, 221, 292, or 661 of SEQ ID NO: 16.

[0021] In view of the above, the present invention further provides a composition comprising an above-described protein, such as HA or NM, or a fragment thereof in an amount sufficient to induce an immune response in an animal and a biologically acceptable carrier.

[0022] Also in view of the above, the present invention provides a method of inducing an immune response to canine influenza virus in an animal. The method comprises administering to the animal the composition comprising a protein or fragment thereof.

[0023] An isolated or purified nucleic acid encoding above-described protein or fragment thereof, optionally as part of a vector, is also provided, as is a composition comprising the isolated or purified nucleic acid, which expresses the protein, such as HA or NM, or a fragment thereof, in an amount sufficient to induce an immune response in an animal and a biologically acceptable carrier.

[0024] Accordingly, the present invention also provides another method of inducing an immune response to canine influenza virus in an animal. The method comprises administering to the animal the composition comprising a nucleic acid.

BRIEF DESCRIPTION OF THE FIGURES

[0025] Fig. 1 is the partial nucleotide sequence (SEQ ID NO: 1; see also GenBank Acc. No. DQ146420) of the coding domain sequence (CDS) of the NM gene from subtype H3N8 of canine influenza virus. In accordance with convention, the sequence is presented from left to right and top to bottom.

[0026] Fig. 2 is the amino acid sequence (SEQ ID NO: 2; see also GenBank Acc. No. DQ146420) encoded by SEQ ID NO: 1. In accordance with convention the sequence is presented in single letter format from left to right and top to bottom.

[0027] Fig. 3 is the complete nucleotide sequence (SEQ ID NO: 3; see also GenBank Acc. No. DQ146419) of the CDS of the HA gene from subtype H3N8 of canine influenza virus.

[0028] Fig. 4 is the amino acid sequence (SEQ ID NO: 4; see also GenBank Acc. No. DQ146419) encoded by SEQ ID NO: 3.

[0029] Fig. 5 is the complete nucleotide sequence (SEQ ID NO: 5) of the CDS of the NP gene from subtype H3N8 of canine influenza virus.

[0030] Fig. 6 is the deduced amino acid sequence (SEQ ID NO: 6) encoded by SEQ ID NO: 5.

[0031] Fig. 7 is the complete nucleotide sequence (SEQ ID NO: 7) of the CDS of the M1 protein gene from subtype H3N8 of canine influenza virus.

[0032] Fig. 8 is the deduced amino acid sequence (SEQ ID NO: 8) encoded by SEQ ID NO: 7.

[0033] Fig. 9 is the complete nucleotide sequence (SEQ ID NO: 9) of the CDS of the NS1 protein gene from subtype H3N8 of canine influenza virus.

[0034] Fig. 10 is the deduced amino acid sequence (SEQ ID NO: 10) encoded by SEQ ID NO: 9.

[0035] Fig. 11 is the complete nucleotide sequence (SEQ ID NO: 11) of the CDS of the PA protein gene from subtype H3N8 of canine influenza virus.

[0036] Fig. 12 is the deduced amino acid sequence (SEQ ID NO: 12) encoded by SEQ ID NO: 11.

[0037] Fig. 13 is the complete nucleotide sequence (SEQ ID NO: 13) of the CDS of the PB1 protein gene from subtype H3N8 of canine influenza virus.

[0038] Fig. 14 is the deduced amino acid sequence (SEQ ID NO: 14) encoded by SEQ ID NO: 13.

[0039] Fig. 15 is the complete nucleotide sequence (SEQ ID NO: 15) of the CDS of the PB2 protein gene from subtype H3N8 of canine influenza virus.

[0040] Fig. 16 is the deduced amino acid sequence (SEQ ID NO: 16) encoded by SEQ ID NO: 15.

DETAILED DESCRIPTION OF THE INVENTION

[0041] The present invention is predicated on the discovery of a strain of influenza virus in canines. The strain was isolated from racing greyhounds in eastern and western Iowa. The strain has been classified as an H3N8 subtype, and has been designated A/canine/Iowa/13628/2005. Accordingly, the present invention provides a virus comprising an HA having SEQ ID NO: 4 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 4, with the proviso that the amino acids at positions 94 and 233 are identical to SEQ ID NO: 4. The virus can further comprise an NM comprising the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 2, with the proviso that the amino acids at positions 68 and 134 are identical to SEQ ID NO: 2. The virus comprising the aforementioned HA, alone or in further combination with the aforementioned NM, can further comprise at least one of the following: an NP having the amino acid sequence of SEQ ID NO: 6 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 6, with the proviso that amino acid 402 is identical to that of SEQ ID NO: 6; an M1 having the amino acid sequence of SEQ ID NO: 8 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 8, with the proviso that amino acid 111 is identical to that of SEQ ID NO: 8; an NS1 having the amino acid sequence of SEQ ID NO: 10; a PA protein having the amino acid sequence of SEQ ID NO: 12 or an amino acid sequence that is greater than 98% (or 99%) identical to SEQ ID NO: 12, with the proviso that amino acids 233, 256, 327, and 561 are identical to SEQ ID NO: 12; a PB1 having the amino acid sequence of SEQ ID NO: 14 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 14, with the proviso that amino acids 200 and 213 are identical to SEQ ID NO: 14; and/or PB2 having the amino acid sequence of SEQ ID NO: 16 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 16, with the proviso that amino acids 107, 221, 292, and 661 are identical to SEQ ID NO: 16. In particular, the present invention provides an isolated canine influenza virus of subtype H3N8 deposited with the American Type Culture Collection, 10801

University Blvd., Manassas, VA 20110-2209, U.S.A., on June 29, 2006, as Patent Deposit No. PTA-7694.

[0042] Influenza virus can be precipitated by subjecting the virus in aqueous medium to one or more insolubilizing steps brought about by the presence of up to 5% by weight of polyethylene glycol (PEG) having a molecular weight between 3,000 and 20,000 or another linear filamentary noncharged polymer in an amount equivalent to the solubilizing power of PEG, separating an insolublized fraction from a non-insolubilized fraction, and recovering virus from one of the fractions (see, e.g., U.S. Pat. No. 3,989,818). Preferably, the temperature does not exceed 35°C, the pH is between 6 and 9, and the ionic strength of the aqueous medium is below the salting out point for the virus. The concentration of the virus in the aqueous medium prior to insolubilizing corresponds to a hemagglutination titer of at least 1 in 32. Aggregated viral particles are obtained, which are believed to provide a better antigenic effect due to the slow release of viral particles after vaccination. If, however, non-aggregated or less aggregated particles are desired, they can be dissociated using any suitable method, such as sonication.

[0043] The virus can be attenuated by passaging in a cell system until the virus has lost its ability to produce disease, while fully retaining its immungenic character. For example, the virus can be serially passaged in a culture of cells originating from a canine species or other suitable species at a temperature of about 37 °C. At each passage, the virus is harvested from one culture and inoculated into a medium containing a fresh cell culture in accordance with methods known in the art. For example, the virus can be collected from tissue cell culture fluids and/or cells. Optionally, during harvesting, the cell culture can be sonicated to promote release of the virus. See, e.g., U.S. Pat. Nos. 5,698,433 and 6,455,298.

[0044] If desired, an influenza strain can be passaged at least once in the allantoic cavity of embryonated eggs, such as chicken eggs, in the presence of serum, to obtain serum-resistant virus (see, e.g., U.S. Pat. No. 3,953,592; Kilbourne et al., J.Exp. Med. 111: 387 (1960); Kilbourne, Science 160: 74-75 (April 1968); and Laver et al., Virology 30: 493-501 (1966)). High potency influenza vaccine with low pyrogenicity and low endotoxicity can be achieved by treating the concentrated allantoic fluid containing an attenuated virus sequentially with butyl acetate and ethyl acetate, followed by flash evaporation (see, e.g., U.S. Pat. No. 4,000,257). Such virus can be administered intranasally as a vaccine.

[0045] Once inoculated into the host, the virus multiplies to some extent so that only a small initial inoculum is required. The virus must be innocuous, and infection of susceptible contacts should be kept to a minimum.

[0046] Alternatively, the virus can be inactivated by abolishing replication and virulence. This can be done by chemical or physical means. Chemical inactivation can be carried out by treatment of the virus with an enzyme, formaldehyde, β-propiolacton or derivative thereof, ethyleneimine or derivative thereof, an organic solvent (e.g., halogenated hydrocarbon), and/or a detergent (e.g., Tween®, Triton X®, sodium desoxycholate, sulfobetain, or cetyltrimethylammonium salts). If necessary, chemically activated compositions can be neutralized. For example, if formaldehyde is used to deactivate the composition, the composition can be neutralized with thio-sulphate. If required, the pH subsequently can be returned to a value of about 7. Alternatively, the virus can be extracted with a mixture of ether and ethanol, the aqueous and organic phases can be separated, and residual ether can be removed from the viral suspension under reduced pressure (see, e.g., U.S. Pat. No. 4,431,633). Physical inactivation advantageously can be carried by subjecting the virus to energy-rich radiation, such as ultraviolet light, γ-radiation, or X-rays. Inactivated forms require a relatively high amount of inoculum and, therefore, a correspondingly large quantity of antigenic material, which has to be manufactured, tested, and distributed.

[0047] In view of the above, the present invention also provides a composition comprising an attenuated or inactivated virus. The virus should be present in an amount sufficient to induce an immune response and, desirably, should provide protection upon challenge. Generally, an adjuvant, such as Tween®, Span®, Freund's complete adjuvant, saponin, *Corynebacterium parvum* (Coparvax®), aluminium phosphate, aluminium hydroxide, or a mixture thereof, is added to the composition, particularly if the composition comprises inactivated virus. Protein hydrolysates and/or amino acids can be added to stabilize the composition (see, e.g., U.S. Pat. No. 4,537,769). Alternatively, the composition can be formulated as an oil-in-water emulsion using oils such as Marcol and/or Arlacel.

[0048] Recombinant influenza strains also can be prepared, such as from the combination of an "over-attenuated" (i.e., the number of passages for attenuation is substantially greater than what is normally required to remove pathogenicity) influenza A parent strain, e.g., A2, with a virulent influenza strain as provided herein (see, e.g., U.S. Pat. No. 3,991,179; also, see U.S. Pat. Nos. 4,009,258; 4,278,662; 4,318,903; 4,338,296; and

4,693,893). A recombinant strain preferably has the growth characteristics of the over-attenuated strain coupled with the antigenic properties, e.g., the HA and NM proteins, of the virulent strain. The selection of strains of influenza virus for vaccine formulation is described in U.S. Pat. No. 5,162,112. Recombinant strains can be formulated as compositions for inducing an immune response.

[0049] Sucrose, arginine monohydrochloride, the monosodium monohydrate of glutamic acid, and gelatin hydrolysate can be used to stabilize an influenza virus composition for storage in a refrigerator. See, e.g., U.S. Pat. App. Pub. No. 2006/0110406.

[0050] In view of the above, the present invention also provides an isolated or purified HA. The HA either has the amino acid sequence of SEQ ID NO: 4 or is derived from an influenza virus and has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 4, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 4 at amino acid positions 94 and 233. A fragment of HA comprising at least nine (such as 9, 12, 15, 18, 21 or 24) contiguous amino acids, at least one of which is identical to the amino acid at position 94 or 233 of SEQ ID NO: 4, is also provided.

[0051] An isolated or purified NM is also provided. The NM comprises the amino acid sequence of SEQ ID NO: 2 or is derived from an influenza virus and comprises an amino acid sequence that is greater than 99% identical to SEQ ID NO: 2, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 2 at amino acid positions 68 and 134. A fragment of NM comprising at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 68 or 134 of SEQ ID NO: 2, is also provided.

