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(57) Abstract: An isolated paramyxovirus, a morbillivirus (FmoPV), isolated nucleic acids encoding the genome of FmoPV, isolated amino acid sequences of FmoPV protein, antibodies to FmoPV and its proteins, and uses thereof are provided. In certain embodiments, the modified FmoPV is a feline morbillivirus. A recombinant FmoPV comprising a modified FmoPV gene or gene segments and the use of such virus are provided. The recombinant FmoPV may be used in the prevention and/or treatment of diseases related to FmoPV or as a delivery vector. A diagnostic assay for the FmoPV is provided. In certain embodiments, the FmoPV causes kidney disease. In certain embodiments, the kidney disease is in felines. In certain embodiments, the kidney disease is tubulo interstitial nephritis ("TIN"). A quantitative assay for the detection of the FmoPV, natural or artificial variants, analogs, or derivatives thereof is provided. In certain embodiments, the quantitative assay is reverse transcription and polymerase chain reaction (RT-PCR). A vaccine and a kit containing the vaccine for the prevention and treatment of FmoPV infection are provided. A diagnosis kit that comprises nucleic acid molecules for the detection of the FmoPV is also provided.



A NOVEL PARAMYXOVIRUS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. patent application Serial No. 61/588,778, filed January 20, 2012, which is hereby incorporated by reference in its entirety.

1. TECHNICAL FIELD

Described herein are isolated paramyxovirus, a morbillivirus (FmoPV), isolated nucleic acids encoding the genome of FmoPV, isolated amino acid sequences of FmoPV proteins, antibodies to FmoPV and its proteins, and uses thereof. In certain embodiments, the modified FmoPV is a feline morbillivirus. Also described herein is a recombinant FmoPV comprising a modified FmoPV gene or gene segments and the use of such a virus. The recombinant FmoPV may be used in the prevention and/or treatment of diseases related to FmoPV or as a delivery vector. Also described herein is a diagnostic assay for the FmoPV. In certain embodiments, the FmoPV causes kidney disease. In certain embodiments, the kidney disease is in felines. In certain embodiments, the kidney disease is tubulointerstitial nephritis ("TIN"). Also described herein is a quantitative assay for the detection of the FmoPV, natural or artificial variants, analogs, or derivatives thereof. In certain embodiments, the quantitative assay is reverse transcription and polymerase chain reaction (RT-PCR). Also described herein is a vaccine and a kit containing the vaccine for the prevention and treatment of FmoPV infection. Described herein is a diagnostic kit that comprises nucleic acid molecules for the detection of the FmoPV.

2. BACKGROUND OF THE INVENTION

Paramyxoviruses are enveloped, negative-sense single-stranded RNA viruses that are divided into two subfamilies, *Paramyxovirinae* and *Pneumovirinae*. Viruses in the subfamily *Paramyxovirinae* have been associated with a number of emerging diseases in humans and various animals in the past two decades (1-9). There are currently five genera within the subfamily *Paramyxovirinae*, namely *Respirovirus*, *Rubulavirus*, *Morbillivirus*, *Henipavirus* and *Avulavirus*, although some members of the subfamily remain unclassified. Among members of *Paramyxovirinae*, measles virus, mumps virus, and human parainfluenza viruses 1 to 4 are most

well known human paramyxoviruses which cause outbreaks of respiratory to systemic infections (10-12). Three novel rubulaviruses, Tuhoko virus 1, 2 and 3, from fruit bats in mainland China and a novel unclassified paramyxovirus, Tailam virus, from Sikkim rats in Hong Kong were recently reported (13,14). Despite the presence of paramyxoviruses in a variety of animals, no paramyxoviruses have been naturally observed in cats, although there is controversial evidence that cats may be infected with parainfluenza 5 virus (15,16).

Cats and dogs are the most common domestic animals and pets worldwide. As a result of their close relatedness, interspecies jumping of viruses among these two kinds of animals is not uncommon. For coronaviruses, feline coronavirus and canine coronavirus are classified under the same species *Alphacoronavirus 1*, and feline coronavirus type II strains were generated by double homologous recombination between feline coronavirus type I strains and canine coronavirus (17). For parvoviruses, the fatal canine parvovirus that emerged in the 1970s also originated from a feline parvovirus, feline panleukopaemia virus (18,19). As for herpesviruses, canid herpesvirus 1 and felid herpesvirus 1 are closely related and are classified under the genus *Varicellovirus* (20). Furthermore, for papillomaviruses, canine oral papillomavirus and feline papillomavirus are also closely related and are classified under the genus *Lambdapapillomavirus* (21). Dogs are well-known hosts of a paramyxovirus, canine distemper virus, in the genus *Morbillivirus* (22), but no paramyxoviruses have ever been discovered in domestic cats.

Many feline diseases have no known causes. For example, the cause of most cases of feline tubulointerstitial nephritis is hitherto unknown and therefore treatment is mainly supportive and prevention is difficult. Tubulointerstitial nephritis ("TIN") involves primary injury to renal tubules and interstitium and is the most common cause of renal failure and one of the leading causes of deaths in cats. However, the cause of most cases of feline TIN remains unknown and therefore treatment is mainly supportive and prevention is difficult. With millions of cats in households around the world, the disease burden from TIN is great. For example, in the United States of America, it is estimated that there are 75 million household cats, while there are an estimated 8 million household cats in United Kingdom (data from Chomel BB, Sun B., Zoo noses in the bedroom. *Emerg Infect Dis.* 2011 Feb;17(2):167-72.). The capability to diagnose, treat or prevent feline kidney or other diseases would have a great benefit.

The citation of any reference herein should not be construed as an admission that such reference is available as "prior art" to the instant application.

3. SUMMARY OF THE INVENTION

In one aspect, provided herein are nucleic acid sequences comprising or consisting of a wild-type or a modified FmoPV gene segment (genomic RNA) or the complement thereof (antigenomic RNA). Also described herein are isolated nucleic acids encoding the genome of FmoPV, polypeptides encoded by portions of the isolated FmoPV, nucleic acids, primers, vectors, host cells, antibodies to FmoPV and to FmoPV polypeptides, immunogenic compositions, diagnostic methods, screening assays, methods of treatment and related uses.

In one aspect, described herein is a novel paramyxovirus in the genus *Morbillivirus*, a feline morbillivirus (hereinafter "FmoPV") from domestic cat (*Felis catus*). Also described herein is that this novel FmoPV virus is associated with tubulointerstitial nephritis (TIN) in cats.

In one aspect, the modified FmoPV gene segment comprises FmoPV nucleic acid sequence and also a heterologous nucleotide sequence. In some embodiments, the first and second heterologous nucleotide sequences encode different peptides or polypeptides. In other embodiments, the first and second heterologous nucleotide sequences encode the same peptide or polypeptides. In specific embodiments, a FmoPV comprising a modified FmoPV gene segment described herein achieves titers of approximately 3×10^5 pfu/ml, 3.5×10^5 pfu/ml, 4×10^5 pfu/ml, 5×10^5 pfu/ml, 1×10^6 pfu/ml, 5×10^6 pfu/ml, 1×10^7 pfu/ml, 5×10^7 pfu/ml, 1×10^8 pfu/ml, 5×10^8 pfu/ml, 1×10^9 pfu/ml or more after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more passages in cells (e.g., MDCK cells) or embryonated chick eggs. In certain embodiments, a FmoPV described herein comprises an attenuating mutation. In one aspect, provided herein are methods of using a FmoPV, wherein the FmoPV comprises a modified FmoPV gene segment.

In one embodiment, provided herein are methods for detecting the presence or expression of FmoPV, natural or artificial variants, analogs, or derivatives thereof, in a biological material, such as cells, blood, serum, plasma, saliva, urine, stool, sputum, nasopharyngeal aspirates, and so forth. The increased or decreased activity or expression of FmoPV in a sample relative to a control sample can be determined by contacting the biological material with an agent which can detect directly or indirectly the presence or expression of FmoPV. In a specific embodiment, the detecting agents are nucleic acid molecules of the present invention.

In a specific embodiment, provided herein is a diagnostic assay for FmoPV, natural or artificial variants, analogs, or derivatives thereof. In particular, provided herein is a quantitative assay for the detection of nucleic acid molecules of FmoPV using reverse transcription and

polymerase chain reaction (RT-PCR). Also provided in the present invention are nucleic acid molecules that are suitable for hybridization to FmoPV nucleic acids such as, including, but not limited to, PCR primers, Reverse Transcriptase primers, probes for Southern analysis or other nucleic acid hybridization analysis for the detection of FmoPV nucleic acids. Said FmoPV nucleic acids consist of or comprise the nucleic acid sequence as described *infra* or a complement, analog, derivative, or fragment thereof, or a portion thereof.

In one aspect, the invention relates to the use of the isolated FmoPV for diagnostic methods. In a specific embodiment, the invention provides a method of detecting mRNA or genomic RNA of FmoPV of the invention in a biological material, such as cells, blood, serum, plasma, saliva, urine, stool, sputum, nasopharyngeal aspirates, and so forth. The increased or decreased level of mRNA or genomic RNA of FmoPV in a sample relative to a control sample can be determined by contacting the biological material with an agent which can detect directly or indirectly the mRNA or genomic RNA of FmoPV. In a specific embodiment, the detecting agents are the nucleic acid molecules of the present invention.

The present invention also relates to a method of identifying a subject infected with FmoPV, natural or artificial variants, analogs, or derivatives thereof. In a specific embodiment, the method comprises obtaining total RNA from a biological sample obtained from the subject; reverse transcribing the total RNA to obtain cDNA; and subjecting the cDNA to PCR assay using a set of primers derived from a nucleotide sequence of FmoPV.

The present invention further relates to a diagnostic kit comprising primers and a nucleic acid probe for the detection of mRNA or genomic RNA of FmoPV. In a specific embodiment, provided herein is a diagnostic kit comprising nucleic acid molecules which are suitable for use to detect FmoPV, natural or artificial variants, analogs, or derivatives thereof. In one embodiment, a kit provided herein comprises, in one or more containers, a nucleic acid sequence described herein. In another embodiment, a kit provided herein, comprises, in one or more containers, a FmoPV described herein.

In another aspect, provided herein are substrates (*e.g.*, host cells and eggs) comprising a nucleic acid sequence described herein.

In one embodiment, provided herein is a method for eliciting an immune response against FmoPV in a subject, wherein the method comprises administering a FmoPV described herein or a composition thereof to the subject. In another embodiment, provided herein is a method of

preventing and/treating FmoPV infection in a subject, wherein the method comprises administering a FmoPV described herein or a composition thereof to the subject. In another embodiment, provided herein is a method for preventing and/or treating an FmoPV disease in a subject, wherein the method comprises administering a FmoPV described herein or a composition thereof to the subject.

In another embodiment, provided herein are methods for eliciting an immune response against an antigen in a subject, comprising administering a FmoPV described herein or a composition thereof to the subject. In another embodiment, provided herein are methods for generating or identifying antibodies that bind to a FmoPV utilizing a FmoPV described herein or a composition thereof.

In another aspect, the FmoPV described herein can be used to assess the antiviral activity of a compound or understand the life cycle of a FmoPV.

3.1 TERMINOLOGY

As used herein, the term “variant” refers either to a naturally occurring genetic mutant of the FmoPV or a recombinantly prepared variation of the FmoPV, each of which contain one or more mutations in its genome compared to the FmoPV having a nucleic acid sequence disclosed in Genbank accession nos. JQ411014, JQ411015 and JQ411016. The term “variant” may also refer to either a naturally occurring variation of a given peptide or a recombinantly prepared variation of a given peptide or protein in which one or more amino acid residues have been modified by amino acid substitution, addition, or deletion.

As used herein, the term “mutant” refers to the presence of mutations in the nucleotide sequence of an organism as compared to a wild-type organism.

As used herein, the terms “antibody” and “antibodies” refer to monoclonal antibodies, bispecific antibodies, multispecific antibodies, human antibodies, humanized antibodies, chimeric antibodies, camelised antibodies, single domain antibodies, single-chain Fvs (scFv), single chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site. Immunoglobulin molecules can be of any

type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), or subclass.

As used herein, the term “antibody fragment” refers to a fragment of an antibody that immunospecifically binds to a FmoPV or any epitope of the FmoPV. Antibody fragments may be generated by any technique known to one of skill in the art. For example, Fab and F(ab')₂ fragments may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the complete light chain, and the variable region, the CH1 region and the hinge region of the heavy chain. Antibody fragments can be also produced by recombinant DNA technologies. Antibody fragments may be one or more complementarity determining regions (CDRs) of antibodies.

As used herein, the term “an antibody or an antibody fragment that immunospecifically binds a polypeptide of the invention” refers to an antibody or a fragment thereof that immunospecifically binds to the polypeptide encoded by the nucleic acid sequence of the FmoPV, or a complement, analog, derivative, or fragment thereof, or a portion thereof, or that immunospecifically binds to the polypeptide of the FmoPV, or a variant, analog, derivative, or fragment thereof, and does not non-specifically bind to other polypeptides. An antibody or a fragment thereof that immunospecifically binds to the polypeptide of the invention may cross-react with other antigens. Preferably, an antibody or a fragment thereof that immunospecifically binds to a polypeptide of the invention does not cross-react with other antigens. An antibody or a fragment thereof that immunospecifically binds to the polypeptide of the invention, can be identified by, for example, immunoassays or other techniques known to those skilled in the art.

As used herein, the term “epitope” refers to a fragment of FmoPV peptide, polypeptide or protein having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a feline. An epitope having immunogenic activity is a fragment of a polypeptide that elicits an antibody response in an animal. An epitope having antigenic activity is a fragment of a polypeptide or protein to which an antibody immunospecifically binds as determined by any method well known in the art, for example, by the immunoassays described herein. Antigenic epitopes need not necessarily be immunogenic.

As used herein, the term “antigenicity” refers to the ability of a substance (*e.g.*, foreign objects, microorganisms, drugs, antigens, proteins, peptides, polypeptides, nucleic acids, DNA,

RNA, etc.) to trigger an immune response in a particular organism, tissue, and/or cell. Sometimes, the term “antigenic” is synonymous with the term “immunogenic”.