[0052] Further provided is an isolated or purified NP. The NP has the amino acid sequence of SEQ ID NO: 6 or is derived from an influenza virus and has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 6, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 6 at amino acid position 402. A fragment of NP comprising at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 402 of SEQ ID NO: 6, is also provided.

[0053] Still further provided is an isolated or purified M1. The M1 has the amino acid sequence of SEQ ID NO: 8 or is derived from an influenza virus and has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 8, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 8 at amino acid position 111. A

fragment of M1 comprising at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 111 of SEQ ID NO: 8, is also provided.

[0054] Even still further provided is an isolated or purified NS1, which has the amino acid sequence of SEQ ID NO: 10.

[0055] An isolated or purified PA protein is also provided. The PA has the amino acid sequence of SEQ ID NO: 12 or is derived from an influenza virus and has an amino acid sequence that is greater than 98% (or 99%) identical to SEQ ID NO: 12, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 12 at amino acid positions 233, 256, 327, and 561. A fragment of PA comprising at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 233, 256, 327, or 561 of SEQ ID NO: 12, is also provided.

[0056] An isolated or purified PB1 is provided. The PB1 has the amino acid sequence of SEQ ID NO: 14 or is derived from an influenza virus and has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 14, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 14 at amino acid positions 200 and 213. A fragment of PB1 comprising at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 200 or 213 of SEQ ID NO: 14, is also provided.

[0057] Provided also is an isolated or purified PB2. The PB2 has the amino acid sequence of SEQ ID NO: 16 or is derived from an influenza virus and has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 16, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 16 at amino acid positions 107, 221, 292, and 661. A fragment of PB2 comprising at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 107, 221, 292, or 661 of SEQ ID NO: 16, is provided as well.

[0058] The above proteins and fragments thereof can be purified (coupled with chemical or physical fragmentation to generate fragments) or synthesized in accordance with methods known in the art. See, e.g., Meienhofer, Hormonal Proteins and Peptides 2: 46, Academic Press, NY (1973), for solid phase protein synthesis, and Schroder et al., The Peptides, vol. 1, Academic Press, NY (1965), for solution phase protein synthesis. Automated systems can be used to carry out such techniques in accordance with manufacturer's instructions. Therapeutic quantities can be recombinantly produced and purified.

[0059] Alternatively, proteins, in particular HA and NM, can be isolated by selective solubilization, while leaving residual subviral particles consisting of the intact lipid/protein membrane enclosing all other non-essential viral components. The difference in size/density of the solubilized proteins and the residual subviral particles allows separation based on differences in physical properties by gradient centrifugation and fractionation, sedimentation, molecular sieve chromatography, or pelleting in an ultracentrifuge. Selective solubilization of HA and NM can be achieved by treatment of the virus with a cationic detergent (see, e.g., U.S. Pat. No. 4,140,762; the '762 patent). The whole virus-containing fluid obtained from cell culture can be treated with a DNA-digesting enzyme followed by addition of a cationic detergent and isolation of surface-antigen proteins (see, e.g., U.S. Pat. No. 5,948,410). The fluid can be subjected to several ultracentrifugation steps, or the virus can be fragmented in the presence of an amphiphilic nonionic detergent followed by filtration to remove undesirable substances (see, e.g., U.S. Pat. No. 6,048,537). Alternatively, membrane filtration and chemical splitting can be used to obtain a viral protein (see, e.g., U.S. Pat. No. 4,327,182). Other procedures are described in U.S. Pat. Nos. 4,064,232 and 4,057,626. Preferably, the virus is multiplied before treatment as exemplified in the '762 patent (col. 2, ll. 10 *et seq*).

[0060] Mapping can be conducted to identify an immune response-inducing epitope of a viral protein, i.e., "epitope mapping." Such mapping involves fragmenting of a protein into overlapping peptides (such as peptides comprising 9, 12, 15, 18, 21 or 24 amino acids). The protein can be fragmented with a proteolytic enzyme. The individual peptides are then tested for their ability to bind to an antibody elicited by the native protein or to induce T-cell or B-cell activation. Alternatively, hydrophilic regions of the protein can be selected, since hydrophilic residues are often on the surface of the protein and, therefore, are accessible to the antibody. X-ray crystallographic analysis of the antigen-antibody complex also can be performed. Potential HLA anchor binding motifs, which are peptide sequences that are known to be likely to bind to MHC molecules, can be identified from the amino acid sequence of a protein. Preferably, the epitope selected is one that shares little to no sequence identity with sequences widely found in the animal to which a composition comprising or expressing a protein fragment will be administered.

[0061] An isolated or purified nucleic acid encoding an above-described protein or fragment thereof, optionally as part of a vector, is also provided. The nucleic acid encoding the HA can comprise the nucleotide sequence of SEQ ID NO: 3 or a fragment thereof encoding at least nine (9, 12, 15, 18, 21 or 24) contiguous amino acids. If desired, a trivalent vaccine based on HA can be prepared, wherein one of the HAs comprises the

amino acid sequence of SEQ ID NO: 4 (see, e.g., U.S. Pat. Nos. 5,762,939 and 6,245,532; see, e.g., U.S. Pat. No. 6,740,325 for a tetravalent vaccine). The nucleic acid encoding the NM can have the nucleotide sequence of SEQ ID NO: 1 or a fragment thereof encoding at least nine contiguous amino acids (see, e.g., U.S. Pat. No. 6,605,457 and U.S. Pat. App. Pub. No. 2003/0129197), whereas the nucleic acid encoding the NP can have the nucleotide sequence of SEQ ID NO: 5 or a fragment thereof encoding at least nine contiguous amino acids, the nucleic acid encoding the M1 protein can have the nucleotide sequence of SEQ ID NO: 7 or a fragment thereof encoding at least nine contiguous amino acids, the nucleic acid encoding the NS1 protein can have the nucleotide sequence of SEQ ID NO: 9, the nucleic acid encoding the PA can have the nucleotide sequence of SEQ ID NO: 11 or a fragment thereof encoding at least nine contiguous amino acids, the nucleic acid encoding the PB1 can have the nucleotide sequence of SEQ ID NO: 13 or a fragment thereof encoding at least nine contiguous amino acids, and the nucleic acid encoding the PB2 can have the nucleotide sequence of SEQ ID NO: 15 or a fragment thereof encoding at least nine contiguous amino acids. One of ordinary skill in the art will appreciate, however, that due to the degeneracy of the genetic code, there are numerous other nucleotide sequences that can encode such amino acid sequences.

[0062] The above nucleic acids, which can be DNA or RNA, and fragments thereof can be synthesized (see, e.g., Oligonucleotide Synthesis, Gait, ed., 1984). Such molecules can include non-naturally occurring nucleotides/bases that encode the desired amino acid sequence. For example, the base or sugar can be methylated. In addition, the backbone of the nucleic acid molecule can be modified, e.g., a phosphorothioate backbone, methylphosphonate, methylphosphorothioate, phosphorodithioate, and combinations thereof.

[0063] Alternatively, isolated vRNA can be subjected to reverse transcriptase to produce an RNA/DNA hybrid, from which the RNA is digested away and the residual DNA is treated to produce a dsDNA having a hairpin end, which is treated with a single-strand-specific nuclease to produce a bimolecular double-stranded copy of the vRNA (see, e.g., U.S. Pat. No. 4,357,421). See, e.g., U.S. Pat. App. Pub. No. 2006/0166321 for the use of tandem transcription cassettes for the preparation of influenza in the absence of helper virus.

[0064] The nucleic acid is optionally part of a DNA vector comprising at least one promoter, in which case each nucleotide sequence is operably linked to a promoter, which can be the same or different. In addition to promoters, other control sequences, such as terminating signals and the like, can be part of the DNA vector.

[0065] For example, the nucleic acid can be introduced into a suitable recombinant expression vector, such as those adapted for bacteria, such as *E. coli* and *Salmonella typhi*,- yeast, such as *Saccharomyces cervisiae* or *Pichia pastoris*, or filamentous fungi, such as *Aspergillus nidulans*. The bacteria, yeast, or fungi can be grown in continuous culture. The polypeptide, which is produced during culture, then can be isolated and purified. Alternatively, the nucleic acid molecule can be introduced into Poxviridae (e.g., fowlpox-based vectors), Herpesviridae (e.g., pseudorabies virus-based vectors, turkey herpes virus-based vectors, feline herpes virus-based vectors, infectious laryngotracheitis virus-based vectors, and bovine herpes virus-based vectors), Adenoviridae (e.g., bovine adenovirus (e.g., serotype 3), human adenovirus (e.g., serotype 4 or 7), and canine adenovirus (e.g., serotype 2; CAV2; see, e.g., U.S. Pat. No. 6,090,393), or an insect virus expression vector, such as recombinant baculovirus (e.g., *Autographa californica* nuclear polyhydrosis virus (AcNPV)), which, in turn, can be used to infect susceptible cultured SF9 cells, which are derived from the insect *Spodotera frugiperda*. Other viral vectors include vaccinia (see, e.g., U.S. Pat. No. 4,722,848), adenovirus, adeno-like virus, adeno-associated virus, retrovirus, and pox (see, e.g., Hruby, Vet. Parasitol. 29: 281-282 (1988); Uiu, "AIDS Research Reviews," Dekker, Inc., 1991, 1: 403-416), which can be administered by a skin scratch or by injection, optionally as a liposomal formulation. Other vectors include Bacille-Calmette-Guerin (BCG; Stover et al., Nature 351: 456-460 (1991)), detoxified anthrax toxin vectors, and the like. Mammalian cells, such as Chinese hamster ovary (CHO) cells, and even plant cells can be used to express the polypeptide from the appropriate construct. One of ordinary skill in the art will appreciate that the choice of host cell will affect the nature of post-translational processing (e.g., glycosylation, folding, and the like), which, in turn, can impact the immunogenicity of the polypeptide, and subsequent purification techniques.

[0066] Expression can be achieved in any appropriate host cell transformed/transfected with the expression vector. Examples of suitable host cells include, but are not limited to, those described above. Thus, the present invention also provides a host cell transformed/transfected with an expression vector.

[0067] Supernatants from host/vector systems that secrete the protein or fragment thereof into culture media can be applied to a purification matrix, such as an affinity column or an ion exchange column. One or more reverse-phase HPLC steps can be employed to purify further the recombinant protein or fragment thereof.

[0068] Production of a protein or fragment thereof as a fusion protein can stabilize production. This can be accomplished by ligating polynucleotide sequences encoding two or more proteins (or fragments thereof) into an appropriate expression vector with or without a peptidic linker. Desirably, the reading frames of the polynucleotides sequences are in phase, so that a single fusion protein that retains the biological activity of each protein (or fragment thereof) is produced. A peptidic linker from 1 to about 50 amino acids can be used to separate the resultant proteins (or fragments thereof) so as to ensure that each protein (or fragment thereof) properly folds into its native secondary, tertiary, and quaternary structures (see, e.g., Maratea et al., Gene 49: 39-46 (1985); Murphy et al., PNAS USA 83: 8258-8262 (1986); U.S. Pat. No. 4,935,233; and U.S. Pat. No. 4,751,180). The ability to adopt a flexible extended conformation, the inability to adopt a secondary structure that could interact with functional amino acids on either one or both of the proteins, and the lack of hydrophobic or charged residues that might react with either one or both of the proteins are factors, which are taken into consideration in selecting a peptide linker. Linkers are not required when the ends of the proteins to be joined do not contain essential regions, such that the ends can be used to separate functional domains and prevent steric interference. Preferred peptide linker sequences contain Gly, Asn, and Ser residues. Other near neutral residues, such as Thr and Ala, also can be used.

[0069] Other additional amino acid sequence(s) can be selected to enhance the expression and/or immunogenicity of the protein or fragment thereof. For example, the protein or fragment thereof can be fused to the heavy chain of immunoglobulin G (IgG) or an antigen-presenting cell (APC) binding protein or a dendritic cell binding protein, such as IL-D, GM-CSF, IL-1, TNF, IL-4, CD40L, CTLA4, CD28, or FLT-3 ligand. Techniques, such as the use of dehydrating agents, e.g., dicyclohexylcarbodiimide (DCCI), or the creation of linkages between sulfhydryl groups, epsilon amino groups, carboxyl groups, and the like, can be used. If desired, a cleavage site can be introduced into the fusion protein to enable separation of the protein (or fragment thereof) from the non-naturally occurring sequence(s). Examples of cleavage sites include a target sequence for a proteolytic enzyme or, if methionine is not present in the protein (or fragment thereof), methionine, which, in turn, is cleaved by cyanogen bromide. Such methods are known in the art. The protein or fragment thereof can be modified by glycosylation or other derivatization (e.g., acetylation or carboxylation), also in accordance with methods known in the art.

[0070] The protein (or fragment thereof) can be expressed *in situ* from a suitable expression system. Any DNA construct, which is effective in producing the encoded

protein or fragment thereof in the desired environment, can be used to express the protein or fragment thereof as described above.

[0071] Alternatively, the nucleic acid molecule can behave as an effective expression system *in situ* when injected into an animal as "naked DNA" (see, e.g., Ulmer et al., Science 259: 1745-1749 (1993); and Cohen, Science 259: 1691-1692 (1993)). DNA delivery also can be facilitated through the use of bupivicaine, polymers, and peptides; alternatively, cationic lipid complexes, particles, or pressure (see, e.g., U.S. Pat. No. 5,922,687) can be used.

[0072] Examples of amino acid sequences that are at least about or greater than 95% identical to, such as at least about or greater than 96%, 97%, 98%, or 99% identical to, SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, or 16 include amino acid sequences that contain one or more substitutions, insertions, additions and/or deletions. Sequence identity can be determined by aligning polypeptide sequences and applying publicly available computer algorithms, such as BLASTP (Pearson et al., PNAS USA 85: 2444-2448 (1988); Pearson, Methods Enzymol. 183: 63-98 (1990); and Altschul et al., Nucl. Acids Res. 25: 3389-3402 (1997)). The software for BLASTP is available on the FTP server of the National Center for Biotechnology Information (NCBI) or NCBI, National Library of Medicine, Building 38A, Room 8N8O5, Bethesda, MD 20894. Once the polypeptide sequences are aligned, the number of identical amino acids over the aligned portions is identified, the number of identical amino acids is divided by the total number of amino acids of the polypeptide of interest, and the result is multiplied by 100 to determine the percentage sequence identity.

[0073] In this regard, one of ordinary skill in the art will appreciate that a fragment of a given amino acid sequence can be at least about or greater than 95% identical to, such as 96%, 97%, 98% or 99% identical to, the amino acid sequence. Thus, fragments are intended to be encompassed by "an amino acid sequence that is at least about or greater than 95% (or 96%, 97%, 98% or 99%) identical to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, or 16." Such fragments desirably retain the immunogenicity of the full-length protein. Functional fragments can be generated by mutational analysis of the nucleic acid encoding the protein and subsequent expression of the resulting mutant protein or by chemical/enzymatic digestion of the protein, itself.

[0074] Modifications, such as substitutions, insertions, additions and/or deletions, can be introduced into the nucleic acid or the protein (or fragment thereof) in accordance with methods known in the art (see, e.g., Adelman et al., DNA 2: 183 (1983), for

oligonucleotide-directed site-specific mutagenesis). Desirably, the modification does not substantially diminish the immunogenicity of the protein fragment; rather, it is preferred that the immunogenicity remains substantially the same or increases relative to the unmodified protein.

[0075] A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, i.e., similar secondary structure and hydropathic nature. Amino acid substitutions can be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids, such as aspartic acid and glutamic acid, can be interchanged, whereas positively charged amino acids, such as lysine and arginine, can be interchanged, and amino acids with uncharged polar head groups having similar hydrophilicity values can be interchanged. In this regard, leucine, isoleucine and valine can be interchanged, glycine and alanine can be interchanged, asparagine and glutamine can be interchanged, and serine, threonine, phenylalanine, and tyrosine can be interchanged. Other groups of amino acids that can be interchanged include: (1) ala, pro, gly, glu, asp, gln, asn, ser and thr; (2) cys, ser, tyr and thr; (3) val, ile, leu, met, ala and phe; (4) lys, arg and his; and (5) phe, tyr, trp, and his.

[0076] In view of the above, a composition comprising the isolated or purified protein/nucleic acid or fragment of either of the foregoing and a biologically acceptable carrier is also provided. The nucleic acid or fragment thereof can be part of a vector. See, for example, U.S. Pat. No. 4,029,763, which is directed to an influenza vaccine comprising, as an active ingredient, NM, and U.S. Pat. No. 4,140,762, which is directed to an influenza vaccine comprising, as active ingredients, HA and NM. U.S. Pat. No. 4,826,687 describes the addition of muramyl dipeptide to a vaccine comprising HA and NM. If desired, polypeptides corresponding substantially to amino acids 148-162, 163-166, and/or 215-239 of M1 can be added to a composition of a protein/nucleic acid or fragment thereof (see, e.g., U.S. Pat. Nos. 5,136,019; 5,616,327; and 5,741,493). Any suitable biologically acceptable carrier can be used in the composition. For example, the protein(s)/nucleic acid(s)/fragments thereof can be resuspended in a diluent, e.g., 0.9% sodium chloride solution, which is optionally buffered with, for example, a phosphate buffer. Any sucrose that remains from purification of the virus can be reduced by dialysis. Dialysis or gel chromatography can be used to remove any remaining cationic detergent. Preferably, the protein or fragment thereof is present in an amount sufficient to induce an immune response (i.e., cellular or humoral) in an animal. A frequently selected carrier for pharmaceuticals and antigens is poly(d,l-lactide-co-glycolide) (PLGA). PLGA is a biodegradable polyester,

and can be used for the controlled release of antigen (Eldridge et al., Curr. Topics Micro. Immuno. 146: 59-66 (1989); see also U.S. Pat. No. 6,090,393). The entrapment of antigens in PLGA microspheres of 1-10 μ in diameter has been shown to have a remarkable adjuvant effect when administered orally.

[0077] If desired, a preserving agent or an inactivating agent, such as formaldehyde, can be added. A conventional amount of preserving/inactivating agent is 1 part per 10,000 parts.

[0078] If desired, one or more proteins (or immunogenic fragments thereof), such as the above-described HA, can be combined with proteosomes. See, e.g., U.S. Pat. No. 6,743,900 and U.S. Pat. App. Pub. No. 2004/0156867.

[0079] Immunogenicity can be improved by inclusion of conventional immunological adjuvants, such as aluminium hydroxide (e.g., about 0.2%) or aluminium phosphate, aluminum (see, e.g., U.S. Pat. Nos. 6,372,223, 6,635,246, 6,861,244 and 7,052,701 and U.S. Pat. App. Pub. Nos. 2004/0096464 and 2006/0147468), chitosan (see, e.g., U.S. Pat. Nos. 6,136,606 and 6,534,065), alum, such as in the form of aluminum hydroxide, aluminum phosphate or aluminum oxide, mineral oils (e.g., Bayol F® and Marcol 52®), Freund's complete adjuvant, Freund's incomplete adjuvant, muramyl dipeptide, monophosphoryl lipid A, and saponins, including the Quil A component. Immunogenicity also can be improved by adding a cytokine, such as an interleukin, or by conjugating proteins or fragments thereof. Preferably, the protein or fragment thereof is conjugated with a macromolecular carrier, such as a protein (e.g., serum albumin, keyhole limpet hemocyanin, imunoglobulin, throglobulin, and ovalbumin), polysaccharide (e.g., latex-functionalized sepharose, agarose, cellulose beads, and the like), phospholipid, polymeric amino acids (e.g., polyglutamic acid, polylysine, and the like), or amino acid co-polymers (see, e.g., U.S. Pat. Nos. 5,136,019 and 5,612,037). Alternatively, the protein or fragment thereof can be encapsulated with a proteoliposome or lipid vesicle.

[0080] The composition, which can induce an immune response, can be prepared in the form of a suspension or can be lyophilized. If lyophilized, it is preferable to add one or more stabilizers. Suitable stabilizers are, for example, sucrose, phosphate, glutamate, and albumin (SPGA; Bovarnick, J. Bacteriol. 59: 509 (1950)), carbohydrates (e.g., sorbitol, mannitol, starch, dextran, and glucose), proteins (e.g., albumin and casein) or degradation products thereof, protein-containing agents (e.g., bovine serum or skim milk), and buffers (e.g., alkali metal phosphates).

[0081] Alternatively, the composition can be formulated as a controlled-release composition. The attenuated/inactivated virus or recombinant vector can be microencapsulated with polymers, such as polycarbonates, polyesters, polyurethanes, polyorthoesters, and polyamides. The particular polymer selected depends on a number of factors including reproducibility of polymer synthesis and microencapsulation, cost of materials and process, toxicological profile, requirements for variable release kinetics, and the physicochemical compatibility of the polymer and the virus/vector.

[0082] The compositions described herein can be used alone or in combination with other active ingredients/compositions. Examples include compositions, which can induce an immune response again canine distemper, infectious canine hepatitis (CAV-1 and CAV-2), rabies, parainfluenza, canine corona virus, measles, leptospirosis, and Bordetella. Polyphenols have been disclosed to inhibit influenza infection in humans (see, e.g., U.S. Pat. No. 5,173,922; the '922 patent). Accordingly, the addition of a polyphenol, such as epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin, free theaflavin, theaflavin monogallate A, theaflavin monogallate B, and/or theaflavin digallate may be beneficial (see the '922 patent). Inhibitors of NM are disclosed in U.S. Pat. No. 5,453,533. The use of cytokines as immunopotentiators and liposomal encapsulation are described in U.S. Pat. No. 5,919,480.

[0083] The amount of nucleic acid in the composition can vary widely. For example, the concentration can range from less than about 0.1% to as much as about 20-50% or more by weight, usually at least about 2%. The concentration of protein in the composition also can vary widely. For example, the concentration can range from less than about 0.1% to as much as about 20-50% or more by weight, usually at least about 2%. Fluid volume and viscosity are taken into consideration when determining the final concentration.

[0084] Accordingly, a method of inducing an immune response to canine influenza virus in an animal is also provided. The susceptibility of an animal to infection can be assessed using the plaque reduction neutralization test (U.S. Pat. No. 4,315,073) or the hemagglutination test. The method comprises administering to the animal an above-described composition comprising an isolated or purified protein/nucleic acid or fragment thereof. If the composition comprises a nucleic acid (or fragment thereof) as part of a vector, preferably the protein (or fragment thereof) is expressed in an amount sufficient to induce an immune response in an animal. For example, a single dose of from about 9 to about 43 international units per kg of animal body weight can be administered. For larger mammals, a single dose can comprise from about 600 to about 3,000 international units per

kg of body weight. For vaccine compositions prepared by culturing virus in the allantoic cavity of fertile eggs, harvesting the virus, and, if desired, stabilizing the harvested virus with a stabilizer, such as a peptone or sucrose, and then distribution into glass vials for subsequent freeze-drying, an effective vaccine dosage unit can contain at least 10^7 EID_{50} (50% egg-infective dose) of virus. In the latter situation, the freeze-dried vaccine is reconstituted by addition of water or another pharmaceutically acceptable diluent prior to administration, such as in the form of a nasal spray or nasal drops. If desired, the vaccine can be administered in two successive dosages at a one-week interval.