As used herein, the term “immunogenicity” refers to the property of a substance (*e.g.*, foreign objects, microorganisms, drugs, antigens, proteins, peptides, polypeptides, nucleic acids, DNA, RNA, etc.) being able to evoke an immune response within an organism. Immunogenicity depends partly upon the size of the substance in question and partly upon how unlike the host molecules is the substance. Highly conserved proteins tend to have rather low immunogenicity.

As used herein, the term “hybridizes under stringent conditions” describes conditions for hybridization and washing under which nucleotide sequences having at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identity to each other typically remain hybridized to each other. Such hybridization conditions are described in, for example but not limited to, *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.; *Basic Methods in Molecular Biology*, Elsevier Science Publishing Co., Inc., N.Y. (1986), pp.75-78, and 84-87; and *Molecular Cloning*, Cold Spring Harbor Laboratory, N.Y. (1982), pp.387-389, and are well known to those skilled in the art. A preferred, non-limiting example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC), 0.5% SDS at about 68°C followed by one or more washes in 2X SSC, 0.5% SDS at room temperature. Another preferred, non-limiting example of stringent hybridization conditions is hybridization in 6X SSC at about 45°C followed by one or more washes in 0.2X SSC, 0.1% SDS at about 50°C to 65°C.

An “isolated” or “purified” peptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or is substantially free of chemical precursors or other chemicals when chemically synthesized. The language “substantially free of cellular material” includes preparations of a polypeptide/protein in which the polypeptide/protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a polypeptide/protein that is substantially free of cellular material includes preparations of the polypeptide/protein having less than about 30%, 20%, 10%, 5%, 2.5%, or 1%, (by dry weight) of contaminating protein. When the polypeptide/protein is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When polypeptide/protein is produced by chemical synthesis, it is

preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly, such preparations of the polypeptide/protein have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the polypeptide/protein fragment of interest. In a preferred embodiment, the polypeptides/proteins are isolated or purified.

As used herein, the term “isolated” virus is one which is separated from other organisms which are present in the natural source of the virus, *e.g.*, biological material such as cells, blood, serum, plasma, saliva, urine, stool, sputum, nasopharyngeal aspirates, and so forth. The isolated virus can be used to infect a subject.

As used herein, the term “having a biological activity of the polypeptides of the invention” refers to the characteristics of the polypeptides or proteins having a common biological activity similar or identical structural domain and/or having sufficient amino acid identity to the polypeptide encoded by the nucleotide sequence of FmoPV or a complement, analog, derivative, or fragment thereof, or a portion thereof, or the polypeptide having the amino acid sequence of FmoPV, or a variant, analog, derivative, or fragment thereof. Such common biological activities of the polypeptides of the invention include antigenicity and immunogenicity.

As used herein, the term “portion” or “fragment” refers to a fragment of a nucleic acid molecule containing at least about 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 11,000, 12,000, 13,000, 14,000, 15,000, 16,000, 17,000 or more contiguous nucleic acids in length of the relevant nucleic acid molecule and having at least one functional feature of the nucleic acid molecule (or the encoded protein has one functional feature of the protein encoded by the nucleic acid molecule); or a fragment of a protein or a polypeptide containing at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 500, 600, 800, 1,000, 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, 9,500 or more amino acid residues in length of the relevant protein or polypeptide and having at least one functional feature of the protein or polypeptide.

As used herein, the term “analogue” (*e.g.*, proteins, polypeptides, peptides, and antibodies) refers to an agent that possesses a similar or identical function as a second agent but does not necessarily comprise a similar or identical amino acid sequence of the second agent, or possess a similar or identical structure of the second proteinaceous agent. In a specific embodiment, antibody analogues immunospecifically bind to the same epitope as the original antibodies from which the analogues were derived. In an alternative embodiment, antibody analogues immunospecifically bind to different epitopes than the original antibodies from which the analogues were derived. An agent that has a similar amino acid sequence refers to a second agent that satisfies at least one of the following: (a) an agent having an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the amino acid sequence of a second agent; (b) an agent encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence encoding a second agent of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, or at least 150 contiguous amino acid residues; and (c) an agent encoded by a nucleotide sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleotide sequence encoding a second agent. An agent with similar structure to a second agent refers to an agent that has a similar secondary, tertiary or quaternary structure to the second agent. The structure of an agent can be determined by methods known to those skilled in the art, including but not limited to, peptide sequencing, X ray crystallography, nuclear magnetic resonance, circular dichroism, and crystallographic electron microscopy.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment

with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length.

The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2264 2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873 5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present invention. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, Nucleic Acids Res. 25:3389 3402. Alternatively, PSI BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, the NCBI website). Another preferred, non limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11 17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

As used herein, the term “derivative” (*e.g.*, proteins, polypeptides, peptides, and antibodies) refers to an agent that comprises an amino acid sequence which has been altered by the introduction of amino acid residue substitutions, deletions, and/or additions. The term “derivative” as used herein also refers to an agent which has been modified, *i.e.*, by the covalent attachment of any type of molecule to the agent. For example, but not by way of limitation, an antibody may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of an agent may be produced by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of an agent may contain one or more non-classical amino acids. A derivative of an agent possesses a similar or identical function as the agent from which it was derived.

As used herein, the term “about” or “approximately” when used in conjunction with a number refers to any number within 1, 5 or 10% of the referenced number.

As used herein, the term “effective amount” in the context of administering a therapy to a subject refers to the amount of a therapy which has a prophylactic and/or therapeutic effect(s). In certain embodiments, in the context of administration of a therapy to a subject, “effective amount” refers to the amount of a therapy which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduction or amelioration in the severity of FmoPV infection, a FmoPV disease or symptom associated therewith; (ii) reduction in the duration of FmoPV infection, a FmoPV disease or symptom associated therewith; (iii) prevention of the progression of a FmoPV infection, a FmoPV disease or symptom associated therewith; (iv) regression of a FmoPV infection, a FmoPV disease or symptom associated therewith; (v) prevention of the development or onset of a FmoPV infection, a FmoPV disease or symptom associated therewith; (vi) prevention of the recurrence of a FmoPV infection, a FmoPV disease or symptom associated therewith; (vii) reduction or prevention of the spread of a FmoPV from one cell to another cell, one tissue to another tissue, or one organ to another organ; (viii) prevention or reduction of the spread/transmission of a FmoPV from one subject to another subject; (ix) reduction in organ failure associated with a FmoPV infection or FmoPV disease; (x) reduction in the hospitalization of a subject; (xi) reduction in the hospitalization length; (xii) an increase in the survival of a

subject with a FmoPV infection or a disease associated therewith; (xiii) elimination of a FmoPV infection or a disease associated therewith; (xiv) inhibition or reduction in FmoPV replication; (xv) inhibition or reduction in the binding or fusion of FmoPV to a host cell(s); (xvi) inhibition or reduction in the entry of an FmoPV into a host cell(s); (xvii) inhibition or reduction of the replication of the FmoPV genome; (xviii) inhibition or reduction in the synthesis of FmoPV proteins; (xix) inhibition or reduction in the assembly of FmoPV particles; (xx) inhibition or reduction in the release of FmoPV particles from a host cell(s); (xxi) reduction in FmoPV titer; (xxii) reduction in the number of symptoms associated with a FmoPVB infection or a FmoPV disease; (xxiii) enhancement, improvement, supplementation, complementation, or augmentation of the prophylactic or therapeutic effect(s) of another therapy; (xxiv) prevention of the onset or progression of a secondary infection associated with a FmoPV infection; and/or (xxv) prevention of the onset or diminution of disease severity of occurring secondary to FmoPV infections. Exemplary doses of an effective amount are provided herein below.

In certain embodiments, the effective amount of a therapy does not result in complete protection from a FmoPV disease, but results in a lower titer or reduced number of FmoPV compared to an untreated subject. In certain embodiments, the effective amount of a therapy results in a 0.5 fold, 1 fold, 2 fold, 4 fold, 6 fold, 8 fold, 10 fold, 15 fold, 20 fold, 25 fold, 50 fold, 75 fold, 100 fold, 125 fold, 150 fold, 175 fold, 200 fold, 300 fold, 400 fold, 500 fold, 750 fold, or 1,000 fold or greater reduction in titer of FmoPV relative to an untreated subject. In certain embodiments, the effective amount of a therapy results in a reduction by 0.5 log, 1 log, 2 logs, 3 logs, 4 logs, 5, logs, 6, logs, 7 logs, or 10 logs or more in titer of FmoPV relative to an untreated subject. Benefits of a reduction in the titer, number or total burden of FmoPV include, but are not limited to, less severe symptoms of the infection, fewer symptoms of the infection, reduction in the length of the disease associated with the infection, and prevention of the onset or diminution of disease severity of infection occurring secondary to FmoPV infections.

As used herein, the term “fragment” in the context of a nucleic acid sequence refers to a nucleotide sequence comprising at least 2 or at least 3 consecutive nucleotides from a parent sequence. In a specific embodiment, the term refers to a nucleotide sequence of 2 to 30, 5 to 30, 10 to 60, 25 to 100, 150 to 300 or more consecutive nucleotides from a parent sequence. In another embodiment, the term refers to a nucleotide sequence of at least 5, 10, 15, 20, 25, 30, 35,

40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 125, 150, 175, 200, 250, 275, 300, 325, 350, 375, 400, 425, 450 or 475 consecutive nucleotides of a parent sequence.

As used herein, the term “fragment” in the context of an amino acid sequence refers to an amino acid sequence comprising at least 2 consecutive amino acid residues from a parent sequence. In a specific embodiment, the term refers to an amino acid sequence of 2 to 30, 5 to 30, 10 to 60, 25 to 100, 150 to 300 or more consecutive amino acid residues from a parent sequence. In another embodiment, the term refers to an amino acid sequence of at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 125, 150, 175, 200, 250, 275, 300, 325, 350, 375, 400, 425, 450 or 475 consecutive amino acid residues of a parent sequence.

As used herein, the term “heterologous” refers to a unit that is not found naturally be associated with another unit. For example, a first nucleotide sequence is said be a heterologous to a second nucleotide sequence if the two nucleotide sequences are not found in nature to be associated with each other.

As used herein, the term “host cell” refers to any type of cell, *e.g.*, a primary cell or a cell from a cell line. In specific embodiments, the term “host cell” refers a cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

As used herein, the term “in combination” in the context of the administration of a therapy(ies) to a subject, refers to the use of more than one therapy. The use of the term “in combination” does not restrict the order in which therapies are administered to a subject. A first therapy can be administered prior to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy to a subject.

As used herein, the term “infection” means the invasion by, multiplication and/or presence of a virus in a cell or a subject. In one embodiment, an infection is an “active”

infection, *i.e.*, one in which the virus is replicating in a cell or a subject. Such an infection is characterized by the spread of the virus to other cells, tissues, and/or organs, from the cells, tissues, and/or organs initially infected by the virus. An infection may also be a latent infection, *i.e.*, one in which the virus is not replicating. In certain embodiments, an infection refers to the pathological state resulting from the presence of the virus in a cell or a subject, or by the invasion of a cell or subject by the virus.

As used herein, the term "FmoPV disease" and phrases referring to a disease associated with a FmoPV infection refer to the pathological state resulting from the presence of a FmoPV in a cell or subject or the invasion of a cell or subject by a FmoPV. In specific embodiments, the term refers to a kidney disease caused by a FmoPV.

As used herein, the term "isolated" in the context of nucleic acids refers to a nucleic acid molecule which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized; however, "isolated" excludes members of a library of clones such as a cDNA library. In a specific embodiment, a nucleic acid described herein is isolated. In another specific embodiment, antibodies described herein are isolated. The language "substantially free of other cellular material" includes preparations of a nucleic acid molecule in which the nucleic acid molecule is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a nucleic acid molecule that is substantially free of cellular material includes preparations having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous nucleic acid molecules or other cellular components. When the nucleic acid molecule is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the nucleic acid molecule preparation. When the nucleic acid molecule is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the nucleic acid molecule. Accordingly such preparations of the nucleic acid molecule have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the nucleic acid molecule of interest.

As used herein, the phrase “multiplicity of infection” or “MOI” is the average number of virus per infected cell. The MOI is determined by dividing the number of virus added (ml added x plaque forming units (pfu)) by the number of cells added (ml added x cells/ml).

As used herein, the terms “nucleic acid” and “nucleotides” refer to deoxyribonucleotides, deoxyribonucleic acids, ribonucleotides, and ribonucleic acids, and polymeric forms thereof, and includes either single- or double-stranded forms. In certain embodiments, such terms include known analogues of natural nucleotides, for example, peptide nucleic acids (“PNA”s), that have similar binding properties as the reference nucleic acid. In some embodiments, such terms refer to deoxyribonucleic acids (*e.g.*, cDNA or DNA). In other embodiments, such terms refer to ribonucleic acids (*e.g.*, mRNA or RNA).

As used herein, the terms “prevent,” “preventing” and “prevention” in the context of the administration of a therapy(ies) to a subject refer to a prophylactic effect that results from the administration of a therapy or a combination of therapies. In a specific embodiment, the terms “prevent,” “preventing” and “prevention” in the context of the administration of a therapy(ies) to a subject to prevent a disease refer to one or more of the following effects resulting from the administration of a therapy or a combination of therapies: (i) the inhibition or reduction in the development or onset of a disease or a symptom thereof; (ii) the inhibition or reduction in the recurrence of a disease or a symptom associated therewith; and (iii) the reduction or inhibition in a pathogen infection and/or replication. In other specific embodiment, the terms “prevent,” “preventing” and “prevention” in the context of the administration of a therapy(ies) to a subject to prevent a FmoPV disease refer to one or more of the following effects resulting from the administration of a therapy or a combination of therapies: (i) the inhibition or reduction in the development or onset of a FmoPV disease or a symptom thereof; (ii) the inhibition or reduction in the recurrence of a FmoPV disease or a symptom associated therewith; and (iii) the reduction or inhibition in FmoPV infection and/or replication.

In another specific embodiment, the terms “prevent,” “preventing” and “prevention” in the context of the administration of a therapy(ies) to a subject to prevent a FmoPV infection refer to one or more of the following effects resulting from the administration of a therapy or a combination of therapies: (i) the reduction or inhibition of the spread of FmoPV from one cell to another cell; (ii) the reduction or inhibition of the spread of FmoPV from one organ or tissue to another organ or tissue; and/or (iii) the reduction or inhibition of the spread of FmoPV from one

region of an organ or tissue to another region of the organ or tissue (*e.g.*, the reduction in the spread of FmoPV from the upper to the lower respiratory tract).

As used herein, the terms “subject” and “patient” are used interchangeably to refer to an animal (*e.g.*, cats, dogs, birds, reptiles, and mammals). In a specific embodiment, a subject is a cat. In another embodiment, a subject is a mammal including a non-primate (*e.g.*, a camel, donkey, zebra, cow, pig, horse, goat, sheep, cat, dog, rat, and mouse) and a primate (*e.g.*, a monkey, chimpanzee, and a human). In another embodiment, a subject is a non-human mammal. In another embodiment, a subject is a human.

As used herein, the terms “therapies” and “therapy” can refer to any protocol(s), method(s), compound(s), composition(s), formulation(s), and/or agent(s) that can be used in the prevention or treatment of a viral infection or a disease or symptom associated therewith. In certain embodiments, the terms “therapies” and “therapy” refer to biological therapy, supportive therapy, and/or other therapies useful in treatment or prevention of a viral infection or a disease or symptom associated therewith known to one of skill in the art. In some embodiments, the term “therapy” refers to an immunogenic composition (*e.g.*, a FmoPV vaccine).

As used herein, the terms “treat,” “treatment,” and “treating” in the context of the administration of a therapy(ies) to a subject refer a beneficial or therapeutic effect resulting from the administration of a therapy or a combination of therapies. In specific embodiments, such terms refer to one, two, three, four, five or more of the following effects resulting from the administration of a therapy or a combination of therapies: (i) reduction or amelioration in the severity of a disease or a symptom associated therewith; (ii) reduction in the duration of a disease or a symptom associated therewith; (iii) prevention of the progression of a disease or symptom associated therewith; (iv) regression of a disease or a symptom associated therewith; (v) prevention of the development or onset of a disease or a symptom associated therewith; (vi) prevention of the recurrence of a disease or a symptom associated therewith; (vii) reduction or prevention of the spread of a pathogen from one cell to another cell, one tissue to another tissue, or one organ to another organ; (viii) prevention or reduction of the spread/transmission of a pathogen from one subject to another subject; (ix) reduction in organ failure associated with a disease; (x) reduction in the hospitalization of a subject; (xi) reduction in the hospitalization length; (xii) an increase in the survival of a subject with a disease associated therewith; (xiii) elimination of a disease; (xiv) inhibition or reduction in pathogen replication; (xv) reduction in

pathogen numbers; (xv) the reduction in the number of symptoms associated with a disease; and (xvi) enhancement, improvement, supplementation, complementation, or augmentation of the prophylactic or therapeutic effect(s) of another therapy.

As used herein, in some embodiments, the term “wild-type” in the context of a virus refers to the types of viruses that are prevalent, circulating and naturally producing typical outbreaks of disease.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the genome organization of FmoPV and other morbilliviruses. The genes are shown as boxes that are drawn to scale. For the P gene, the first line above the box labeled “P” with the letter V at the end of the line represents the region of V CDS and the second line with the letter C at the end of the line represents the C CDS.

Fig. 2 is the 16050 bp nucleotide sequence of FmoPV 761U Cats/Hong Kong/2009.

Fig. 3 is the 16050 bp nucleotide sequence of FmoPV 776U Cats/Hong Kong/2009.

Fig. 4 is the 16050 bp nucleotide sequence of FmoPV M252A Cats/Hong Kong/2009.

Fig. 5 Multiple alignments of N proteins of FmoPV and other morbilliviruses. The conserved MA(S,T)L motif in morbilliviruses and the three conserved motifs in paramyxoviruses are marked in open boxes with solid line border and reported consensus sequences are indicated above the alignment (where x represents any amino acid residue and Ø represents an aromatic amino acid residue). Amino acid residue numbers for each protein are shown to the right of each sequence. Dots indicate identical residues and dashes indicate gaps. The NES are in open boxes with dotted line border and the NLS in open box with dashed line border.

Fig. 6A-D. Panel A shows the cytopathic effects of FmoPV on CRFK cells. The open squares show the formation of giant cells. Panels B and C, show indirect immunofluorescent antigen detection in uninfected and infected CRFK cells using serum from guinea pig immunized with recombinant N protein of FmoPV, showing specific apple green cytoplasmic fluorescence in FmoPV infected CRFK cells. Panel D is an electron microscopic examination of infected CRFK cell culture supernatant showing enveloped virus with burst envelope and typical “herring bone” appearance of helical N in paramyxoviruses.

Fig. 7 is a phylogenetic analysis of the N, P, M, F, A and L amino acid sequences of FmoPV. The trees were constructed by maximum likelihood method with bootstrap values

calculated from 1000 trees and rooted on midpoint. The scale bars indicate the branch length that corresponds to 0.5 substitutions per site. Three strains from FmoPV were named as 761U, 776U, M252A. Names and accession numbers of the other viruses are listed in Table 3.

Fig. 8 Phylogenetic analysis of amino acid sequences of 72-bp fragment of L gene of paramyxoviruses identified from cats in the present study. The tree was constructed by neighbor-joining method. The scale bar indicates the branch length that corresponds to 2 amino acid differences per sequence. The three strains from stray cats numbered 761U, 776U and M252A with genome sequences determined are shown in bold. RSV, respiratory syncytial virus (U39661); DmoPV, Dolphin morbillivirus ([NC_005283](#)); PprPV, Peste-des-petits ruminants virus ([NC_006383](#)); MeaPV, Measles virus ([NC_001498](#)); CdiPV, Canine distemper virus ([NC_001921](#)); MosPV, Mossman virus ([NC_005339](#)); NarPV, Nariva virus (FJ362497); ThkPV3, Tuhoko virus 3 (GU128082); ThkPV2, Tuhoko virus 2 (GU128081); ThkPV, Tuhoko virus 1 (GU128080); JPV, J-virus ([NC_007454](#)); BeiPV, Beilong virus ([NC_007803](#)); NipPV, Nipah virus ([NC_002728](#)); HenPV, Hendra virus ([NC_001906](#)); FdlPV, Fer-de-lance virus ([NC_005084](#)); SenPV, Sendai virus ([NC_001552](#)); HpiPV-1, Human parainfluenza virus 1 ([NC_003461](#)).

Fig. 9 shows a Western blot analysis with stray cat sera against the purified (His)₆-tagged recombinant FmoPV N protein antigen. Results of RT-PCR of the corresponding urine samples for FmoPV are also shown.

Fig. 10A-F. Panels A and B show histological section of kidneys stained by H & E from a stray cat with FmoPV detected in urine and a normal cat, showing aggregates of inflammatory cells in the interstitium and renal tubular degeneration in the infected cat. Panels C and D show immunohistochemical staining of kidney sections of a stray cat with FmoPV detected in urine using guinea pig serum positive for anti-FmoPV N protein antibody and preimmune guinea pig serum, showing positive renal tubular cells. Panels E and F show immunohistochemical staining of lymph node sections of a stray cat positive for FmoPV using guinea pig serum positive for anti-FmoPV N protein antibody and preimmune guinea pig serum, showing positive mononuclear cells.

Fig. 11A-B presents representative images of cauxin-immunohistochemical stained paraffin-embedded renal sections of cats without and with histological evidence of TIN in Panels A and B, respectively.

Fig. 12A-C shows double staining of the lymph node of an FmoPV infected stray cat for (A) mouse anti-human myeloid/histocyte antigen and then labeled with Texas-red conjugated goat anti-mouse IgG; (B) guinea pig antiserum against the N protein of FmoPV, followed by FITC conjugated rabbit anti-guinea pig IgG; (C) the merged photo showed that both antigens co-localized in cytoplasm of the cells.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1 NUCLEIC ACIDS

In one aspect, provided herein are nucleic acid sequences comprising or consisting of a wild-type or a modified feline morbillivirus ("FmoPV"). Also provided are modified FmoPV gene segment (genomic RNA) or the complement thereof (antigenomic RNA).

In one aspect, described herein is the entire nucleotide sequence of the FmoPV. In certain embodiments, the nucleotide sequences are Genbank accession numbers: JQ411014, JQ411015 and JQ411016. The JQ411014 nucleotide sequence is shown in Fig. 2, labeled as FmoPV 761U Cats/Hong Kong/2009. The JQ411015 nucleotide sequence is shown in Fig. 3, labeled as FmoPV 776U Cats/Hong Kong/2009. The JQ411016 nucleotide sequence is shown in Fig. 4, labeled as FmoPV M252A Cats/Hong Kong/2009.

In other aspects, described herein are a complement, analog, derivative, or fragment thereof, or a portion of the FmoPV nucleotide sequence. In certain embodiments, described herein are nucleic acid molecules that hybridizes to any portion of the genome of the FmoPV, under stringent conditions. In specific embodiment, described herein are nucleic acid molecules which are suitable for use as primers consisting of or comprising the nucleic acid sequence of the FmoPV. In another embodiment, described herein are nucleic acid molecules that are suitable for use as hybridization probes for the detection of FmoPV. The primers and probes are contained in a kit for the detection of nucleic acid molecules or proteins from wild-type, natural or artificial variants, analogs, or derivatives of FmoPV.

Described herein is a natural variant of FmoPV having a sequence that is different from the genomic sequence of Genbank accession numbers: JQ411014, JQ411015 and JQ411016 due to one or more naturally occurred mutations, including, but not limited to, point mutations, rearrangements, insertions, deletions, etc., to the genomic sequence that may or may not result in a phenotypic change. Preferably, the variants include 1-5, 6-10, 11-10, 20-40, 40-60, 60-100,

100-500, 500-1000, 1000-2000 nucleic acid changes in the genome. In certain embodiments, the mutation of the genomic sequence of the FmoPV resulted in rearrangements, insertions, and/or deletions relative to the wild-type genomic sequence of FmoPV.

In certain embodiments, a nucleic acid sequence described herein is part of or incorporated into a vector. In a specific embodiment, a nucleic acid sequence described herein is part of or incorporated into a vector that facilitates the production of a modified FmoPV gene segment or the complement thereof. In one embodiment, a nucleic acid sequence described herein is part of or incorporated into the pDZ vector (see, *e.g.*, Quinlivan et al., 2005, J. of Virology 79: 8431–8439 for information relating to the pDZ vector). In another embodiment, a nucleic acid sequence described herein is part of or incorporated into the pHW2000 vector (see, *e.g.*, Hoffmann et al., 2000, Proc Natl Acad Sci U S A. 97(11):6108-13 for information relating to the pHW2000 vector). In another embodiment, a nucleic acid sequence described herein is part of or incorporated into the pAD3000 vector (see, *e.g.*, Hoffmann et al., 2000, Proc Natl Acad Sci U S A. 97(11):6108-13 for information relating to the pAD3000 vector). In another embodiment, a nucleic acid sequence described herein is part of or incorporated into the pAD4000 vector (see, *e.g.*, Wang et al., 2007, J. of Virology 4: 102 for information relating to the pAD4000 vector). In one embodiment, a nucleic acid sequence described herein is part of or incorporated into the vector in Section 6 *infra*.

Techniques for the production or use of the nucleic acids will employ, unless otherwise indicated, routine conventional techniques of molecular biology and recombinant DNA manipulation and production. Any cloning technique known to the skilled artisan can be used to assemble the nucleic acids described herein and to mutate nucleotides where necessary. Such techniques are well-known and are available to the skilled artisan in laboratory manuals such as Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (2001). In particular, polymerase chain reaction, restriction enzymes, ligase enzyme, mutagenic primers, and amplification of nucleic acid fragments in vectors can be used to generate the individual elements of the nucleic acids described herein and then to assemble them.

In some embodiments, a nucleic acid sequence described herein is introduced (*e.g.*, transfected) into a substrate, such as a host cell or an embryonated egg. Thus, in some embodiments, provided herein is a substrate (*e.g.*, host cells or eggs) comprising a nucleic acid

sequence described herein. In other embodiments, a nucleic acid sequence described herein that is part of or incorporated into a vector is introduced (*e.g.*, transfected) into a substrate, such as a host cell or an embryonated egg. Thus, in some embodiments, provided herein is a substrate (*e.g.*, host cells or eggs) comprising a nucleic acid sequence described herein that is part of or incorporated into a vector. In certain embodiments, provided herein is a cell line that is transformed with the vector containing FmoPV nucleic acid sequences. In certain embodiments, provided herein is a transgenic animal containing a vector comprising FmoPV nucleic acid sequences.

In certain embodiments, the FmoPV nucleic acid is used in a diagnostic assay for the FmoPV infection. In particular, the diagnostic assay is a quantitative assay for the detection of the FmoPV, natural or artificial variants, analogs, or derivatives thereof. In certain embodiments, the quantitative assay is PCR or RT-PCR. In certain embodiments, the FmoPV nucleic acid is in separate containers in a diagnostic kit.

5.2 PROTEINS

The open reading frames of FmoPV gene segments can be determined using standard molecular biology and virology techniques. Provided herein are FmoPV polypeptides expressed by the FmoPV nucleic acid molecule comprising the FmoPV nucleic acid sequences. In certain embodiments, the FmoPV proteins are. In certain embodiments, FmoPV antigens are fragments or full length N, P/V/C(P), P/V/C/(V), P/VC(C), M, F, H and L proteins. Also described herein are recombinant or chimeric viruses encoded by viral vectors derived from the genome of FmoPV or natural variants thereof.

In another specific embodiment, described herein is a chimeric FmoPV virus which further comprises a heterologous nucleotide sequence. In certain embodiments, a chimeric virus may be encoded by a nucleotide sequence in which heterologous nucleotide sequences have been added to the genome or in which endogenous or native nucleotide sequences have been replaced with heterologous nucleotide sequences.