[0085] The composition can be administered to puppies as a single dose at the age of 12 weeks, or repeatedly starting from the age of 6 weeks (e.g., at 6, 9 and 12 weeks), or weekly from 4 weeks on. The effective dosage and route of administration are determined by the nature of the composition, the nature of the expression product, LD_{50}, and, if recombinant vector is used, the expression level of the vector, as well as the breed of dog and its age, sex, weight, and condition. Dosages of expressed product can range from a few to a few hundred micrograms, e.g., 5-500 μg. Preferred dosages of virus or recombinant vector can range from about 10^3 to about 10^6 pfu. The dose for the live attenuated strain can be at least about 10^3 $TCID_{50}$.

[0086] The compositions can be administered parenterally (i.e., by injection (e.g., intradermal, subcutaneous, or intramuscular) or by the route of infection, such as nasally) or enterally (i.e., by oral administration). The use of a gelling agent and a muco- or bio-adhesive to enhance the immune response against an intradermally administered immunogenic composition is described in U.S. Pat. App. Pub. No. 2005/0255121. If desired, the composition for inducing an immune response can be administered through drinking water or syrup in accordance with Chu et al. (U.S. Pat. App. Pub. No. 2006/0171960, which was published on August 3, 2006). Oral administration is advantageous inasmuch as it avoids time-consuming and labor-intensive intramuscular injection, which, in turn, can create stress for the animal and discomfort. Discomfort, in turn, can affect the performance of race dogs. Alternatively, the composition comprising a recombinant vector expressing at least one immune response-inducing epitope can be applied directly to the skin for localized expression and induction of an immune response.

[0087] Efficacy of the composition, which can induce an immune response, can be demonstrated by exposing puppies to a virulent strain of canine influenza virus. Untreated dogs should develop clinical signs characteristic of canine influenza viral infection, whereas treated dogs should not.

[0088] The recombinant vectors and the products expressed from them can be used to produce antibodies, such as polyclonal antibodies (pAb) and monoclonal antibodies (mAb), in accordance with methods known in the art (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1988); Harlow and Lane, *Using Antibodies: A Laboratory Manual* (1998), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1998); Shepherd and Dean, *Monoclonal Antibodies: A Practical Approach*, Oxford University Press, U.S.A. (2000)); and Harris and Adair, *Antibody Therapeutics*, CRC Press, Inc., Boca Raton, FL (1997)). The antibodies, in particular mAbs, can be used in binding assays and diagnostic kits/tests to determine the presence/absence of an antigen of canine influenza virus or whether or not an immune response to the virus has been stimulated. The antibodies also can be used to recover material by immuno-adsorption chromatography.

[0089] Antibodies also can provide passive immunization. For example, partially purified immune sera from host animals or from hybridoma cell lines can be injected into an animal. The antibodies provide a therapeutic effect by binding to and neutralizing an infectious influenza virus.

[0090] A composition comprising an anti-idiotypic antibody having an internal image of an epitope of an above-described protein, such as a protein consisting of the amino acid sequence SEQ ID NO: 1 or SEQ ID NO: 3, is also provided.

[0091] One of ordinary skill in the art will appreciate that an anti-idiotypic antibody, which bears an internal image of an epitope, such as those described herein, can be prepared. See, e.g., Herlyn et al., Science 232: 100-102 (1986)). Methods of preparing monoclonal and polyclonal anti-idiotypic antibodies, which bear the internal image of the polypeptide, are described in U.S. Pat. No. 5,053,224, for example. Briefly, polyclonal anti-idiotypic antibodies can be produced by immunizing animals with monoclonal idiotypic antibodies raised against the polypeptide and screened for reactivity with the polypeptide and screening for antisera, which react with idiotypic antibodies to the polypeptide. Monoclonal antibodies (mAbs) also can be prepared from such animals using standard techniques of immortalizing the antibody-secreting cells of the animal and screening cultures with idiotypic antibodies in competition with the polypeptide. While mAbs are preferred, polyclonal antibodies (pAbs), which are prepared in a variety of mammalian systems, also can be used.

[0092] Another method for inducing an immune response to CIV in a canine is also provided. This method comprises administering to the canine an effective amount of a composition comprising an anti-idiotypic antibody as described above.

[0093] The isolated or purified nucleic acid molecules or vectors comprising them can be used to generate DNA for probes/primers, which can be used to detect the presence or absence of hybridizable DNA or to amplify DNA, such as cDNA.

[0094] Labeled proteins or fragments thereof, as well as labeled nucleic acids or fragments thereof, can be used in assays. Assay methods include fluoroimmunoassays (smith et al., Ann. Clin. Biochem. 18: 253-275 (1981)), radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), and enzyme-multiplied immunoassay technique (EMIT; see *Enzyme Immunoassay*, Maggio, ed., CRC Press, Inc., Boca Raton, FL, 1980. pp. 141-150; 234-235, and 242-243). Such methods can be used to detect the presence of the virus and to diagnose the state of infection.

[0095] The virus, itself, can be used as a vector. The use of viruses as vectors is within the skill in the art.

EXAMPLE

[0096] The following example serves to illustrate the present invention. The example is not intended to limit the scope of the invention in any way. The example describes the identification and partial characterization of a canine influenza virus.

[0097] Outbreaks of acute respiratory disease, characterized by cough, fever, rapid respiration, and hemorrhagic nasal discharge, occurred among greyhounds within two race track compounds located in eastern and western Iowa in April 2005. While a large percentage of affected dogs recovered, many succumbed to hemorrhagic pneumonia.

[0098] Lungs of affected dogs exhibited extensive red to red-black discoloration with moderate to marked palpable firmness and mild fibrinous pleuritis. Lung sections were characterized by severe hemorrhagic interstitial to bronchointerstitial pneumonia. Patchy interstitial change with alveolar septal thickening, coagulums of debris in alveoli, and associated atelectasis were evident. Focally extensive pyogranulomatous bronchointerstitial pneumonia with dilatation of airways by degenerate cells and debris was observed. Scattered vasculitis and vasular thrombi were apparent.

[0099] Microbiological testing for conventional viral and bacterial agents did not reveal any significant pathogens except *Streptococcus equi* subsp. *zooepidemicus*, which was present in lung tissues from all animals examined. Two of four lung samples tested positive for influenza virus using real-time reverse transcriptase-polymerase chain reaction (RT-PCR; Harmon et al., Development of a PCR-based differential test for H1N1 and H3N2 swine influenza viruses. In: *Proceedings of the 42nd Annual Meeting of American Association of Veterinary Laboratory Diagnosticians*. San Diego, CA. October 1999. p. 44.). Immunohistochemistry using monoclonal antibody (mAb) specific for the NP of influenza virus (Vincent et al., J. Vet. Diagn. Invest. 9: 191-195 (1997)) was also positive within viral pneumonic lesions of both lungs as was antigen-capturing ELISA (Directgen™ Flu A, Becton/Dickinson, Sparks, MD) testing on the samples. Bronchioalveolar lavage samples from the two positive lungs tested positive for influenza virus by PCR.

[00100] Virus isolation was attempted because the detection of influenza virus in canine lungs was an unexpected observation, since only a single report of influenza virus infection in dogs existed (Dubovi et al., Isolation of equine influenza virus from racing greyhounds with fatal hemorrhagic pneumonia. In: *Proceedings of the 47th Annual Meeting of American Association of Veterinary Laboratory Diagnosticians*. Greensboro, NC. October

2004. p. 158.). A virus that was able to agglutinate rooster red blood cells was isolated in Madin-Darby canine kidney (MDCK) cells from lung and bronchioalveolar lavage fluid of one of the two animals in which influenza virus was detected by immunohistochemical (IHC) assay and PCR. The isolate was determined by PCR to be influenza virus of H3 subtype. The virus isolate was subtyped as H3N8 using HA-inhibition and NM-inhibition assays. The virus isolate was recognized by antisera raised against various H3 equine influenza viruses, including Miami ((A/Eq/MI/1/63-H3N8) 640-1280), AK((A/Eq/AK/29759/91-H3N8) 320-640), and Kentucky ((A/Eq/Kentucky/81-H3N8) 160-320).

[00101] Sequencing of HA and NA genes of both isolates revealed 100% and 99.8% identity, respectively, between the two isolates. Phylogenetically, the HA gene of the isolates was genetically close (96-98% nucleotide homology) to the HA gene of recent H3N8 equine influenza viruses (Macken et al., The value of a database in surveillance and vaccine selection. In: *Options for the Control of Influenza IV.* Osterhaus et al., eds. Elsevier Science, Amsterdam. 2001. pp. 103-106.). The NA gene of the isolates also showed 96-98% homology with the NA gene of recent H3N8 equine influenza viruses. Since greyhounds in two different race tracks, which are geographically remote in Iowa, simultaneously succumbed to the disease without the involvement of sick horses indicates that the influenza virus isolate is a canine-adapted strain that can perpetuate in and spread among dogs. *S. zooepidemicus*, which has been implicated in respiratory disease and septicemia-associated problems in many different animal species (Wood et al., J. Clin. Microbiol. 43: 120-126 (2005); and Gillespie et al., The General Staphylococcus and Streptococcus. In: *Hagan and Bruner's Infectious Diseases of Domestic Animals.* 7th ed. Comstock/Cornell University Press. Ithaca, NY. 1981. pp. 164-180)), probably contributed to the severity of the disease.

[00102] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[00103] The use of the terms "a," "an," "the," and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within

the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to illuminate better the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[00104] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. It should be understood that the illustrated embodiments are exemplary only, and should not be taken as limiting the scope of the invention.

WHAT IS CLAIMED IS:

1. An isolated canine influenza virus of subtype H3N8 comprising a hemagglutinin (HA) having SEQ ID NO: 4 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 4, with the proviso that the amino acids at positions 94 and 233 are identical to SEQ ID NO: 4.

2. The isolated canine influenza virus of claim 1, which comprises a neuraminidase (NM) comprising the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 2, with the proviso that the amino acids at positions 68 and 134 are identical to SEQ ID NO: 2.

3. The isolated canine influenza virus of claim 1 or 2, which further comprises at least one of the following: a nucleoprotein (NP) having the amino acid sequence of SEQ ID NO: 6 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 6, with the proviso that amino acid 402 is identical to that of SEQ ID NO: 6; a matrix 1 protein (M1) having the amino acid sequence of SEQ ID NO: 8 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 8, with the proviso that amino acid 111 is identical to that of SEQ ID NO: 8; a nonstructural protein 1 (NS1) having the amino acid sequence of SEQ ID NO: 10; a PA protein having the amino acid sequence of SEQ ID NO: 12 or an amino acid sequence that is greater than 98% identical to SEQ ID NO: 12, with the proviso that amino acids 233, 256, 327, and 561 are identical to SEQ ID NO: 12; a PB1 having the amino acid sequence of SEQ ID NO: 14 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 14, with the proviso that amino acids 200 and 213 are identical to SEQ ID NO: 14; and/or PB2 having the amino acid sequence of SEQ ID NO: 16 or an amino acid sequence that is greater than 99% identical to SEQ ID NO: 16, with the proviso that amino acids 107, 221, 292, and 661 are identical to SEQ ID NO: 16.

4. The isolated canine influenza virus of claim 1, which is attenuated.

5. A composition comprising the isolated canine influenza virus of claim 4 in an amount sufficient to induce an immune response.