In certain embodiments, the chimeric viruses are encoded by the vectors which further comprise a heterologous nucleotide sequence. In accordance with the present invention a chimeric virus is encoded by a viral vector that may or may not include nucleic acids that are non-native to the viral genome. In accordance with the invention a chimeric virus is encoded by

a viral vector to which heterologous nucleotide sequences have been added, inserted or substituted for native or non-native sequences. In accordance with the present invention, the chimeric virus may be encoded by nucleotide sequences derived from different strains or variants of FmoPV. In particular, the chimeric virus is encoded by nucleotide sequences that encode antigenic polypeptides derived from different strains or variants of FmoPV.

A chimeric virus may be of particular use for the generation of recombinant vaccines protecting against two or more viruses (Tao *et al.*, *J. Virol.* 72:2955-2961; Durbin *et al.*, 2000, *J. Virol.* 74:6821-6831; Skiadopoulos *et al.*, 1998, *J. Virol.* 72:1762-1768; Teng *et al.*, 2000, *J. Virol.* 74:9317-9321). For example, it can be envisaged that a vector expressing one or more proteins of FmoPV and FmoPV variants, will protect a subject vaccinated with such vector against infections by both the FmoPV and FmoPV variant. Attenuated and replication-defective viruses may be of use for vaccination purposes with live vaccines.

In accordance with the present invention the heterologous sequence to be incorporated into the viral vectors encoding the recombinant or chimeric viruses of the invention include sequences obtained or derived from different strains or variants of the FmoPV.

In certain embodiments, the chimeric or recombinant viruses of the invention are encoded by viral vectors derived from viral genomes wherein one or more sequences, intergenic regions, termini sequences, or portions or entire ORF have been substituted with a heterologous or non-native sequence. In certain embodiments of the invention, the chimeric viruses of the invention are encoded by viral vectors derived from viral genomes wherein one or more heterologous sequences have been inserted or added to the vector.

Any nucleotide sequence heterologous to FmoPV may be included in a modified FmoPV gene segment described herein. In certain embodiments, the heterologous nucleotide sequence is 8 to 100 nucleotides in length, 15 to 100 nucleotides in length, 25 to 100 nucleotides in length, 50 to 200 nucleotide in length, 50 to 400 nucleotide in length, 200 to 500 nucleotide in length, or 400 to 600 nucleotides in length, 500 to 800 nucleotide in length. In other embodiments, the heterologous nucleotide sequence is 750 to 900 nucleotides in length, 800 to 100 nucleotides in length, 850 to 1000 nucleotides in length, 900 to 1200 nucleotides in length, 1000 to 1200 nucleotides in length, 1000 to 1500 nucleotides or 10 to 1500 nucleotides in length. In some embodiments, the heterologous nucleotide encodes a peptide or polypeptide that is 5 to 10 amino acids in length, 10 to 25 amino acids in length, 25 to 50 amino acids in length, 50 to 100 amino

acids in length, 100 to 150 amino acids in length, 150 to 200 amino acids in length, 200 to 250 amino acids in length, 250 to 300 amino acids in length, 300 to 400 amino acids in length, or 500 or more amino acids in length. In some embodiments, the heterologous nucleotide encodes a polypeptide that does not exceed 500 amino acids in length. In specific embodiments the heterologous nucleotide sequence does not contain a stop codon. In certain embodiments, the heterologous nucleotide sequence is codon-optimized. Techniques for codon optimization are known in the art and can be applied to codon optimize a heterologous nucleotide sequence.

In one embodiment, a heterologous nucleotide sequence encodes an antigen of any infectious pathogen or an antigen associated with any disease that is capable of eliciting an immune response. In a specific embodiment, the antigen is a glycoprotein. In certain embodiments, a heterologous nucleotide sequence encodes a viral antigen. In other embodiments, the viral antigen is an antigen from a virus other than a FmoPV.

In specific embodiments, a FmoPV described herein is attenuated. In a particular embodiment, the FmoPV is attenuated such that the virus remains, at least partially, infectious and can replicate *in vivo*, but only generate low titers resulting in subclinical levels of infection that are non-pathogenic. Such attenuated viruses are especially suited for embodiments described herein wherein the virus or an immunogenic composition thereof is administered to a subject to induce an immune response.

In some embodiments, a FmoPV described herein comprises one or more attenuating mutations in a modified FmoPV gene segment. In some embodiments, a FmoPV described herein comprises one or more attenuating mutations in a complementing FmoPV gene segment. In certain embodiments, a FmoPV described herein comprises one or more attenuating mutations in two, three or more complementing FmoPV gene segments. In some embodiments, a FmoPV described herein comprises one or more attenuating mutations in a modified FmoPV gene segment and one or more attenuating mutations in a complementing FmoPV gene segment.

The selection of the viral vector may depend on the species of the subject that is to be treated or protected from a viral infection. If the subject is a feline, then an attenuated FmoPV can be used to provide the antigenic sequences.

In accordance with the present invention, the viral vectors can be engineered to provide antigenic sequences which confer protection against infection by the FmoPV, natural or artificial variants, analogs, or derivatives thereof. The viral vectors may be engineered to provide one,

two, three or more antigenic sequences. In accordance with the present invention the antigenic sequences may be derived from the same virus, from different strains or variants of the same type of virus, or from different viruses.

The expression products and/or recombinant or chimeric virions obtained in accordance with the invention may advantageously be utilized in vaccine formulations. The expression products and chimeric virions of may be engineered to create vaccines against a broad range of pathogens, including viral and bacterial antigens, tumor antigens, allergen antigens, and auto antigens involved in autoimmune disorders. In particular, the chimeric virions of the present invention may be engineered to create vaccines for the protection of a subject from infections with the FmoPV, natural or artificial variants, analogs, or derivatives thereof.

In another aspect, the mutation of the genomic sequence of the FmoPV resulted in changes in the FmoPV proteins. In certain embodiments, the mutation of the genomic sequence of the FmoPV resulted in less than 25, 20, 15, 10, 5, 4, 3, or 2 amino acid substitutions in the FmoPV proteins.

Either conservative or non-conservative amino acid substitutions can be made at one or more amino acid residues. In preferred embodiments, the variants have conservative amino acid substitutions that are made at one or more predicted non-essential amino acid residues (*i.e.*, amino acid residues which are not critical for the expression of the biological activities of the virus, *e.g.*, infectivity, replicability, protein synthesis ability, assembling ability, and cytotoxic effect). In other embodiments, the variants have non-conservative amino acid substitutions that are made at one or more predicted non-essential amino acid residues (*i.e.*, amino acid residues which are not critical for the biological activities of the virus, *e.g.*, infectivity, replication ability, protein synthesis ability, assembling ability, and cytotoxic effect). In other embodiments, the amino acid substitutions are made at essential amino acid residues (*i.e.*, amino acid residues which are critical for the biological activities of the virus, *e.g.*, infectivity, replicability, protein synthesis ability, assembling ability, and cytotoxic effect).

A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. A “non-conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a side chain with an opposite charge. Families of amino acid residues having side chains with similar charges have been defined in the art. Genetically encoded amino

acids are can be divided into four families: (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine, histidine; (3) nonpolar = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar = glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. In similar fashion, the amino acid repertoire can be grouped as (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine histidine, (3) aliphatic = glycine, alanine, valine, leucine, isoleucine, serine, threonine, with serine and threonine optionally be grouped separately as aliphatic-hydroxyl; (4) aromatic = phenylalanine, tyrosine, tryptophan; (5) amide = asparagine, glutamine; and (6) sulfur -containing = cysteine and methionine. (See, for example, Biochemistry, 4th ed., Ed. by L. Stryer, WH Freeman and Co.: 1995).

The invention further relates to mutant FmoPV peptides. In one embodiment, mutations can be introduced randomly along all or part of the coding sequence of the FmoPV or variants thereof, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Techniques for mutagenesis known in the art can also be used, including but not limited to, point-directed mutagenesis, chemical mutagenesis, *in vitro* site-directed mutagenesis, using, for example, the QuikChange Site-Directed Mutagenesis Kit (Stratagene), etc. Non-limiting examples of such modifications include substitutions of amino acids to cysteines toward the formation of disulfide bonds; substitution of amino acids to tyrosine and subsequent chemical treatment of the polypeptide toward the formation of dityrosine bonds, as disclosed in detail herein; one or more amino acid substitutions and/or biological or chemical modification toward generating a binding pocket for a small molecule (substrate or inhibitor), and/or the introduction of side-chain specific tags (*e.g.*, to characterize molecular interactions or to capture protein-protein interaction partners). In a specific embodiment, the biological modification comprises alkylation, phosphorylation, sulfation, oxidation or reduction, ADP-ribosylation, hydroxylation, glycosylation, glucosylphosphatidylinositol addition, ubiquitination. In another specific embodiment, the chemical modification comprises altering the charge of the recombinant virus. In yet another embodiment, a positive or negative charge is chemically added to an amino acid residue where a charged amino acid residue is modified to an uncharged residue.

5.3 CONSTRUCTION OF RECOMBINANT FMOPV

Techniques known to one skilled in the art may be used to produce a recombinant FmoPV containing a modified FmoPV gene segment described herein. For example, reverse genetics techniques may be used to generate such a FmoPV. Briefly, reverse genetics techniques generally involve the preparation of synthetic recombinant viral RNAs that contain the non-coding regions of the negative-strand, viral RNA which are essential for the recognition by viral polymerases and for packaging signals necessary to generate a mature virion. The recombinant RNAs are synthesized from a recombinant DNA template and reconstituted *in vitro* with purified viral polymerase complex to form recombinant ribonucleoproteins (RNPs) which can be used to transfect cells. A more efficient transfection is achieved if the viral polymerase proteins are present during transcription of the synthetic RNAs either *in vitro* or *in vivo*. The synthetic recombinant RNPs can be rescued into infectious virus particles.

Alternatively, helper-free plasmid technology may be used to produce a recombinant FmoPV containing a modified FmoPV gene segment. Briefly, full length cDNAs of viral segments are amplified using PCR with primers that include unique restriction sites, which allow the insertion of the PCR product into the plasmid vector. The plasmid vector is designed so that an exact negative (vRNA sense) transcript is expressed. For example, the plasmid vector may be designed to position the PCR product between a truncated human RNA polymerase I promoter and a hepatitis delta virus ribozyme sequence such that an exact negative (vRNA sense) transcript is produced from the polymerase I promoter. Separate plasmid vectors comprising each viral segment as well as expression vectors comprising necessary viral proteins may be transfected into cells leading to production of recombinant viral particles. In another example, plasmid vectors from which both the viral genomic RNA and mRNA encoding the necessary viral proteins are expressed may be used.

5.4. PROPAGATION OF FMOPV

The FmoPV described herein can be propagated in any substrate that allows the virus to grow to titers that permit the uses of the viruses described herein. In one embodiment, the substrate allows the FmoPV described herein to grow to titers comparable to those determined for the corresponding wild-type viruses.

The FmoPV described herein may be grown in host cells (*e.g.*, cat, avian cells, chicken cells, etc.) that are susceptible to infection by the viruses, embryonated eggs or animals (*e.g.*, birds). Specific examples of host cells include Vero cells, MDCK cells, MBCK cells, COS cells, 293 cells, 293T cells, A549 cells, MDBK cells, etc. Such methods are well-known to those skilled in the art. In a specific embodiment, the FmoPV described herein may be propagated in cell lines. In another embodiment, the FmoPV described herein described herein are propagated in chicken cells or embryonated eggs. Representative chicken cells include, but are not limited to, chicken embryo fibroblasts and chicken embryo kidney cells.

For virus isolation, the FmoPV described herein can be removed from cell culture and separated from cellular components, typically by well known clarification procedures, *e.g.*, such as gradient centrifugation and column chromatography, and may be further purified as desired using procedures well known to those skilled in the art, *e.g.*, plaque assays.

5.5 COMPOSITIONS & ROUTES OF ADMINISTRATION

The FmoPV described herein may be incorporated into compositions. In a specific embodiment, the compositions are pharmaceutical compositions, such as immunogenic compositions (*e.g.*, vaccine formulations). The pharmaceutical compositions provided herein can be in any form that allows for the composition to be administered to a subject. In a specific embodiment, the pharmaceutical compositions are suitable for veterinary and/or human administration. The compositions may be used in methods of preventing and/or treating an FmoPV infection. The compositions may also be used in methods of preventing and/or treating FmoPV disease. The composition may be used in methods of eliciting an immune response to a particular antigen(s) or in methods of delivering a certain protein to a subject.

In one embodiment, a pharmaceutical composition comprises a FmoPV in an admixture with a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical composition may comprise one or more other therapies in addition to a FmoPV. In specific embodiments, a FmoPV described herein that is incorporated into a pharmaceutical composition (*e.g.*, an immunogenic composition such as a vaccine) is a live virus. An immunogenic composition comprising a live FmoPV for administration to a subject may be preferred because multiplication of the virus in the subject may lead to a prolonged stimulus of similar kind and

magnitude to that occurring in natural infections, and therefore, confer substantial, long lasting immunity.

In some embodiments, a FmoPV described herein that is incorporated into a pharmaceutical composition (*e.g.*, an immunogenic composition such as a vaccine) is inactivated. Techniques known to one of skill in the art may be used to inactivate FmoPV described herein.

In specific embodiments, immunogenic compositions described herein are monovalent formulations. In other embodiments, immunogenic compositions described herein are multivalent formulations.

As used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeiae for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the pharmaceutical composition is administered. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

In certain embodiments, biodegradable polymers, such as ethylene vinyl acetate, polyanhydrides, polyethylene glycol (PEGylation), polymethyl methacrylate polymers, polylactides, poly(lactide-co-glycolides), polyglycolic acid, collagen, polyorthoesters, and polylactic acid, may be used as carriers. Liposomes or micelles can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

In a specific embodiment, pharmaceutical compositions are formulated to be suitable for the intended route of administration to a subject. For example, the pharmaceutical composition may be formulated to be suitable for parenteral, oral, intradermal, intranasal, transdermal, pulmonary, colorectal, intraperitoneal, and rectal administration. In a specific embodiment, the pharmaceutical composition may be formulated for intravenous, oral, intraperitoneal, intranasal, intratracheal, subcutaneous, intramuscular, topical, intradermal, transdermal or pulmonary administration.

In certain embodiments, the compositions described herein comprise, or are administered in combination with, an adjuvant. The adjuvant for administration in combination with a composition described herein may be administered before, concomitantly with, or after administration of the composition. In specific embodiments, an inactivated virus immunogenic composition described herein comprises one or more adjuvants. In some embodiments, the term “adjuvant” refers to a compound that when administered in conjunction with or as part of a composition described herein augments, enhances and/or boosts the immune response to a FmoPV virus, but when the compound is administered alone does not generate an immune response to the virus. In some embodiments, the adjuvant generates an immune response to a FmoPV and does not produce an allergy or other adverse reaction. Adjuvants can enhance an immune response by several mechanisms including, *e.g.*, lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages.

Specific examples of adjuvants include, but are not limited to, aluminum salts (alum) (such as aluminum hydroxide, aluminum phosphate, and aluminum sulfate), 3 De-O-acylated monophosphoryl lipid A (MPL) (see GB 2220211) and QS21 (see Kensil et al., in *Vaccine Design: The Subunit and Adjuvant Approach* (eds. Powell & Newman, Plenum Press, NY, 1995); U.S. Pat. No. 5,057,540). In some embodiments, the adjuvant is Freund’s adjuvant (complete or incomplete). Other adjuvants are oil in water emulsions (such as squalene or peanut oil), optionally in combination with immune stimulants, such as monophosphoryl lipid A (see Stoute et al., *N. Engl. J. Med.* 336, 86-91 (1997)). Another adjuvant is CpG (*Bioworld Today*, Nov. 15, 1998). Such adjuvants can be used with or without other specific immunostimulating agents such as MPL or 3-DMP, QS21, polymeric or monomeric amino acids such as polyglutamic acid or polylysine.

The pharmaceutical compositions described herein can be included in a container, pack, or dispenser together with instructions for administration.

In a particular embodiment, the recombinant N proteins of the present invention have antigenicity, making them suitable for use in immunogenic compositions. The antigenicity of these recombinant N proteins is demonstrated in Example 7. Among tested sera from the 56 cats that were RT-PCR positive and 401 cats that were RT-PCR negative for FmoPV, 49 (76.7%) and 78 (19.4%), respectively, were positive for IgG against N protein of FmoPV by Western blot analysis ($P < 0.0001$). See Fig. 9 and see Table 6 below. Among tested sera from the 56 cats that

were RT-PCR positive for FmoPV, only 5 (8.9%) were positive for IgM against N protein of FmoPV.

In Fig. 9, a Western blot analysis with stray cat sera against the purified (His)₆-tagged recombinant FmoPV N protein antigen, prominent immunoreactive protein bands of about 69 kDa, consistent with the expected size of 68.7 kDa of the recombinant protein, were detected in three of the six cat serum samples shown, indicating antigen-antibody interactions between the recombinant FmoPV N protein and serum antibodies. Results of RT-PCR of the corresponding urine samples for FmoPV are also shown. Table 6 shows the FmoPV viral load and antibody level of RT-PCR positive stray cats in this study.

Table 6

Cat no.	Date of sample collection	Type of positive sample(s)	FmoPV	
			Viral load (copies/ml)	Western blot
543	14.05.2009	Urine	1.4×10^4	+
545	14.05.2009	Faecal swab	3.8×10^5	-
557	12.06.2009	Urine	9.5×10^2	+
572	24.06.2009	Urine	1.2×10	+
587	02.07.2009	Urine	4.88	++
591	08.07.2009	Urine	2.7×10^4	+++
592	08.07.2009	Urine	3.0×10^3	+++
670	27.08.2009	Urine	2.7×10^3	+
680	31.08.2009	Urine	8.8×10^3	+++
688	03.09.2009	Urine	7.1×10^3	+++
725	03.11.2009	Faecal swab	2.7×10^3	++
761	24.11.2009	Urine	5.9×10^5	+++
773	01.12.2009	Urine	6.4×10^4	+
776	04.12.2009	Urine	2.3×10^3	+++
802	24.12.2009	Urine	2.76	-
810	29.12.2009	Urine	1.6×10^2	-
818	12.01.2010	Urine	1.06	-
835	22.01.2010	Urine	2.4×10^4	+
850	29.01.2010	Urine	2.1×10^4	++
851	29.01.2010	Urine	2.6	++
		Faecal swab	5.0×10^2	
858	26.02.2010	Urine	6.9×10^4	+
898	23.03.2010	Urine	2.4×10^4	+
900	23.03.2010	Urine	1.6×10^4	+
906	23.03.2010	Urine	9.8×10^3	++
		Faecal swab	2.0×10^4	
908	25.03.2010	Urine	8.8×10^2	+
909	25.03.2010	Urine	2.6×10^3	-
938	29.04.2010	Urine	2.1×10^2	++
962	06.05.2010	Urine	8.0×10^3	+++
968	10.05.2010	Urine	4.7×10^2	+++
970	10.05.2010	Blood	3.1×10^4	+++
979	17.05.2010	Urine	5.3×10^3	+++
990	24.05.2010	Urine	1.4×10^3	++
997	31.05.2010	Urine	5.0×10^3	++
1012	10.06.2010	Urine	9.5×10^3	+
1036	28.06.2010	Urine	1.6×10^4	++
1055	02.08.2010	Urine	1.0×10^3	+
1057	02.08.2010	Urine	9.7×10^3	++
1078	09.09.2010	Urine	2.0×10^5	+
1091	24.09.2010	Urine	7.0×10^3	+
1096	27.09.2010	Urine	2.0×10^1	+++

Cat no.	Date of sample collection	Type of positive sample(s)	FmoPV	
			Viral load (copies/ml)	Western blot
1107	07.10.2010	Urine	4.6×10^3	++
1148	25.10.2010	Urine	1.4×10^5	++
1155	28.10.2010	Urine	3.2×10^{-1}	+
1189	25.11.2010	Urine	6.9×10^3	+
1226	06.01.2011	Urine	3.7×10^{-2}	+++
1297	28.02.2011	Urine	2.7×10^2	+
1312	09.03.2011	Urine	3.8	++
1314	09.03.2011	Urine	1.6×10^3	-
1325	14.03.2011	Urine	2.3×10^3	++
1327	24.03.2011	Urine	3.7×10^4	+
1336	31.03.2011	Urine	5.4×10^5	++
1357	28.04.2011	Urine	1.0×10^2	++
1359	28.04.2011	Urine	2.0×10^5	+
1392	30.05.2011	Urine	5.2×10^4	-
1407	13.06.2011	Urine	3.5×10	++
1409	16.06.2011	Urine	1.4×10^6	+

Specific apple green finely granular and diffuse cytoplasmic fluorescence was also observed using serum from guinea pig immunized with recombinant N protein of FmoPV or corresponding serum of the infected cat (Fig. 6).

5.6 IMMUNOGENIC COMPOSITIONS COMPRISING LIVE VIRUSES

In one embodiment, provided herein are immunogenic compositions (e.g., vaccines) comprising one or more live FmoPV described herein. In some embodiments, the live virus is attenuated. In some embodiments, an immunogenic composition comprises two, three, four or more live viruses.

In certain embodiments, provided herein are immunogenic compositions (e.g., vaccines) comprising about 10^5 to about 10^{10} fluorescent focus units (FFU) of live attenuated FmoPV described herein, about 0.1 to about 0.5 mg monosodium glutamate, about 1.0 to about 5.0 mg hydrolyzed porcine gelatin, about 1.0 to about 5.0 mg arginine, about 10 to about 15 mg sucrose, about 1.0 to about 5.0 mg dibasic potassium phosphate, about 0.5 to about 2.0 mg monobasic potassium phosphate, and about 0.001 to about 0.05 $\mu\text{g/ml}$ gentamicin sulfate per dose. In some embodiments, the immunogenic compositions (e.g., vaccines) are packaged as pre-filled sprayers containing single 0.2 ml doses.

In a specific embodiment, provided herein are immunogenic compositions (e.g., vaccines) comprising $10^{6.5}$ to $10^{7.5}$ FFU of live attenuated FmoPV described herein, 0.188 mg monosodium glutamate, 2.0 mg hydrolyzed porcine gelatin, 2.42 mg arginine, 13.68 mg sucrose, 2.26 mg dibasic potassium phosphate, 0.96 mg monobasic potassium phosphate, and $< 0.015 \mu\text{g/ml}$

gentamicin sulfate per dose. In some embodiments, the immunogenic compositions (e.g., vaccines) are packaged as pre-filled syringes containing single 0.2 ml doses.

In a specific embodiment, the live virus is propagated in embryonated chicken eggs before its use in an immunogenic composition described herein. In another specific embodiment, the live virus is not propagated in embryonated chicken eggs before its use in an immunogenic composition described herein. In another specific embodiment, the live virus is propagated in mammalian cells before its use in an immunogenic composition described herein.

An immunogenic composition comprising a live virus for administration to a subject may be preferred because multiplication of the virus in the subject may lead to a prolonged stimulus of similar kind and magnitude to that occurring in natural infections, and, therefore, confer substantial, long lasting immunity.

5.7 GENERATION OF ANTIBODIES

The FmoPV described herein may be used to elicit antibodies against FmoPV or a heterologous nucleotide sequence. In a specific embodiment, a FmoPV described herein or a composition thereof may be administered to a non-human subject (e.g., mouse, rabbit, rat, guinea pig, cat, etc.) to induce an immune response that includes the production of antibodies which may be isolated using techniques known to one of skill in the art (e.g., immunoaffinity chromatography, centrifugation, precipitation, etc.).

Alternatively, a virus described herein may be used to screen for antibodies from antibody libraries. For example, a FmoPV may be immobilized to a solid support (e.g., a silica gel, a resin, a derivatized plastic film, a glass bead, cotton, a plastic bead, a polystyrene bead, an alumina gel, or a polysaccharide, a magnetic bead), and screened for binding to antibodies. As an alternative, the antibodies may be immobilized to a solid support and screened for binding to a FmoPV described herein. Any screening assay, such as a panning assay, ELISA, surface plasmon resonance, or other antibody screening assay known in the art may be used to screen for antibodies that bind to a FmoPV. The antibody library screened may be a commercially available antibody library, an *in vitro* generated library, or a library obtained by identifying and cloning or isolating antibodies from a subject infected with FmoPV. In particular embodiments, the antibody library is generated from a survivor of an FmoPV outbreak. Antibody libraries may be generated in accordance with methods known in the art. In a particular embodiment, the

antibody library is generated by cloning the antibodies and using them in phage display libraries or a phagemid display library.

Antibodies elicited or identified in accordance with the methods described herein may be tested for specificity for FmoPV antigens and the ability to neutralize FmoPV using the biological assays known in the art or described herein. In one embodiment, an antibody identified or isolated from a non-human animal antibody specifically binds to a FmoPV antigen.

Antibodies elicited or identified in accordance with the methods described herein may be tested for specificity to, and the ability to neutralize, a peptide or polypeptide antigen encoded by a heterologous nucleotide sequence described herein using the biological assays known in the art or described herein. In one embodiment, an antibody identified or isolated from a non-human animal antibody specifically binds to a peptide or polypeptide antigen encoded by a heterologous nucleotide sequence described herein. In one embodiment, the neutralizing antibody neutralizes the viral, bacterial, fungal or other pathogen, or a tumor that expresses the peptide or polypeptide antigen encoded by a heterologous nucleotide sequence described herein.

Antibodies elicited or identified using a FmoPV described herein include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds to a hemagglutinin polypeptide. The immunoglobulin molecules may be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. Antibodies include, but are not limited to, monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, chimeric antibodies, single-chain Fvs (scFv), single chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies elicited or identified using a method described herein), and epitope-binding fragments of any of the above.

Antibodies elicited or identified using a FmoPV described herein may be used in diagnostic immunoassays, passive immunotherapy, and generation of antiidiotypic antibodies. The antibodies before being used in passive immunotherapy may be modified, *e.g.*, the antibodies may be chimerized or humanized. See, *e.g.*, U.S. Patent Nos. 4,444,887 and 4,716,111; and International Publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741, each of which is incorporated herein by reference in its entirety, for reviews on the generation of chimeric and humanized

antibodies. In addition, the ability of the antibodies to neutralize FmoPV and the specificity of the antibodies for FmoPV antigens may be tested prior to using the antibodies in passive immunotherapy. Antibodies against FmoPV antigens are used to detect the presence of FmoPV in a subject. In specific embodiments, FmoPV antibodies are used to diagnose FmoPV infections in feline. In specific embodiments, FmoPV antibodies are used to diagnose TIN in feline.

The antibodies elicited or identified using a FmoPV described herein may be incorporated into compositions. In a specific embodiment, the compositions are pharmaceutical compositions. In some embodiments, a pharmaceutical composition may comprise one or more other therapies in addition to an antibody. The pharmaceutical compositions provided herein can be in any form that allows for the composition to be administered to a subject. In a specific embodiment, the pharmaceutical compositions are suitable for veterinary and/or human administration. In another specific embodiment, the antibody compositions are formulated for the intended route of administration (*e.g.*, parenteral, intranasal, or pulmonary administration). The antibody compositions may be used in methods of preventing and/or treating a FmoPV infection. The antibody compositions may also be used in methods of preventing and/or treating FmoPV disease.

Antibodies elicited or identified using a FmoPV described herein may be used to monitor the efficacy of a therapy and/or disease progression. Any immunoassay system known in the art may be used for this purpose including, but not limited to, competitive and noncompetitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assays), "sandwich" immunoassays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays and immunoelectrophoresis assays, to name but a few.