6. The isolated canine influenza virus of claim 1, which is inactivated.

7. A composition comprising the isolated canine influenza virus of claim 6 in an amount sufficient to induce an immune response.

8. An isolated canine influenza virus of subtype H3N8 deposited with the American Type Culture Collection as Patent Deposit No. PTA-7694.

9. An isolated or purified HA, which (i) has the amino acid sequence of SEQ ID NO: 4 or (ii) is derived from an influenza virus and which has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 4, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 4 at amino acid positions 94 and 233, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 94 or 233 of SEQ ID NO: 4.

10. A composition comprising the isolated or purified HA or fragment thereof of claim 9 in an amount sufficient to induce an immune response in an animal and a biologically acceptable carrier.

11. A method of inducing an immune response to canine influenza virus in an animal, which method comprises administering to the animal the composition of claim 10, whereupon an immune response to canine influenza virus is induced in the animal.

12. An isolated or purified nucleic acid encoding the HA or fragment thereof of claim 9, optionally as part of a vector.

13. The isolated or purified nucleic acid of claim 12, wherein the nucleic acid encoding the HA has the nucleotide sequence of SEQ ID NO: 3 or a fragment thereof encoding at least nine contiguous amino acids.

14. A composition comprising the isolated or purified nucleic acid of claim 12 or 13, which expresses HA or a fragment thereof in an amount sufficient to induce an immune response in an animal, and a biologically acceptable carrier.

15. A method of inducing an immune response to canine influenza virus in an animal, which method comprises administering to the animal the composition of claim 14, whereupon an immune response to canine influenza virus is induced in the animal.

16. An isolated or purified NM, which (i) comprises the amino acid sequence of SEQ ID NO: 2 or (ii) is derived from an influenza virus and which comprises an amino acid sequence that is greater than 99% identical to SEQ ID NO: 2, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 2 at amino acid positions 68 and 134, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 68 or 134 of SEQ ID NO: 2.

17. A composition comprising the isolated or purified NM or fragment thereof of claim 16 in an amount sufficient to induce an immune response in an animal and a biologically acceptable carrier.

18. A method of inducing an immune response to canine influenza virus in an animal, which method comprises administering to the animal the composition of claim 17, whereupon an immune response to canine influenza virus is induced in the animal.

19. An isolated or purified nucleic acid encoding the NM or fragment thereof of claim 16, optionally as part of a vector.

20. The isolated or purified nucleic acid of claim 19, wherein the nucleic acid encoding the NM comprises the nucleotide sequence of SEQ ID NO: 1 or a fragment thereof encoding at least nine contiguous amino acids.

21. A composition comprising the isolated or purified nucleic acid of claim 19 or 20, which expresses NM or a fragment thereof in an amount sufficient to induce an immune response, and a biologically acceptable carrier.

22. A method of inducing an immune response to canine influenza virus in an animal, which method comprises administering to the animal the composition of claim 21, whereupon an immune response to canine influenza virus is induced in the animal.

23. An isolated or purified NP, which (i) has the amino acid sequence of SEQ ID NO: 6 or (ii) is derived from an influenza virus and which has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 6, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 6 at amino acid position 402, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 402 of SEQ ID NO: 6.

24. An isolated or purified nucleic acid encoding the NP or fragment thereof of claim 23, optionally as part of a vector.

25. The isolated or purified nucleic acid of claim 24, wherein the nucleic acid encoding the NP has the nucleotide sequence of SEQ ID NO: 5 or a fragment thereof encoding at least nine contiguous amino acids.

26. An isolated or purified M1, which (i) has the amino acid sequence of SEQ ID NO: 8 or (ii) is derived from an influenza virus and which has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 8, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 8 at amino acid position 111, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 111 of SEQ ID NO: 8.

27. An isolated or purified nucleic acid encoding the M1 or fragment thereof of claim 26, optionally as part of a vector.

28. The isolated or purified nucleic acid of claim 27, wherein the nucleic acid encoding the M1 has the nucleotide sequence of SEQ ID NO: 7 or a fragment thereof encoding at least nine contiguous amino acids.

29. An isolated or purified NS1, which has the amino acid sequence of SEQ ID NO: 10.

30. An isolated or purified nucleic acid encoding the NS1 of claim 29, optionally as part of a vector.

31. The isolated or purified nucleic acid of claim 30, wherein the nucleic acid encoding the NS1 has the nucleotide sequence of SEQ ID NO: 9.

32. An isolated or purified PA protein, which (i) has the amino acid sequence of SEQ ID NO: 12 or (ii) is derived from an influenza virus and which has an amino acid sequence that is greater than 98% identical to SEQ ID NO: 12, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 12 at amino acid positions 233, 256, 327, and 561, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 233, 256, 327, or 561 of SEQ ID NO: 12.

33. An isolated or purified nucleic acid encoding the PA or fragment thereof of claim 32, optionally as part of a vector.

34. The isolated or purified nucleic acid of claim 33, wherein the nucleic acid encoding the PA has the nucleotide sequence of SEQ ID NO: 11 or a fragment thereof encoding at least nine contiguous amino acids.

35. An isolated or purified PB1, which (i) has the amino acid sequence of SEQ ID NO: 14 or (ii) is derived from an influenza virus and which has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 14, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 14 at amino acid positions 200 and 213, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 200 or 213 of SEQ ID NO: 14.

36. An isolated or purified nucleic acid encoding the PB1 or fragment thereof of claim 35, optionally as part of a vector.

37. The isolated or purified nucleic acid of claim 36, wherein the nucleic acid encoding the PB1 has the nucleotide sequence of SEQ ID NO: 13 or a fragment thereof encoding at least nine contiguous amino acids.

38. An isolated or purified PB2, which (i) has the amino acid sequence of SEQ ID NO: 16 or (ii) is derived from an influenza virus and which has an amino acid sequence that is greater than 99% identical to SEQ ID NO: 16, with the proviso that the amino acid sequence is identical to that of SEQ ID NO: 16 at amino acid positions 107, 221, 292, and 661, or a fragment of (i) or (ii), wherein the fragment comprises at least nine contiguous amino acids, at least one of which is identical to the amino acid at position 107, 221, 292, or 661 of SEQ ID NO: 16.

39. An isolated or purified nucleic acid encoding the PB2 or fragment thereof of claim 38, optionally as part of a vector.

40. The isolated or purified nucleic acid of claim 39, wherein the nucleic acid encoding the PB2 has the nucleotide sequence of SEQ ID NO: 15 or a fragment thereof encoding at least nine contiguous amino acids.

NM
AGTTTAAAATGAATCCAAATCAAAAGATAATAGCAATTGGATTTGCATCATTGGG
GATATTAATCATTAATGTCATTCTCCATGTAGTCAGCATTATAGTAACAGTACTG
GTCCTCAATAACAATAGAACAGATCTGAACTGCAAAGGGACGATCATAAGAGAA
TACAATGAAACAGTAAGAGTAGAAAAACTTACTCAATGGTATAATACCAGTACA
ATTAAGTACATAGAGAGACCTTCAAATGAATACTACATGAATAACACTGAACCA
CTTTGTGAGGCCCAAGGCTTTGCACCATTTTCCAAAGATAATGGAATACGAATTG
GGTCGAGAGGCCATGTTTTTGTGATAAGAGAACCTTTTGTATCATGTTCGCCCTC
AGAATGTAGAACCTTTTTCCTCACACAGGGCTCATTACTCAATGACAAACATTCT
AACGGCACAATAAAGGATCGAAGCCCGTATAGGACTTTGATGAGTGTCAAAATA
GGGCAATCACCCAATGTATATCAAGCTAGGTTTGAATCGGTGGCATGGTCAGCA
ACAGCATGCCATGATGGAAAAAAATGGATGACAGTTGGAGTCACAGGGCCCGAC
AATCAAGCAATTGCAGTAGTGAACTATGGAGGTGTTCCGGTTGATACTATTAATT
CATGGGCAGGGGATATTTTAAGAACCCAAGAATCATCATGCACCTGCATTAAAG
GAGACTGTTATTGGGTAATGACTGATGGACCGGCAAATAGGCAAGCTAAATATA
GGATATTCAAAGCAAAAGATGGAAGAGTAATTGGACAAACTGATATAAGTTTCA
ATGGGGGACACATAGAGGAGTGTTCTTGTTACCCCAATGAAGGGAAGGTGGAAT
GCATATGCAGGGACAATTGGACTGGAACAAATAGACCAATTCTGGTAATATCTTC
TGATCTATCGTACACAGTTGGATATTTGTGTGCTGGCATTCCCACTGACACTCCTA
GGGGAGAGGATAGTCAATTCACAGGCTCATGTACAAGTCCTTTGGGAAATAAAG
GATACGGTGTAAAAGGCTTCGGGTTTCGACAAGGAACTGACGTATGGGCCGGAA
GGACAATTAGTAGGACTTCAAGATCAGGATTCGAAATAATAAAAATCAGGAATG
GTTGGACACAGAACAGTAAGGACCAAATCAGGAGGCAAGTGATTATCGATGACC
CAAATTGGTCAGGATATAGCGGTTCTTTCACATTGCCGGTTGAACTGACAAAAAA
GGGATGTTTGGTCCCCTGTTTCTGGGTTGAAATGATTAGAGGTAAACCTGAAGAA
ACAACAATATGGACCTCTAGCAGCTCCATTGTGATGTGTGGAGTAGATCATAAAA
TTGCCAGTTGGTCATGGCACGATGGAGCTATTCTTCCCTTTGACATCGATAAGAT
GTAATTTACGAAAAAAACTCCTTGTTTCTACTA (SEQ ID NO: 1)

FIG. 1

NM - Amino

MNPNQKIIAIGFASLGILIINVILHVVSIIVTVLVLNNNRTDLNCKGTIIREYNETVRVEK
LTQWYNTSTIKYIERPSNEYYMNNTEPLCEAQGFAPFSKDNGIRIGSRGHVFVIREPFV
SCSPSECRTFFLTQGSLLNDKHSNGTIKDRSPYRTLMSVKIGQSPNVYQARFESVAWS
ATACHDGKKWMTVGVTGPDNQAIAVVNYGGVPVDTINSWAGDILRTQESSCTCIKG
DCYWVMTDGPANRQAKYRIFKAKDGRVIGQTDISFNGGHIEECSCYPNEGKVECICR
DNWTGTNRPILVISSDLSYTVGYLCAGIPTDTPRGEDSQFTGSCTSPLGNKGYGVKGF
GFRQGTDVWAGRTISRTSRSGFEIIKIRNGWTQNSKDQIRRQVIIDDPNWSGYSGSFTL
PVELTKKGCLVPCFWVEMIRGKPEETTIWTSSSSIVMCGVDHKIASWSWHDGAILPF
DIDKM (SEQ ID NO: 2)

FIG. 2

HA:

AGCAAAAGCAGGGGATATTTCTGTCAATCATGAAGACAACCATTATTTTAATACT
ACTGACCCATTGGGCCTACAGTCAAAACCCAATCAGTGGCAATAACACAGCCAC
ACTGTGTCTGGGACACCATGCAGTAGCAAATGGAACATTGGTAAAAACAATGAG
TGATGATCAAATTGAGGTGACAAATGCTACAGAATTAGTTCAGAGCATTTCAATG
GGGAAAATATGCAACAAATCATATAGAATTCTAGATGGAAGAAATTGCACATTA
ATAGATGCAATGCTAGGAGACCCCCACTGTGACGCCCTTCAGTATGAGAGTTGG
GACCTCTTTATAGAAAGAAGCAGCGCTTTCAGCAATTGCTACCCATATGACATCC
CTGACTATGCATCGCTCCGATCCATTGTAGCATCCTCAGGAACAGTTGAATTCAC
AGCAGAGGGATTCACATGGACAGGTGTAACTCAAAACGGAAGAAGTGGAGCCTG
CaaAAGGGGATCAGCCGATAGTTTCTTTAGCCGACTGAATTGGCTAACAAAATCT
GGAAGCTCTTACCCCACATTGAATGTGACAATGCCTAACAATAAAAATTTCGACA
AGCTATACATCTGGGGGATTCATCACCCGAGCTCAAATCAAGAGCAGACAAAAT
TGTACATCCAAGAATCAGGACGAGTAACAGTCTCAACAAAAAGAAGTCAACAAA
CAATAATCCCTAACATCGAATCTAGACCGTTGGTCAGAGGTCAATCAGGCAGGA
TAAGCATATACTGGACCATTGTAAAACCTGGAGATATCCTAATGATAAACAGTA
ATGGCAACTTAGTTGCACCGCGGGGATATTTTAAATTGAACACAGGGAAAAGCT
CTGTAATGAGATCCGATGTACCCATAGACATTTGTGTGTCTGAATGTATTACACC
AAATGGAAGCATCTCCAACGACAAGCCATTCCAAAATGTGAACAAAGTTACATA
TGGAAAATGCCCCAAGTATATCAGGCAAAACACTTTAAAGCTGGCCACTGGGAT
GAGGAATGTACCAGAAAAGCAAACCAGAGGAATCTTTGGAGCAATAGCGGGATT
CATCGAAAACGGCTGGGAAGGAATGGTTGATGGGTGGTATGGGTTCCGATATCA
AAACTCTGAAGGAACAGGGCAAGCTGCAGATCTAAAGAGCACTCAAGCAGCCAT
TGACCAGATTAATGGAAAGTTAAACAGAGTGATTGAAAGAACCAATGAGAAATT
CCATCAAATAGAGAAGGAATTCTCAGAAGTAGAAGGAAGAATTCAGGACTTGGA
GAAATATGTAGAAGACACCAAAATAGACCTATGGTCCTACAATGCAGAATTGCT
GGTGGCTCTAGAAAATCAACATACAATTGACTTAACAGATGCAGAAATGAATAA
ATTATTTGAGAAGACTAGACGCCAGTTAAGAGAAAACGCAGAAGACATGGGAGG
TGGATGTTTCAAGATTTACCACAAATGTGATAATGCATGCATTGAATCAATAAGA
ACTGGGACATATGACCATTACATATACAGAGATGAAGCATTAAACAACCGATTT
CAGATCAAAGGTGTAGAGTTGAAATCAGGCTACAAAGATTGGATACTGTGGATT
TCATTCGCCATATCATGCTTCTTAATTTGCGTTGTTCTATTGGGTTTCATTATGTGG
GCTTGCCAAAAAGGCAACATCAGATGCAACATTTGCATTTGAGTAAACTGATAGT
TAAAAACACCCTTGTTTCTACT (SEQ ID NO:3)

FIG. 3

HA - Amino

MKTTIILILLTHWAYSQNPISGNNTATLCLGHHAVANGTLVKTMSDDQIEVTNATEL
VQSISMGKICNKSYRILDGRNCTLIDAMLGDPHCDALQYESWDLFIERSSAFSNCYPY
DIPDYASLRSIVASSGTVEFTAEGFTWTGVTQNGRSGACKRGSADSFFSRLNWLTKS
GSSYPTLNVTMPNNKNFDKLYIWGIHHPSSNQEQTKLYIQESGRVTVSTKRSQQTIIP
NIESRPLVRGQSGRISIYWTIVKPGDILMINSNGNLVAPRGYFKLNTGKSSVMRSDVPI
DICVSECITPNGSISNDKPFQNVNKVTYGKCPKYIRQNTLKLATGMRNVPEKQTRGIF
GAIAGFIENGWEGMVDGWYGFRYQNSEGTGQAADLKSTQAAIDQINGKLNRVIERT
NEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDAEMN
KLFEKTRRQLRENAEDMGGGCFKIYHKCDNACIESIRTGTYDHYIYRDEALNNRFQI
KGVELKSGYKDWILWISFAISCFLICVVLLGFIMWACQKGNIRCNICI (SEQ ID NO: 4)

FIG. 4

NP

CAGGGAGCAAAAGCAGGGTAGATAATCACTCACTGAGTGACATCAAAGTCATGG
CGTCTCAAGGCACCAAACGATCCTATGAACAGATGGAAACTGATGGGGAACGCC
AGAATGCAACTGAAATCAGAGCATCTGTCGGAAGGATGGTGGGAGGAATCGGAC
GGTTTTATGTCCAGATGTGTACTGAGCTTAAACTAAACGACCATGAAGGGCGGCT
GATTCAGAACAGCATAACAATAGAAAGGATGGTACTTTCAGCATTCGACGAAAG
AAGAAACAAGTATCTCGAGGAGCATCCCAGTGCTGGGAAAGACCCTAAGAAAAC
GGGAGGCCCGATATACAGAAGAAAAGATGGGAAATGGATGAGGGAACTCATCC
TCCATGATAAAGAAGAAATCATGAGAATCTGGCGTCAGGCCAACAATGGTGAAG
ACGCTACTGCTGGTCTTACTCATATGATGATCTGGCACTCCAATCTCAATGACAC
CACATACCAAAGAACAAGGGCTCTTGTTCGGACTGGGATGGATCCCAGAATGTG
CTCTCTGATGCAAGGCTCAACCCTCCCACGGAGATCTGGAGCCGCTGGTGCTGCA
GTAAAAGGTGTTGGAACAATGGTAATGGAACTCATCAGGATGATCAAACGCGGA
ATAAATGATCGGAATTTCTGGAGAGGTGAAAATGGTCGAAGAACCAGAATTGCT
TATGAAAGAATGTGCAATATCCTCAAAGGGAAATTTCAGACAGCAGCACAACGG
GCTATGATGGACCAGGTGAGGGAAGGCCGCAATCCTGGAAACGCTGAGATTGAG
GATCTCATTTTCTTGGCACGATCAGCACTTATTTTGAGAGGATCAGTAGCCCATA
AATCATGCCTACCTGCCTGTGTTTATGGCCTTGCAGTAACCAGTGGGTATGACTTT
GAGAAGGAAGGATACTCTCTGGTTGGAATTGATCCTTTCAAACTACTCCAGAACA
GTCAAATTTTCAGTCTAATCAGACCAAAAGAAAACCCAGCACACAAAAGCCAGT
TGGTGTGGATGGCATGCCATTCTGCAGCATTTGAGGATCTGAGAGTTTTAAATTT
CATTAGAGGAACCAAAGTAATCCCAAGAGGACAGTTAACAACCAGAGGAGTTCA
AATTGCTTCAAATGAAAACATGGAGACAATAAATTCTAGCACACTTGAACTGAG
AAGCAAATATTGGGCAATAAGGACCAGAAGCGGAGGAAACACCAGTCAACAGA
GAGCATTTGCAGGACAGATAAGTGTGCAACCTACTTTCTCAGTACAGAGAAATCT
TCCCTTTGAGAGAGCAACCATTATGGCTGCATTCACTGGTAACACTGAAGGGAGG
ACTTCCGACATGAGAACGGAAATCATAAGGATGATGGAAAATGCCAAATCAGAA
GATGTGTCTTTCCAGGGGCGGGGAGTCTTCGAGCTCTCGGACGAAAAGGCAACG
AACCCGATCGTGCCTTCCTTTGACATGAGCAATGAAGGGTCTTATTTCTTCGGAG
ACAATGCTGAGGAGTTTGACAGTTAAAGAAAAATACCCTTGTTTCTACTAATACG
AGACGATAT (SEQ ID NO: 5)

FIG. 5

NP - Amino

MASQGTKRSYEQMETDGERQNATEIRASVGRMVGGIGRFYVQMCTELKLNDHEGR
LIQNSITIERMVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRRKDGKWMRELILHD
KEEIMRIWRQANNGEDATAGLTHMMIWHSNLNDTTYQRTRALVRTGMDPRMCSL
MQGSTLPRRSGAAGAAVKGVGTMVMELIRMIKRGINDRNFWRGENGRRTRIAYER
MCNILKGKFQTAAQRAMMDQVREGRNPGNAEIEDLIFLARSALILRGSVAHKSCLPA
CVYGLAVTSGYDFEKEGYSLVGIDPFKLLQNSQIFSLIRPKENPAHKSQLVWMACHS
AAFEDLRVLNFIRGTKVIPRGQLTTRGVQIASNENMETINSSTLELRSKYWAIRTRSG
GNTSQQRAFAGQISVQPTFSVQRNLPFERATIMAAFTGNTEGRTSDMRTEIIRMMEN
AKSEDVSFQGRGVFELSDEKATNPIVPSFDMSNEGSYFFGDNAEEFDS (SEQ ID NO:
6)

FIG. 6

M1

TATTCGTCTCAGGGAGCAAAAGCAGGTAGATATTTAAAGATGAGTCTTCTAACCG
AGGTCGAAACGTACGTTCTCTCTATCGTACCATCAGGCCCCCTCAAAGCCGAGAT
CGCGCAGAGACTTGAAGATGTCTTTGCGGGAAAGAACACCGATCTTGAGGCACT
CATGGAATGGCTAAAGACAAGACCAATCCTGTCACCTCTGACTAAAGGGATTTTA
GGATTTGTATTCACGCTCACCGTGCCCAGTGAGCGAGGACTGCAGCGTAGACGCT
TTGTCCAAAATGCCCTTAGTGGAAACGGAGATCCAAACAACATGGACAGAGCAG
TAAAACTGTACAGGAAGCTTAAAAGAGAAATAACATTCCATGAGGCAAAAGAGG
TGGCACTCAGCTATTCCACTGGTGCACTAGCCAGCTGCATGGGACTCATATACAA
CAGAATGGGAACTGTTACAACCGAAGTGGCATTTGGCCTGGTATGCGCCACATGT
GAACAGATTGCTGATTCCCAGCATCGATCTCACAGGCAGATGGTGACAACAACC
AACCCATTAATCAGACATGAAAACAGAATGGTATTAGCCAGTACCACGGCTAAA
GCCATGGAACAGATGGCAGGATCGAGTGAGCAGGCAGCAGAGGCCATGGAGGT
TGCTAGTAGGGCTAGGCAGATGGTACAGGCAATGAGAACCATTGGGACCCACCC
TAGCTCCAGTGCCGGTTTGAAAGATGATCTCCTTGAAAATTTACAGGCCTACCAG
AAACGGATGGGAGTGCAAATGCAGCGATTCAAGTGATCCTCTCGTTATTGCAGC
AAGTATCATTGGAATCTTGCACTTGATATTGTGGATTCTTGATCGTCTTTTCTTCA
AATTCATTTATCGTCGCCTTAAATACGGGTTGAAAAGAGGGCCTTCTACGGAAGG
AGTACCTGAGTCTATGAGGGAAGAATATCGGCAGGAACAGCAGAATGCTGTGGA
TGTTGACGATGGTCATTTTGTCAACATAGAGCTGGAGTAAAAAACTACCTTGTTT
CTACTAATACGAGACGATAT (SEQ ID NO: 7)

FIG. 7

M1 - Amino

MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEALMEWLKTRPILSPLTKG
ILGFVFTLTVPSERGLQRRRFVQNALSGNGDPNNMDRAVKLYRKLKREITFHEAKEV
ALSYSTGALASCMGLIYNRMGTVTTEVAFGLVCATCEQIADSQHRSHRQMVTTTNP
LIRHENRMVLASTTAKAMEQMAGSSEQAAEAMEVASRARQMVQAMRTIGTHPSSS
AGLKDDLLENLQAYQKRMGVQMQRFK (SEQ ID NO: 8)