5.8 PROPHYLACTIC & THERAPEUTIC USES

In one aspect, provided herein are methods for inducing an immune response in a subject utilizing a FmoPV described herein or an immunogenic composition thereof. In a specific embodiment, a method for inducing an immune response to a FmoPV in a subject comprises administering to a subject in need thereof an effective amount of a FmoPV or an immunogenic

composition thereof. In certain embodiments, the FmoPV or immunogenic composition thereof expresses FmoPV proteins from two or more types, subtypes or strains of FmoPV, and thus, may be used to induce an immune response to two or more types, subtypes or strains of FmoPV. In a specific embodiment, a method for inducing an immune response to a FmoPV in a subject comprises administering to a subject in need thereof a FmoPV described herein as a live virus vaccine. In particular embodiments, the live virus vaccine comprises an attenuated virus. In another embodiment, a method for inducing an immune response to FmoPV in a subject comprises administering to a subject in need thereof a FmoPV described herein as an inactivated virus vaccine.

In another aspect, provided herein are methods for preventing and/or treating a FmoPV infection in a subject utilizing a FmoPV described herein or a pharmaceutical composition thereof. In one embodiment, a method for preventing or treating a FmoPV infection in a subject comprises administering to a subject in need thereof an effective amount of a FmoPV or a composition thereof. In another embodiment, a method for preventing or treating an FmoPV infection in a subject comprises administering to a subject in need thereof an effective amount of a FmoPV or a pharmaceutical composition thereof and one or more other therapies. In another embodiment, a method for preventing or treating a FmoPV infection in a subject comprises administering to a subject in need thereof a FmoPV described herein as a live virus vaccine. In particular embodiments, the live virus vaccine comprises an attenuated virus. In another embodiment, a method for preventing or treating a FmoPV infection in a subject comprises administering to a subject in need thereof a FmoPV described herein as an inactivated virus vaccine.

In another aspect, provided herein are methods for preventing and/or treating a FmoPV in a subject utilizing a FmoPV described herein or a pharmaceutical composition thereof. In a specific embodiment, a method for preventing or treating a FmoPV disease in a subject comprises administering to a subject in need thereof an effective amount of a FmoPV or a pharmaceutical composition thereof. In another embodiment, a method for preventing or treating a FmoPV in a subject comprises administering to a subject in need thereof an effective amount of a FmoPV or a pharmaceutical composition thereof and one or more other therapies. In another embodiment, a method for preventing or treating a FmoPV disease in a subject comprises administering to a subject in need thereof a FmoPV described herein as a live virus vaccine. In

particular embodiments, the live virus vaccine comprises an attenuated virus. In another embodiment, a method for preventing or treating a FmoPV disease in a subject comprises administering to a subject in need thereof a FmoPV described herein as an inactivated virus vaccine.

5.9 DOSAGE AND FREQUENCY OF ADMINISTRATION

A FmoPV, an antibody or a composition described herein may be delivered to a subject by a variety of routes. These include, but are not limited to, intranasal, intratracheal, oral, intradermal, intramuscular, topical intraperitoneal, transdermal, intravenous, pulmonary, conjunctival and subcutaneous routes. In some embodiments, a composition is formulated for topical administration, for example, for application to the skin. In specific embodiments, the composition is formulated for nasal administration, *e.g.*, as part of a nasal spray. In certain embodiments, a composition is formulated for intramuscular administration. In some embodiments, a composition is formulated for subcutaneous administration. In specific embodiments for live virus vaccines, the vaccine is formulated for administration by a route other than injection. In some embodiments it may be desirable to introduce the pharmaceutical compositions into the lungs by any suitable route. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent for use as a spray.

In some embodiments, when a FmoPV or a composition thereof is administered to a non-human subject (*e.g.*, a cat), the virus or composition is administered orally to the subject in the subject's food. In other embodiments, when a FmoPV or a composition thereof is administered to a subject (*e.g.*, cat), the virus or composition is administered orally to the subject in the subject's water. In other embodiments, when a FmoPV or a composition thereof is administered to a non-human subject, the virus or composition is administered by spraying the subject with the virus or composition.

The amount of a FmoPV, an antibody or composition described herein which will be effective in the treatment and/or prevention of a FmoPV infection or a FmoPV disease will depend on the nature of the disease, and can be determined by standard techniques. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the infection or disease caused by it, and should be decided according to the

judgment of the practitioner and each subject's circumstances. For example, effective doses may also vary depending upon means of administration, target site, physiological state of the subject (including age, body weight, health), whether the subject is human or an animal, whether other medications are administered, and whether treatment is prophylactic or therapeutic. Similarly, the amount of a FmoPV or a composition thereof that will be effective as a delivery vector will vary and can be determined by standard techniques. Treatment dosages are optimally titrated to optimize safety and efficacy.

In certain embodiments, an *in vitro* assay is employed to help identify optimal dosage ranges. Effective doses may be extrapolated from dose response curves derived from *in vitro* or animal model test systems.

Exemplary doses for live FmoPV may vary from 10-100, or more, virions per dose. In some embodiments, suitable dosages of a live FmoPV virus are 10^2 , 5×10^2 , 10^3 , 5×10^3 , 10^4 , 5×10^4 , 10^5 , 5×10^5 , 10^6 , 5×10^6 , 10^7 , 5×10^7 , 10^8 , 5×10^8 , 1×10^9 , 5×10^9 , 1×10^{10} , 5×10^{10} , 1×10^{11} , 5×10^{11} or 10^{12} pfu, and can be administered to a subject once, twice, three or more times with intervals as often as needed. In another embodiment, a live FmoPV is formulated such that a 0.2-mL dose contains $10^{6.5}$ – $10^{7.5}$ fluorescent focal units of live FmoPV. In another embodiment, an inactivated vaccine is formulated such that it contains about 15 μ g to about 100 μ g, about 15 μ g to about 75 μ g, about 15 μ g to about 50 μ g, or about 15 μ g to about 30 μ g of a FmoPV protein.

In certain embodiments, a FmoPV described herein or a composition thereof is administered to a subject as a single dose followed by a second dose 3 to 6 weeks later. In accordance with these embodiments, booster inoculations may be administered to the subject at 6 to 12 month intervals following the second inoculation. In certain embodiments, the booster inoculations may utilize a different FmoPV strain or a composition thereof. In some embodiments, the administration of the same FmoPV strain or a composition thereof may be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or at least 6 months.

For passive immunization with an antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 50 mg/kg or 0.1 to 15 mg/kg, of the subject body weight. For example, dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg or in other words, 70 mg or 700 mg or within the range of 70-700 mg, respectively,

for a 70 kg patient. An exemplary treatment regime entails administration once per every two weeks or once a month or once every 3 to 6 months for a period of one year or over several years, or over several year-intervals. In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the FmoPV in the patient.

5.10 SCREENING ASSAYS

In one aspect, a FmoPV described herein may be used to study the life cycle of a FmoPV. For example, a FmoPV described herein that expresses a detectable heterologous sequence (*e.g.*, a detectable substance such as described above) is introduced into a host cell and the life cycle of the virus is monitored by the assessing the expression of the detectable heterologous sequence. A FmoPV described herein that expresses a detectable heterologous sequence may also be administered to a non-human animal and the infection monitored by assessing the expression of the detectable heterologous sequence.

In another aspect, provided herein are high throughput screening assays for the identification or validation of compounds that modulate the replication of negative-sense, single-stranded RNA viruses, in particular FmoPV. In a specific embodiment, the high throughput screening assay to identify a compound that modulates the replication of a negative-sense, single-stranded RNA virus (in particular FmoPV) comprises: (a) contacting a compound or a member of a library of compounds with a host cell infected with a FmoPV described herein that expresses a detectable heterologous nucleotide sequence; and (b) measuring the expression or activity of a product encoded by the detectable heterologous nucleotide sequence. In another embodiment, the high throughput screening assay to identify a compound that modulates the replication of a negative-sense, single-stranded RNA virus (in particular FmoPV) comprises: (a) infecting a host cell with a FmoPV described herein that expresses a detectable heterologous nucleotide sequence in the presence of a compound or a member of a library of compounds; and (b) measuring the expression or activity a product encoded by the detectable heterologous nucleotide sequence. In another embodiment, the high throughput screening assay to identify a

compound that modulates the replication of a negative-sense, single-stranded RNA virus (in particular FmoPV) comprises: (a) contacting a host cell with a compound or a member of a library of compounds; (b) infecting the host cell with a FmoPV described herein that expresses a detectable heterologous nucleotide sequence; and (c) measuring the expression or activity a product encoded by the detectable heterologous nucleotide sequence.

Any method known to one of skill in the art can be used measure the expression or activity of a product encoded by the detectable heterologous nucleotide sequence. In one embodiment, the product encoded by the detectable heterologous nucleotide sequence is RNA and a technique known to one of skill in the art, such as RT-PCR or Northern blot analysis, is used to measure the expression of the RNA product. In another embodiment, the product encoded by the detectable heterologous nucleotide sequence is protein and a technique known to one of skill in the art, such as western blot analysis or an ELISA, is used to measure the expression of the protein product. In another embodiment, the product encoded by the detectable heterologous nucleotide sequence is protein and the activity of the protein is measured using a technique known to one of skill in the art.

Any screening assay described herein can be performed individually, *e.g.*, just with the test compound, or with appropriate controls. For example, a parallel assay without the test compound, or other parallel assays without other reaction components (*e.g.*, virus) can be performed. In one embodiment, a parallel screening assay as described above is performed except that a negative control and/or a positive control are used in place of a test compound. In another embodiment, to eliminate cytotoxic compounds that appear as false positives, a counter screen is performed in which uninfected cells are transfected with a nucleic acid construct (*e.g.*, a plasmid) comprising a detectable heterologous nucleotide sequence and the expression or activity of a product encoded by the detectable heterologous nucleotide sequence is measured. Alternatively, it is possible to compare assay results to a reference, *e.g.*, a reference value, *e.g.*, obtained from the literature, a prior assay, and so forth. Appropriate correlations and art known statistical methods can be used to evaluate an assay result.

In another aspect, the antiviral effect of a compound on FmoPV can be assessed in a non-human animal using a FmoPV described herein. In one embodiment, the antiviral effect of a compound on FmoPV can be assessed by a method comprising: (a) administering (for example, parenterally, subcutaneously, intranasally, or intraperitoneally) to a non-human subject,

concurrently, subsequently or prior to administration of a compound, an effective amount of a FmoPV described herein; b) waiting for a time interval following the administration of the FmoPV; and d) detecting the FmoPV in the subject or in a biological specimen from the subject.

5.11 KITS

In one aspect, provided herein is a kit comprising, in one or more containers, one or more nucleic acid sequences described herein. In a specific embodiment, a kit comprises, in a container, a FmoPV gene segment or a complement thereof. In another embodiment, a kit comprises, in one, two or more containers, a nucleic acid sequence encoding a FmoPV gene segments or a complement thereof. The kit may further comprise one or more of the following: host cells suitable for rescue of the virus, reagents suitable for transfecting plasmid DNA into a host cell, helper virus, plasmids encoding one or more types of FmoPV gene segments, one or more expression plasmids encoding viral proteins, and/or one or more primers specific for a FmoPV gene segment or a complement thereof, or nucleic acid sequences encoding the same.

In another aspect, provided herein is a kit comprising one or more containers filled with one or more of the one or more FmoPV described herein or a composition thereof. In a specific embodiment, provided herein is a pharmaceutical pack or kit comprising, in one or more containers, a composition comprising one or more FmoPV described herein. In another aspect, provided herein is a kit comprising, in one or more containers, primers specific for a particular FmoPV gene segment.

In another aspect, provided herein is a kit comprising one or more containers filled with one or more antibodies generated or identified using a FmoPV described herein. In one embodiment, a kit comprises an antibody described herein, preferably an isolated antibody, in one or more containers. In a specific embodiment, a kit encompassed herein contains an isolated FmoPV antigen that the antibodies encompassed herein react with as a control. In a specific, a kit provided herein further comprise a control antibody which does not react with a FmoPV antigen that an antibody encompassed herein reacts with. In another specific embodiment, a kit provided herein contains a means for detecting the binding of an antibody to a FmoPV antigen that an antibody encompassed herein reacts with (*e.g.*, the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody

may be conjugated to a detectable substrate). In specific embodiments, a kit may include a recombinantly produced or chemically synthesized FmoPV antigen. The FmoPV antigen provided in the kit may also be attached to a solid support. In a more specific embodiment the detecting means of the above described kit includes a solid support to which a FmoPV antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the FmoPV antigen can be detected by binding of the said reporter-labeled antibody.

Optionally associated with such a kit can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Example 2 demonstrates a diagnostic test done by RT-PCR. Another diagnostic test demonstrated in Example 3, is a viral load test using real-time quantitative RT-PCR using the above genomic information obtained from sequencing.

5.12 COMPLETE GENOME SEQUENCING AND ANALYSIS

Three complete genomes of FmoPV, from two urine (761U, 776U) and one rectal swab (M252A) samples, were amplified and sequenced using RNA extracted directly from the specimens as templates with a strategy described in our previous publications (13,14). Genome analysis was performed as described in our previous publications (13,14,23,24,25). Phylogenetic trees were constructed by maximum likelihood method using PhyML 3.0 (26).

The complete genome sequences of three strains of FmoPV designated 761U, 776U and M252A were determined. The genome sequence for FmoPV strain 761U was deposited at GenBank and given accession number JQ411014. The FmoPV 761U nucleotide sequence is shown in Fig. 2. The genome sequence for FmoPV strain 776U was deposited at GenBank and given accession number JQ411015. The FmoPV 776U nucleotide sequence is shown in Fig. 3. The genome sequence for FmoPV strain M252A was deposited at GenBank and given accession number JQ411016. The FmoPV M252A nucleotide sequence is shown in Fig. 4.