FIG. 8

NS1

GGAGCAAAAGCAGGGTGACAAAAACATAATGGATTCCAACACTGTGTCAAGCTT
TCAGGTAGACTGTTTTCTTTGGCATGTCCGCAAACGATTCGCAGACCAAGAACTG
GGTGATGCCCCATTCCTTGACCGGCTTCGCCGAGACCAGAAGTCCCTAAGGGGA
AGAGGTAGCACTCTTGGTCTGGACATCGAAACAGCCACTCATGCAGGAAAGCAG
ATAGTGGAGCAGATTCTGGAAAAGGAATCAGATGAGGCACTTAAAATGACCATT
GCCTCTGTTCCTGCTTCACGCTACTTAACTGACATGACTCTTGATGAGATGTCAAG
AGACTGGTTCATGCTCATGCCCAAGCAAAAAGTAACAGGCTCCCTATGTATAAG
AATGGACCAAGCAATCATGGATAAGAACATCATACTTAAAGCAAACTTTAGTGT
GATTTTCGAAAGGCTGGAAACACTAATACTACTTAGAGCCTTCACCGAAGAAGG
AGCAGTCGTTGGCGAAATTTCACCATTACCTTCTCTTCCAGGACATACTAATGAG
GATGTCAAAAATGCAATTGGGGTCCTCATCGGAGGACTTAAATGGAATGATAAT
ACGGTTAGAATCTCTGAAACTCTACAGAGATTCGCTTGGAGAAGCAGTCATGAA
AATGGGAGACCTTCATTCCCTTCAAAGCAGAAACGAAAAATGGAGAGAACAATT
AAGCCAGAAATTTGAAGAAATAAGATGGTTGATTGAAGAAGTGCGACATAGATT
GAAAAATACAGAAAATAGTTTTGAACAAATAACATTTATGCAAGCCTTACAACT
ATTGCTTGAAGTAGAACAAGAGATAAGAACTTTCTCGTTTCAGCTTATTTAATGA
T (SEQ ID NO: 9)

FIG. 9

NS1 - Amino

MDSNTVSSFQVDCFLWHVRKRFADQELGDAPFLDRLRRDQKSLRGRGSTLGLDIET
ATHAGKQIVEQILEKESDEALKMTIASVPASRYLTDMTLDEMSRDWFMLMPKQKVT
GSLCIRMDQAIMDKNIILKANFSVIFERLETLILLRAFTEEGAVVGEISPLPSLPGHTNE
DVKNAIGVLIGGLKWNDNTVRISETLQRFAWRSSHENGRPSFPSKQKRKMERTIKPEI
(SEQ ID NO: 10)

FIG. 10

PA

TAAATGGAAGACTTTGTGCGACAGTGCTTCAATCCAATGATCGTCGAGCTTGCGG
AAAAGGCAATGAAAGAATATGGAGAGAACCCGAAAATCGAAACAAACAAATTT
GCAGCAATATGCACTCACTTGGAAGTCTGCTTCATGTACTCGGATTTCCACTTTAT
AAATGAACTGGGTGAGTCAGTGGTCATAGAGTCTGGTGACCCAAATGCTCTTTTG
AAACACAGATTTGAAATCATTGAGGGGAGAGATCGAACAATGGCATGGACAGTA
GTAAACAGCATCTGCAACACCACAAGAGCTGAAAAACCTAAATTTCTTCCAGATT
TATACGACTATAAGGAGAACAGATTTGTTGAAATTGGTGTGACAAGGAGAGAAG
TTCACATATACTACCTGGAGAAAGCCAACAAAATAAAGTCTGAGAAAACACATA
TCCACATTTTCTCATTTACAGGAGAAGAAATGGCTACAAAAGCGGACTATACTCT
TGATGAAGAGAGTAGAGCCAGGATCAAGACCAGACTATTCACTATAAGACAAGA
AATGGCCAGTAGAGGCCTCTGGGATTCCTTTCGTCAGTCCGAGAGAGGCGAAGA
GACAATTGAAGAAAGATTTGAAATCACAGGAACGATGCGCAAGCTTGCCAATTA
CAGTCTCCCACCGAACTTCTCCAGCCTTGAAAATTTTAGAGTCTATATAGATGGA
TTCGAACCGAACGGCTGCATTGAGAGTAAGCTTTCTCAAATGTCCAAAGAAGTA
AATGCCAAAATCGAACCATTTTCAAAGACAACACCCCGACCACTCAAAATGCCA
GGTGGTCCACCCTGCCATCAGCGATCCAAATTCTTGCtAATGGATGCTCTGAAACT
GAGCATTGAGGACCCAAGTCACGAGGGAGAGGGGATACCACTATATGATGCAAT
CAAATGCATGAAAACTTTCTTTGGATGGAAAGAGCCCAGTATTGTTAAACCACAT
AAAAAGGGTATAAACCCGAACTATCTCCAAACTTGGAAGCAAGTATTAGAAGAA
ATACAAGACCTTGAGAACGAAGAAAGGACCCCCAAGACCAAGAATATGAAAAA
AACAAGCCAATTGAAATGGGCACTAGGTGAAAATATGGCACCAGAGAAAGTGG
ATTTTGAGGATTGTAAAGACATCAATGATTTAAAACAATATGACAGTGATGAGCC
AGAAGCAAGGTCTCTTGCAAGTTGGATTCAAAGTGAGTTCAACAAGGCTTGTGA
GCTGACAGATTCAAGCTGGATAGAGCTCGATGAAATTGGGGAGGATGTCGCCCC
AATAGAATACATTGCGAGCATGAGGAGAAATTATTTTACTGCTGAGATTTCCCAT
TGTAGAGCAACAGAATATATAATGAAAGGAGTATACATCAACACTGCTCTACTC
AATGCATCCTGTGCTGCGATGGATGAATTTCAATTAATTCCGATGATAAGTAAAT
GCAGGACCAAAGAAGGGAGAAGGAAAACAAATTTATATGGATTCATAATAAAG
GGAAGGTCCCATTTAAGAAATGATACTGACGTGGTGAACTTTGTAAGTATGGAAT
TTTCTCTCACTGATCCAAGATTTGAGCCACACAAATGGGAAAAATACTGCGTTCT
AGAAATTGGAGACATGCTTCTAAGAACTGCTGTAGGTCAAGTGTCAAGACCCAT
ATTTTTGTATGTAAGGACAAATGGAACCTCTAAAATTAAAATGAAATGGGGAAT
GGAAATGAGACGCTGCCTCCTTCAGTCTCTGCAACAGATTGAAAGCATGATCGA
AGCTGAGTCCTCAGTCAAAGAAAAGGACATGACCAAAGAATTTTTTGAGAACAA
ATCAGAGACATGGCCTATAGGAGAGTCCCCCAAAGGAGTGGAAGAGGGCTCAAT
CGGGAAGGTTTGCAGGACCTTATTAGCAAAATCTGTGTTTAACAGTTTATATGCA
TCTCCACAACTGGAAGGATTTTCAGCTGAATCTAGGAAATTACTTCTCATTGTTC
AGGCTCTTAGAGATGACCTGGAACCTGGAACCTTTGATATTGGGGGGTTATATGA
ATCAATTGAGGAGTGCCTGATTAATGATCCCTGGGTTTTGCTTAATGCATCTTGGT
TCAACTCCTTCCTCACACATGCACTGAAGTAGTTGTGGCAATGCTACTATTTGTTA
TCCATACTGTCCA (SEQ ID NO: 11)

FIG. 11

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PA - Amino

MEDFVRQCFNPMIVELAEKAMKEYGENPKIETNKFAAICTHLEVCFMYSDFHFINEL
GESVVIESGDPNALLKHRFEIIEGRDRTMAWTVVNSICNTTRAEKPKFLPDLYDYKEN
RFVEIGVTRREVHIYYLEKANKIKSEKTHIHIFSFTGEEMATKADYTLDEESRARIKTR
LFTIRQEMASRGLWDSFRQSERGEETIEERFEITGTMRKLANYSLPPNFSSLENFRVYI
DGFEPNGCIESKLSQMSKEVNAKIEPFSKTTPRPLKMPGGPPCHQRSKFLLMDALKLS
IEDPSHEGEGIPLYDAIKCMKTFFGWKEPSIVKPHKKGINPNYLQTWKQVLEEIQDLE
NEERTPKTKNMKKTSQLKWALGENMAPEKVDFEDCKDINDLKQYDSDEPEARSLAS
WIQSEFNKACELTDSSWIELDEIGEDVAPIEYIASMRRNYFTAEISHCRATEYIMKGVY
INTALLNASCAAMDEFQLIPMISKCRTKEGRRKTNLYGFIIKGRSHLRNDTDVVNFVS
MEFSLTDPRFEPHKWEKYCVLEIGDMLLRTAVGQVSRPIFLYVRTNGTSKIKMKWG
MEMRRCLLQSLQQIESMIEAESSVKEKDMTKEFFENKSETWPIGESPKGVEEGSIGKV
CRTLLAKSVFNSLYASPQLEGFSAESRKLLLIVQALRDDLEPGTFDIGGLYESIEECLIN
DPWVLLNASWFNSFLTHALK (SEQ ID NO: 12)