The genome size of these FmoPV nucleotide sequences are 16050 bases and G+C contents 35.1% to 35.3%, with FmoPV having the largest genome among all morbilliviruses with genome sequences available (see Fig. 1). The genome of FmoPV conforms to the rule of six as

in other paramyxovirus genomes. It contains a 12-nt complementary 3' leader and 5' trailer sequence. The 3' leader sequence is 55 nt. In contrast to other morbilliviruses which only have 5' trailer sequences of 40 or 41 nt, the genome of FmoPV has a trailer sequence of 400 nt, accounting for its bigger genome size. Such long trailer sequences of >400 nt have only been observed in avian paramyxoviruses 3 (681-707 nt) and 5 (552 nt) and tupaia paramyxovirus (590 nt).

Similar to other morbilliviruses, the genome of FmoPV contains six genes (3'-N-P/V/C-M-F-H-L-5') (see Fig. 1). Pairwise alignment of the predicted gene products among FmoPV and other paramyxoviruses showed the highest amino acid identities with members of the genus *Morbillivirus*, with the N, P/V/C(P), P/V/C(V), P/V/C(C), M, F, H and L of FmoPV having 54.3-56.8%, 25.6-31.7%, 20.7-25.7%, 18.3-25.4%, 57.6-60.0%, 35.8-45.1%, 20.4-24.1% and 55.2-57.3% amino acid identities to those of other morbilliviruses (see Table 1). The lengths and characteristics of the major structural genes and intergenic regions (IGRs) are summarized in Table 2.

TABLE 1

Pairwise amino acid identities of predicted gene products of FmoPV compared to other paramyxoviruses

Paramyxoviruses	Percentage of amino acid sequence identity																			
	761U	776U	M252A		761U	776U	M252A		761U	776U	M252A		761U	776U	M252A		761U	776U	M252A	
<i>Morbillivirus</i>																				
FmoPV 761U	-	99.2	96.0	-	97.4	89.2	-	98.8	95.8	-	98.9	98.9	96.3	-	99.0	96.3	-	99.4	97.0	
FmoPV 776U	99.2	-	96.1	97.4	-	88.6	98.8	-	96.4	98.9	-	95.9	96.3	99.0	-	95.3	99.4	-	97.3	
FmoPV M252A	96.0	96.1	-	89.2	88.6	-	95.8	96.4	-	96.3	95.9	-	96.3	95.3	-	95.3	97.0	97.3	-	
CdIPV	56.8	56.5	56.2	29.7	29.3	27.5	58.8	58.5	58.8	36.0	35.8	36.1	20.4	20.6	20.6	55.5	55.4	55.4	55.4	
DmoPV	54.5	54.3	55.0	26.4	25.8	25.6	59.3	59.6	59.5	43.0	42.2	42.4	24.1	24.1	23.9	56.4	56.4	56.5	56.5	
McaPV	55.4	55.2	54.6	29.0	28.8	27.8	60.0	59.7	59.0	44.0	44.0	43.9	20.7	20.9	20.7	56.0	55.9	55.8	55.8	
PpPV	55.6	55.8	55.8	31.6	31.7	31.6	58.2	58.3	58.8	42.8	42.8	43.2	21.6	21.8	21.6	57.3	57.3	57.2	57.2	
RuPV	55.5	55.5	55.6	28.4	28.4	26.8	59.5	59.2	58.2	44.6	44.3	45.1	21	21.1	22.4	55.4	55.3	55.2	55.2	
PdPV	56.6	56.2	56.2	30.6	30.4	28.8	57.9	57.9	57.6	42.7	42.9	42.9	20.6	20.8	20.9	55.4	55.4	55.7	55.7	
<i>Avulavirus</i>																				
AviPV-6	29.1	29.3	28.1	18.3	17.9	18.6	23.5	23.5	22.9	26.8	27.0	26.6	17.7	17.6	17.3	28.5	28.6	29.1	29.1	
NdPV	27.1	27.1	28.0	19.8	18.8	18.1	19.5	20.0	20.9	25.8	25.8	25.4	17.5	17.2	16.2	27.7	27.5	27.6	27.6	
<i>Henipavirus</i>																				
HenPV	33.6	33.2	32.8	21.1	22.2	23.3	43.9	44.2	44.2	32.4	32.6	33.0	17.8	17.8	18.9	43.4	43.4	43.4	43.4	
NipPV	33.8	33.4	33.0	22.4	21.8	22.8	43.1	43.3	43.4	33.0	33.2	33.3	18.2	18.2	18.9	44.9	44.8	45.2	45.2	
<i>Respirovirus</i>																				
BpPV-3	25.7	25.9	25.4	18.0	18.7	19.2	33.9	33.6	34.2	28.5	28.2	28.5	18.9	18.9	18.2	38.4	38.6	38.9	38.9	
SenPV	24.2	25.2	25.0	19.0	18.5	20.5	34.7	34.7	34.4	27.1	26.5	26.5	20.5	20.3	21.1	39.1	39.3	39.4	39.4	
<i>Rubulavirus</i>																				
HpiPV-2	27.5	27.7	27.9	19.4	19.6	16.8	22.8	23.1	21.8	25.0	24.4	25.0	18.0	18.0	18.3	30.4	30.2	30.2	30.2	
MunPV	27.6	27.8	27.7	19.6	19.9	17.2	20.4	20.0	20.7	26.4	26.2	25.7	18.1	17.8	18.7	30.0	30.1	29.9	29.9	
Unclassified																				
<i>Paramyxovirinae</i>																				
AsaPV	29.8	29.8	28.4	16.4	17.2	17.1	34.7	34.7	34.7	30.9	30.6	30.8	20.1	20.1	19.0	40.0	40.1	40.4	40.4	
TinPV	35.7	36.4	36.0	22.7	22.7	23.0	48.0	48.3	48.8	32.7	32.9	33.0	16.1	15.8	16.6	46.4	46.4	46.4	46.4	
BeiPV	36.6	36.2	36.3	23.5	23.3	23.9	47.4	47.7	48.5	32.9	32.7	32.7	16.1	15.8	15.8	46.3	46.3	46.6	46.6	
FdIPV	28.4	28.4	28.5	19.9	21.1	20.8	34.9	34.5	33.9	29.0	29.2	29.7	19.9	19.9	19.2	40.0	40.0	39.7	39.7	
JPV	34.0	34.0	34.0	23.1	23.3	22.3	47.8	48.1	48.4	31.8	31.8	32.9	14.8	15.0	14.4	46.9	46.9	47.2	47.2	
MeaPV	38.6	38.6	37.6	22.9	22.9	22.0	47.5	48.2	46.9	36.3	36.3	35.8	19.0	19.2	19.9	48.6	48.8	48.8	48.8	
TupPV	33.4	32.7	32.1	23.2	23.2	23.9	43.9	43.2	42.5	35.0	35.4	35.0	14.7	14.4	15.6	47.3	47.2	47.1	47.1	
NatPV	37.2	36.9	38.1	22.7	23.8	22.6	51.5	51.5	50.6	33.0	32.5	32.6	18.6	18.4	18.7	47.7	48.0	47.9	47.9	

TABLE 2

Molecular features and predicted gene products of FmoPV and other morbilliviruses

Virus	Gene	mRNA features (nt)					Intergenic regions (nt)	Deduced protein			
		Total length	5' UTR	ORF	3' UTR	hexamer phase		Size (aa)	MW (kDa)	pI	Coding frame
FmoPV 761U	Leader	55					(TTT)				
	N	1659	52	1560	47	2	CTT	519	57.01	5.27	3
	P/V/C(P)	1637	63	1476	98	2	CTT	491	53.12	5.20	2
	P/V/C(V)	1638	63	831	744	2	CTT	276	29.97	4.85	2
	P/V/C(C)	1637	94	513	1030	2	CTT	170	19.90	9.69	3
	M	1378	31	1014	333	4	CTA	337	38.05	9.29	2
	F	2191	215	1632	344	5	CTT	543	60.26	8.80	1
	H	1934	30	1788	116	3	CTT	595	68.11	6.25	3
	L	6781	22	6609	150	2	(CTT)	2202	252.87	8.32	3
	Trailer	400									
FmoPV 776U	Leader	55					(TTT)				
	N	1659	52	1560	47	2	CTT	519	57.06	5.15	3
	P/V/C(P)	1637	63	1476	98	2	CTT	491	53.19	5.33	2
	P/V/C(V)	1638	63	831	744	2	CTT	276	29.99	4.91	2
	P/V/C(C)	1637	94	513	1030	2	CTT	170	19.87	9.69	3
	M	1378	31	1014	333	4	CTA	337	38.02	9.29	2
	F	2191	215	1632	344	5	CTT	543	60.21	8.79	1
	H	1934	30	1788	116	3	CTT	595	68.24	6.03	3
	L	6781	22	6609	150	2	(CTT)	2202	253.01	8.23	3
	Trailer	400									
FmoPV M252A	Leader	55					(TTT)				
	N	1659	52	1560	47	2	CTT	519	57.08	5.34	3
	P/V/C(P)	1637	63	1476	98	2	CTT	491	53.41	5.44	2
	P/V/C(V)	1638	63	831	744	2	CTT	276	29.94	5.13	2
	P/V/C(C)	1637	94	513	1030	2	CTT	170	19.86	9.69	3
	M	1378	31	1014	333	4	CTA	337	38.06	9.29	2
	F	2191	215	1632	344	5	CTT	543	60.19	8.80	1
	H	1934	30	1788	116	3	CTT	595	68.18	6.25	3
	L	6781	22	6609	150	2	(CTT)	2202	252.91	8.28	3
	Trailer	400									
MeaPV	Leader	55					(CTT)				
	N	1689	52	1578	59	2	CTT	525	58.02	5.11	3

Virus	Gene	mRNA features (nt)						Deduced protein			
		Total length	5' UTR	ORF	3' UTR	hexamer phase	Intergenic regions (nt)	Size (aa)	MW (kDa)	pI	Coding frame
CdiPV	P/V/C(P)	1655	59	1524	72	2	CTT	507	53.90	4.99	1
	P/V/C(V)	1656	59	900	697	2	CTT	299	31.85	4.66	1
	P/V/C(C)	1655	81	561	1013	2	CTT	186	21.11	10.36	2
	M	1466	32	1008	426	4	CTT	335	37.71	9.07	3
	F	2373	583	1653	137	3	CTT	550	59.53	8.78	1
	H	1958	20	1854	84	3	CGT	617	69.17	7.88	2
	L	6643	22	6552	69	2	(CTT)	2183	247.74	8.43	3
	Trailer	40									
	Leader	55					(CTT)				
	N	1683	52	1572	59	2	CTT	523	58.14	5.20	3
	P/V/C(P)	1655	59	1524	72	2	CTT	507	54.75	5.03	1
	P/V/C(V)	1656	59	900	697	2	CTT	299	33.11	4.66	1
	P/V/C(C)	1655	81	525	1049	2	CTT	174	20.26	10.30	2
	M	1447	32	1008	407	4	CTT	335	37.77	8.87	3
DmoPV	F	2206	85	1989	132	2	CTT	662	72.95	9.18	3
	H	1946	20	1815	111	3	CTA	604	67.99	6.74	2
	L	6642	22	6555	65	2	(CAA)	2184	248.19	8.39	3
	Trailer	41									
	Leader	55					(CTT)				
	N	1683	52	1572	59	2	CTT	523	57.49	5.14	3
	P/V/C(P)	1655	59	1521	75	2	CTT	506	55.26	5.09	1
	P/V/C(V)	1656	59	912	685	2	CTT	303	33.69	4.75	1
	P/V/C(C)	1655	81	534	1040	2	CTT	177	20.41	10.19	2
	M	1453	32	1008	413	4	CTT	335	37.97	8.97	3
	F	2212	421	1659	132	2	CTT	552	59.87	8.81	3
	H	1946	20	1815	111	3	CTT	604	68.04	6.18	2
	L	6643	22	6552	69	2	(CAA)	2183	248.07	8.52	3
	Trailer	40									
PprPV	Leader	55					(CTT)				
	N	1689	52	1578	59	2	CTT	525	57.78	5.21	3
	P/V/C(P)	1655	59	1530	66	2	CTT	509	54.79	5.14	1
	P/V/C(V)	1656	59	897	700	2	CTT	298	31.34	4.58	1
	P/V/C(C)	1655	81	534	1040	2	CTT	177	19.93	9.92	2
	M	1483	32	1008	443	4	CTT	335	37.95	8.97	3
	F	2411	634	1641	136	2	CTT	546	59.12	8.71	3
	H	1957	20	1830	107	4	CTT	609	68.76	6.64	3
	L	6643	22	6552	69	2	(CTA)	2183	247.27	7.73	3
	Trailer	40									
	Leader	55					(CTT)				
	N	1689	52	1578	59	2	CTT	525	58.04	5.08	3
	P/V/C(P)	1655	59	1524	72	2	CTT	507	54.36	4.82	1
	P/V/C(V)	1656	59	900	697	2	CTT	299	32.57	4.56	1
RinPV	P/V/C(C)	1655	81	534	1040	2	CTT	177	19.93	10.29	2
	M	1460	32	1008	420	4	CTT	335	37.54	9.15	3
	F	2367	589	1641	137	3	CTT	546	58.73	8.43	1
	H	1958	20	1830	108	3	CGT	609	67.90	6.61	2
	L	6643	22	6552	69	2	(CTT)	2183	248.21	8.48	3
	Trailer	40									

The conserved N-terminal motif MA(T/S)L in morbilliviruses was absent in the N protein of FmoPV, which contained the sequence MSSSL as a result of A→S (G→U at first codon position) substitution at the second amino acid (Fig. 5). Similar to the nuclear localization signal (NLS) of the N proteins in CdiPV, MeaPV and RinPV but different from the classical NLS sequence (PMID: 16716375), a leucine/isoleucine-rich motif at amino acid positions 70-77 is identified in the N protein of FmoPV (see SI Fig. 7). Similar to the nuclear export signal (NES) of the N proteins in CdiPV and RinPV, a leucine-rich motif at amino acid positions 4-11 is also identified in the N protein of FmoPV (see Fig. 5).