FIG. 12

PB1

GAAAGCAGGCAAACCATTTGAATGGATGTCAATCCGACTCTACTTTTCTTAAAGG
TGCCAGCGCAAAATGCTATAAGCACAACATTCCCTTATACTGGAGATCCTCCCTA
CAGTCATGGAACAGGGACAGGATACACCATGGATACTGTCAACAGAACACACCA
ATATTCAGAAAAAGGGAAATGGACAACAAACACTGAGATTGGAGCACCACAACT
TAATCCAATCGATGGACCACTTCCTGAAGACAATGAACCAAGTGGGTACGCCCA
AACAGATTGTGTATTGGAAGCAATGGCTTTCCTTGAAGAATCCCATCCCGGAATC
TTTGAAAATTCGTGTCTTGAAACGATGGAGGTGATTCAGCAGACAAGAGTGGAC
AAACTAACACAAGGCCGACAAACTTATGATTGGACCTTGAATAGGAATCAACCT
GCCGCAACAGCACTTGCTAATACGATTGAAGTATTCAGATCAAATGGTCTGACTT
CCAATGAATCGGGGAGATTGATGGACTTCCTCAAAGATGTCATGGAGTCCATGA
ACAAGGAGGAAATGGAAATAACAACACACTTCCAACGGAAGAGAAGAGTAAGA
GACAACATGACAAAGAGAATGATAACACAGAGAACCATAGGGAAGAAAAAACA
ACGATTAAGCAGAAAGAGCTATCTAATCAGAACATTAACCCTAAACACAATGAC
CAAGGACGCTGAAAGAGGGAAATTGAAACGACGAGCAATCGCTACCCCAGGGA
TGCAGATAAGAGGATTTGTATATTTTGTTGAAACACTAGCTCGAAGAATATGTGA
AAAGCTTGAACAATCAGGATTGCCAGTTGGCGGTAATGAGAAAAAGGCCAAACT
GGCTAATGTCGTCAGAAAAATGATGACTAATTCCCAAGACACTGAACTCTCCTTC
ACCATCACTGGGGACAATACCAAATGGAATGAAAATCAGAACCCACGCATATTC
CTGGCAATGATCACATACATAACTAGAAATCAGCCAGAATGGTTCAGAAATGTT
CTAAGCATTGCACCGATTATGTTCTCAAATAAAATGGCAAGACTGGGGAAAGGA
TATATGTTTGAAAGCAAAAGTATGAAATTGAGAACTCAAATACCAGCAGAAATG
CTAGCAAGCATTGACCTAAAATATTTCAATGATTCAACAAAAAAGAAAATTGAA
AAGATACGACCACTCCTGGTTGACGGGACTGCTTCACTGAGTCCTGGCATGATGA
TGGGAATGTTCAACATGTTGAGCACTGTGCTGGGTGTATCCATATTAAACCTGGG
CCAGAGGAAATATACAAAGACCACATACTGGTGGGATGGTCTGCAATCATCCGA
TGACTTTGCTTTGATAGTGAATGCGCCTAATCATGAAGGAATACAAGCTGGAGTA
GACAGATTCTATAGAACTTGCAAACTGGTCGGGATCAACATGAGCAAAAAGAAG
TCCTACATAAATAGAACTGGAACATTCGAATTCACAAGCTTTTTCTACCGGTATG
GTTTTGTAGCCAATTTCAGCATGGAACTACCCAGTTTTGGGGTTTCCGGAATAAA
TGAATCTGCAGACATGAGCATTGGAGTGACAGTCATCAAAAACAACATGATAAA
TAATGATCTCGGTCCTGCCACGGCACAAATGGYACTCCAACTCTTCATTAAGGAT
TATCGGTACACATACCGGTGCCATAGAGGTGATACCCAGATACAAACCAGAAGA
TCTTTTGAGTTGAAGAAACTGTGGGAACAGACTCGATCAAAGACTGGTCTACTGG
TATCAGATGGGGGTCCAAACCTATATAACATCAGAAACCTACACATCCCGGAAG
TCTGTTTAAAATGGGAGCTAATGGATGAAGATTATAAGGGGAGGCTATGCAATC
CATTGAATCCTTTCGTTAGTCACAAAGAAATTGAATCAGTCAACAGTGCAGTAGT
AATGCCTGCGCATGGCCCTGCCAAAAGCATGGAGTATGATGCTGTtGCAACAACA
CATTCTTGGATCCCCAAGAGGAACCGGTCCATATTGAACACAAGCCAAAGGGGA
ATACTAGAAGATGAGCAGATGTATCAGAAATGCTGCAACCTGTTTGAAAAATTCT
TCCCCAGCAGCTCATACAGAAGACCAGTCGGAATTTCTAGTATGGTTGAGGCCAT
GGTATCCAGGGCCCGCATTGATGCACGAATTGACTTCGAATCTGGACGGATAAA
GAAGGATGAGTTCGCTGAGATCATGAAGATCTGTTCCACCATTGAAGAGCTCAG
ACGGCAAAAATAGTGAA (SEQ ID NO: 13)

FIG. 13

PB1 - Amino

MDVNPTLLFLKVPAQNAISTTFPYTGDPPYSHGTGTGYTMDTVNRTHQYSEKGKWT
TNTEIGAPQLNPIDGPLPEDNEPSGYAQTDCVLEAMAFLEESHPGIFENSCLETMEVIQ
QTRVDKLTQGRQTYDWTLNRNQPAATALANTIEVFRSNGLTSNESGRLMDFLKDV
MESMNKEEMEITTHFQRKRRVRDNMTKRMITQRTIGKKKQRLSRKSYLIRTLTLNT
MTKDAERGKLKRRAIATPGMQIRGFVYFVETLARRICEKLEQSGLPVGGNEKKAKL
ANVVRKMMTNSQDTELSFTITGDNTKWNENQNPRIFLAMITYITRNQPEWFRNVLSI
APIMFSNKMARLGKGYMFESKSMKLRTQIPAEMLASIDLKYFNDSTKKKIEKIRPLLV
DGTASLSPGMMMGMFNMLSTVLGVSILNLGQRKYTKTTYWWDGLQSSDDFALIVN
APNHEGIQAGVDRFYRTCKLVGINMSKKKSYINRTGTFEFTSFFYRYGFVANFSMELP
SFGVSGINESADMSIGVTVIKNNMINNDLGPATAQMXLQLFIKDYRYTYRCHRGDTQ
IQTRRSFELKKLWEQTRSKTGLLVSDGGPNLYNIRNLHIPEVCLKWELMDEDYKGRL
CNPLNPFVSHKEIESVNSAVVMPAHGPAKSMEYDAVATTHSWIPKRNRSILNTSQRGI
LEDEQMYQKCCNLFEKFFPSSSYRRPVGISSMVEAMVSRARIDARIDFESGRIKKDEF
AEIMKICSTIEELRRQK (SEQ ID NO: 14)

FIG. 14

PB2

TATTGGTCTCAGGGAGCGAAAGCAGGTCAAATATATTCAATATGGAGAGAATAA
AAGAACTGAGAGATCTGATGTTACAATCCCGCACCCGCGAGATACTAACAAAAA
CTACTGTGGACCACATGGCCATAATCAAGAAATACACATCAGGAAGACAAGAGA
AGAACCCTGCACTTAGGATGAAATGGATGATGGCAATGAAATACCCAATTACAG
CAGATAAGAGGATAATGGAGATGATTCCTGAGAGAAATGAACAGGGACAAACC
CTTTGGAGCAAAACGAACGATGCTGGCTCAGACCGCGTAATGGTATCACCTCTGG
CAGTGACATGGTGGAATAGGAATGGACCAACAACGAACACAATTCATTATCCGA
AAGTCTACAAAACTTATTTTGAAAAGGTTGAAAGATTGAAACACGGAACCTTTG
GCCCCGTTCATTTTAGGAATCAAGTCAAGATAAGACGAAGAGTTGATGTAAACC
CTGGTCACGCGGACCTCAGTGCTAAAGAAGCACAAGATGTGATCATGGAAGTTG
TTTTCCCAAATGAAGTGGGAGCCAGAATTCTAACATCAGAATCACAACTAACAAT
AACCAAAGAGAAAAAGGAAGAACTTCAGGACTGCAAAATTGCTCCCTTGATGGT
AGCATACATGCTAGAAAGAGAGTTGGTCCGAAAAACAAGGTTCCTCCCAGTAGT
AGGCGGAACAAGCAGTGTATACATTGAAGTGTTGCATCTGACTCAGGGAACATG
CTGGGAGCAAATGTACACCCCAGGAGGAGAAGTTAGAAACGATGATATTGATCA
AAGTTTAATTATTGCAGCCCGGAACATAGTGAGAAGAGCAACAGTATCAGCAGA
TCCACTAGCATCCCTACTGGAAATGTGCCACAGTACACAGATTGGTGGAACAAG
GATGGTAGACATCCTTAAGCAGAACCCAACAGAGGAACAAGCTGTGGATATATG
CAAAGCAGCAATGGGATTGAGAATTAGCTCATCATTCAGCTTTGGTGGATTCACC
TTCAAAAGGACAAGTGGATCATCAGTCAAGAGAGAAGAAGAAATGCTTACGGGC
AACCTTCAAACATTGAAAATAAGAGTGCATGAGGGCTATGAAGAATTCACAATG
GTCGGAAGAAGAGCAACAGCCATTATCAGAAAGGCAACCAGAAGATTGATTCAA
TTGATAGTAAGTGGGAGAGATGAACAATCAATTGCTGAAGCAATAATTGTAGCC
ATGGTGTTTTCGCAAGAAGATTGCATGATAAAAGCAGTTCGAGGCGATTTGAACT
TTGTTAATAGAGCAAATCAGCGTTTGAACCCCATGCATCAACTCTTGAGGCATTT
CCAAAAAGATGCAAAAGTGCTTTTCCAAAATTGGGGAATTGAACCCATCGACAA
TGTAATGGGGATGATTGGAATATTGCCTGACATGACCCCAAGCACCGAGATGTC
ATTGAGAGGAGTGAGAGTCAGCAAAATGGGAGTGGATGAGTACTCCAGCACTGA
GAGAGTGGTGGTGAGCATTGACCGTTTTTTTAAGAGTTCGGGATCAAAGGGGAAA
CATACTACTGTCCCCTGAAGAAGTCAGTGAAACACAAGGAACGGAAAAGCTGAC
AATAATTTATTCGTCATCAATGATGTGGGAGATTAATGGTCCCGAATCAGTGTTG
GTCAATACTTATCAATGGATCATCAGAAACTGGGAAATTGTAAAAATTCAGTGGT
CACAGGACCCCACAATGTTATACAATAAGATAGAATTTGAACCATTCCAATCCCT
GGTCCCTAGGGCCACCAGAAGCCAATACAGCGGTTTCGTAAGAACCCTGTTTCAG
CAAATGCGAGATGTACTTGGAACATTTGATACTGCTCAAATAATAAAACTCCTCC
CTTTTGCCGCTGCTCCTCCGGAACAGAGTAGGATGCAGTTCTCTTCTTTGACTGTT
AATGTAAGAGGTTCGGGAATGAGGATACTTGTAAGAGGCAATTCCCCGGTGTTC
AACTACAATAAAGTCACTAAAAGGCTCACAGTCCTCGGAAAGGATGCAGGTGCG
CTTACTGAGGACCCAGATGAAGGTACGGCTGGAGTAGAATCTGCTGTTCTAAGA
GGGTTTCTCATTTTAGGTAAAGAAAACAAGAGATATGGCCCAGCACTAAGCATC
AATGAACTTAGCAAACTTGCAAAAGGGGAGAAAGCCAATGTACTAATTGGGCAA
GGGGACGTAGTGTTGGTAATGAAACGGAAACGTGACTCTAGCATACTTACTGAC
AGCCAGACAGCGACCAAAAGGATTCGGATGGCCATCAATTAGTGTTGAATTGTTT
AAAAACGACCTTGTTTCTACTAATACGAGACCATAT (SEQ ID NO: 15)

FIG. 15

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PB2 - Amino

MERIKELRDLMLQSRTREILTKTTVDHMAIIKKYTSGRQEKNPALRMKWMMAMKY
PITADKRIMEMIPERNEQGQTLWSKTNDAGSDRVMVSPLAVTWWNRNGPTTNTIHY
PKVYKTYFEKVERLKHGTFGPVHFRNQVKIRRRVDVNPGHADLSAKEAQDVIMEVV
FPNEVGARILTSESQLTITKEKKEELQDCKIAPLMVAYMLERELVRKTRFLPVVGGTS
SVYIEVLHLTQGTCWEQMYTPGGEVRNDDIDQSLIIAARNIVRRATVSADPLASLLE
MCHSTQIGGTRMVDILKQNPTEEQAVDICKAAMGLRISSSFSFGGFTFKRTSGSSVKR
EEEMLTGNLQTLKIRVHEGYEEFTMVGRRATAIIRKATRRLIQLIVSGRDEQSIAEAII
VAMVFSQEDCMIKAVRGDLNFVNRANQRLNPMHQLLRHFQKDAKVLFQNWGIEPI
DNVMGMIGILPDMTPSTEMSLRGVRVSKMGVDEYSSTERVVVSIDRFLRVRDQRGNI
LLSPEEVSETQGTEKLTIIYSSSMMWEINGPESVLVNTYQWIIRNWEIVKIQWSQDPT
MLYNKIEFEPFQSLVPRATRSQYSGFVRTLFQQMRDVLGTFDTAQIIKLLPFAAAPPE
QSRMQFSSLTVNVRGSGMRILVRGNSPVFNYNKVTKRLTVLGKDAGALTEDPDEGT
AGVESAVLRGFLILGKENKRYGPALSINELSKLAKGEKANVLIGQGDVVLVMKRKR
DSSILTDSQTATKRIRMAIN (SEQ ID NO: 16)

FIG. 16