As in other morbilliviruses, the P/V/C gene of FmoPV contains two initiation codons, the first one for translation of P and V and the second for translation of C. Similar to most members of *Paramyxovirinae*, the P/V/C gene of FmoPV contains a UC-rich editing site that allows the addition of non-templated G residues to mRNA products during P/V/C gene transcription,

resulting in the production of different proteins with a common N-terminal region. In all three strains of FmoPV, this common N-terminal region consists of 226 amino acids.

To determine the exact location of P gene editing site and the number and frequency of G-residue insertions, a small cDNA fragment including the UC-rich region was amplified, cloned and sequenced using mRNA extracted from FmoPV infected CRFK cells. Among 23 independent clones sequenced, 13 contained the sequence TTAAAAGGGG (without G insertion, encoding P protein) and 10 contained the sequence TTAAAAGGGGG (one G inserted, encoding V protein). The sequence TTA_nG_n is conserved as in other paramyxovirus editing sites except for those of rubulaviruses. In contrast to other morbilliviruses in which the sequence is TTA₅G₃ (33), the TTA_nG_n sequence in FmoPV is TTA₄G₄.

Different from all other known morbilliviruses, the F protein of FmoPV has a single-basic protein cleavage site, whereas the cleavage sites in other morbilliviruses are multi-basic (34). Cellular trypsin-like protease cleaves the F protein into F1 and F2 before cell fusion occurs, which facilitates the isolation of these viruses in cell lines. Two heptad repeat sequences similar to those in F proteins of other paramyxoviruses were also identified in the F₁ of FmoPV. The F protein of FmoPV also contains the 10 Cys residues that are highly conserved in other morbilliviruses and 5 potential N-glycosylation sites, most of which located in the F₂ peptide.

Phylogenetic trees constructed using the predicted amino acid sequences of N, P, M, F, H and L genes of FmoPV and other members of *Paramyxoviridae* are shown in Fig. 7. In all six trees, the three viruses were clustered with morbilliviruses, with high bootstrap supports, forming a distinct subgroup (see Fig. 7). The trees were constructed by maximum likelihood method with bootstrap values calculated from 1000 trees and rooted on midpoint. The scale bars in Figure 2 indicate the branch length that corresponds to 0.5 substitutions per site. Three strains from FmoPV were named as 761U, 776U, M252A. Names and accession numbers of the other viruses in Figure 2 are listed in Table 3 below.

TABLE 3

Viruses and GenBank accession numbers

Abbreviation	Virus name	GenBank accession no.
AsaPV	Atlantic Salmon paramyxovirus	EU156171
AviPV-5	Avian paramyxovirus 5	GU206351

Abbreviation	Virus name	GenBank accession no.
AviPV-6	Avian paramyxovirus 6	NC_003043
AviPV-7	Avian paramyxovirus 7	FJ231524
BeiPV	Beilong virus	NC_007803
BpiPV-3	Bovine parainfluenza virus 3	NC_002161
CdiPV	Canine distemper virus	NC_001921
DmoPV	Dolphin morbillivirus	NC_005283
FdiPV	Fer-de-lance virus	NC_005084
GooPV	Goose paramyxovirus SF02	NC_005036
HenPV	Hendra virus	NC_001906
HpiPV-1	Human parainfluenza virus 1	NC_003461
HpiPV-2	Human parainfluenza virus 2	NC_003443
HpiPV-3	Human parainfluenza virus 3	NC_001796
HpiPV-4a	Human parainfluenza virus 4a	BAJ11741
HuRSV	Human respiratory syncytial virus	NC_001781
JPV	J-virus	NC_007454
MeaPV	Measles virus	NC_001498
MosPV	Mossman virus	NC_005339
MumPV	Mumps virus	NC_002200
NarPV	Nariva virus	FJ362497
NdiPV	Newcastle disease virus	NC_002617
NipPV	Nipah virus	NC_002728
PdiPV	Phocine distemper virus	P35944, P35939, BAA01205, BAA01206, CAA12080, CAA70843
PprPV	Peste-des-petits-ruminants virus	NC_006383
RinPV	Rinderpest virus	NC_006396
SenPV	Sendai virus	NC_001552
SpiPV-3	Swine parainfluenza virus 3	EU439429
ThkPV-1	Tuhoko virus 1	GU128080
ThkPV-2	Tuhoko virus 2	GU128081
ThkPV-3	Tuhoko virus 3	GU128082
TlmPV	Tailam virus	JN689227
TupPV	Tupaia paramyxovirus	NC_002199

5.13 DETECTION OF FMOPV INFECTION IN FELINES

Infection of a feline by FmoPV can be detected in sera by the use of immunofluorescent antibodies as demonstrated in Example 9, or by the detection of neutralizing antibodies as demonstrated in Example 10. Of the 27 cat sera samples tested in Example 9, immunofluorescent antibody was detected from 7 cats with titer from 1:40 to 1:640. Table 4 below shows the association between TIN and evidence of FmoPV infection.

TABLE 4

Association between TIN and evidence of FmoPV infection

Sample No.	TIN	Evidence of FmoPV infection							
		Urine RT-PCR	Western Blot	Antibody response					
				IF (IgG) with serum dilution					
				1:10	1:40	1:160	1:640	1:2560	1:10240
1357	+	+	++	+	+	+	+	-	-
1359	+	+	+	+	+	+	-	-	-
1363	-	-	+	-	-	-	-	-	-
1364	-	-	-	-	-	-	-	-	-
1365	-	-	+	-	-	-	-	-	-
1366	+	-	-	-	-	-	-	-	-
1367	-	-	-	-	-	-	-	-	-
1368	-	-	-	-	-	-	-	-	-
1391	-	-	-	-	-	-	-	-	-
1392	+	+	-	+	+	-	-	-	-
1393	+	-	+	+	+	-	-	-	-
1394	-	-	-	-	-	-	-	-	-
1395	-	-	-	-	-	-	-	-	-
1396	-	-	-	-	-	-	-	-	-
1397	-	-	++	+	+	-	-	-	-
1402	-	-	+	-	-	-	-	-	-
1403	-	-	+	-	-	-	-	-	-
1404	-	-	-	-	-	-	-	-	-
1405	-	-	-	-	-	-	-	-	-
1406	-	-	-	-	-	-	-	-	-
1407	+	+	++	+	+	+	-	-	-
1408	+	-	-	-	-	-	-	-	-
1409	+	+	+	+	+	+	-	-	-
1417	+	-	+	-	-	-	-	-	-
1418	-	-	-	-	-	-	-	-	-
1419	-	-	-	-	-	-	-	-	-
1420	-	-	-	-	-	-	-	-	-

The same 27 cat sera samples used in Example 9 were tested for the presence of neutralization antibody in Example 10. Neutralization antibody was detected from 6 cats with titer from 1:20 to 1:40, shown below in Table 5, of which all are positive for immunofluorescent antibody (Table 4).

TABLE 5

Neutralizing antibody detected from cats' sera

Sample No.	Neutralizing IF (IgG) with serum dilution								
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
1357	+	+	-	-	-	-	-	-	-
1359	+	+	-	-	-	-	-	-	-
1363	-	-	-	-	-	-	-	-	-
1364	-	-	-	-	-	-	-	-	-
1365	-	-	-	-	-	-	-	-	-
1366	-	-	-	-	-	-	-	-	-
1367	-	-	-	-	-	-	-	-	-
1368	-	-	-	-	-	-	-	-	-
1391	-	-	-	-	-	-	-	-	-
1392	+	+	-	-	-	-	-	-	-
1393	+	+	-	-	-	-	-	-	-
1394	-	-	-	-	-	-	-	-	-
1395	-	-	-	-	-	-	-	-	-
1396	-	-	-	-	-	-	-	-	-
1397	-	-	-	-	-	-	-	-	-
1402	-	-	-	-	-	-	-	-	-
1403	-	-	-	-	-	-	-	-	-
1404	-	-	-	-	-	-	-	-	-
1405	-	-	-	-	-	-	-	-	-
1406	-	-	-	-	-	-	-	-	-
1407	+	+	-	-	-	-	-	-	-
1408	-	-	-	-	-	-	-	-	-
1409	+	+	+	-	-	-	-	-	-
1417	-	-	-	-	-	-	-	-	-
1418	-	-	-	-	-	-	-	-	-
1419	-	-	-	-	-	-	-	-	-
1420	-	-	-	-	-	-	-	-	-

6. EXAMPLES

Described herein is a novel feline paramyxovirus, FmoPV, from stray cats in Hong Kong, which represents the first documentation of paramyxoviruses found in the domestic cat (*Felis catus*). Woo et al. (2012) "Feline morbillivirus, a novel paramyxovirus associated with

tubulointerstitial nephritis in domestic cats.” PNAS (in press), which is incorporated herein by reference in its entirety.

A molecular epidemiology study was carried out in stray cats in Hong Kong and on diseased cats from mainland China from which the novel feline paramyxovirus, FmoPV, was isolated and characterized as shown in the following examples.

To summarize, FmoPV was detected in the urine samples of 53 of 457 stray cats and in the rectal swab and blood samples of four and one of these cats, respectively. Western blot analysis revealed a seroprevalence of 27.8% among tested cats for IgG against recombinant N protein and the presence of antibody is highly associated with the presence of virus. Analysis of the complete genomes of three FmoPV strains, described earlier in Section 5.12, showed that they formed a distinct cluster among the morbilliviruses in all six phylogenetic trees constructed using the N, P, M, F, H and L genes (Fig. 7). Immunohistochemistry also showed that, similar to other morbilliviruses such as measles virus, FmoPV infects both mononuclear cells and parenchymal cells (Fig. 10). The three strains of FmoPV exhibited high sequence similarity and identical genome organization, suggesting a single species of FmoPV and a high degree of species specificity in FmoPV. Although no recombination was identified in the present FmoPV strains (data not shown), other viruses from cats, such as feline coronaviruses and feline papillomavirus, have been shown to be closely related to or recombine with their canine counterparts in dogs, suggesting that feline viruses may have the potential to cross species barrier in animals of similar living habitat (17,35).

Some recent studies suggested that feline TIN is mediated by an autoimmune mechanism because cats vaccinated with CRFK cell lysates developed antibodies to both CRFK and kidney cell lysates (36-38). Half of these cats sensitized to CRFK lysates on multiple occasions developed tubulointerstitial nephritis at 2 weeks post-sensitization. Sera from CRFK inoculated cats were confirmed to recognize annexin A2 and alpha-enolase by Western blot. In humans, alpha-enolase antibodies are nephritogenic and alpha-enolase and annexin A2 antibodies have been associated with autoimmune diseases. It is therefore possible that a feline nephrotropic virus, such as FmoPV, may trigger off a self-sustained immunopathological process after this acute insult. Notably, some morbilliviruses, such as Peste des Petits Ruminants virus, Rinderpest virus and canine distemper virus, have also been found in kidney and/or urine (39-41). Further

studies would delineate if these viruses are also associated with renal pathologies in these animals.

Although domestic cats have been associated with humans for almost 10,000 years, they usually pose little physical hazards to humans. However, as a result of cat bites or via other routes, cats can transmit a range of bacteria (e.g. *Bartonella henselae*), protozoa (e.g. *Toxoplasma gondii*), and uncommonly viruses (e.g. rabies virus), causing diseases in humans. Apart from the present novel paramyxovirus, viruses of at least 15 families have been found in cats, including the recent discovery of the first picornavirus in cats (23). Moreover, the domestic cats have also been shown to be susceptible to infection by highly pathogenic avian influenza viruses H5N1 and H7N7 and SARS coronavirus, suggesting that they can be susceptible to viruses associated with serious infections (42-44). A previous survey in Hong Kong (http://www.aud.gov.hk/pdf_e/e54ch04.pdf) showed that one in every eight households was keeping pets with 22.3% keeping cats. The number of locally licensed pet shops selling cats and dogs in Hong Kong has increased from 77 in 2000 to 155 in 2009. In many households, owners having pets share their beds with their pets, and the pet owners often kiss or are being licked by their pets. Such behavior may allow significant exposure to zoonotic agents carried by the pet or parasitizing arthropods (45). Continuous surveillance of viruses in these animals is important to understand their potential for causing emerging infectious diseases in other mammals, including humans.

6.1 EXAMPLE 1

Sample collection

The Agriculture Fisheries and Conservation Department (AFCD), Hong Kong provided samples collected from 457 stray cats captured from various locations in Hong Kong over a 2-year period (March 2009 to February 2011) as part of a surveillance program. Tracheal and rectal swabs, urine and blood were collected using procedures described previously (23). In addition, oral and rectal swabs from 16 diseased cats from mainland China were also collected. The study was approved by the Committee on the Use of Live Animals in Teaching and Research, The University of Hong Kong. Samples were collected immediately after euthanasia as routine policies for disposal of locally captured stray cats.

Necropsies of FmoPV-infected stray cats

To identify possible diseases associated with FmoPV, necropsies were performed on two euthanized stray cats positive for FmoPV by RT-PCR. Tissue samples were collected from the lungs; brain; heart; prescapular, retropharyngeal, submandibular and thoracic lymph nodes; spleen; liver; kidneys; urinary bladder; gall bladder; thymus; salivary gland; eyeball; nasal turbinate; intestine; pancreas; foot pads; testicles or ovary; tonsil and adrenal gland. Half of each tissue sample was fixed in 10% neutral buffered formalin for histological processing and the other half was submerged in viral transport medium for RNA extraction and virus isolation.

Since the kidneys of the two stray cats showed histopathological features compatible with TIN, the kidneys, urine and plasma were obtained from a total of 27 strayed cats, including the two cats with necropsies performed, and were subject to RT-PCR, histopathology and antibody detection by western blot and immunofluorescence to examine for possible association between FmoPV infection (RT-PCR and/or antibody positive) and TIN.

6.2 EXAMPLE 2

RT-PCR of L gene of morbilliviruses and DNA sequencing.

Viral RNA was extracted from tracheal and rectal swabs, urine and blood using EZ1 Virus Mini Kit (QIAgen) and from tissue samples using QIAamp Viral RNA Mini Kit (QIAgen). *Morbillivirus* detection was performed by amplifying a 155-bp fragment of L gene of morbilliviruses using conserved primers (LPW12490 5'- CAGAGACTTAATGAAATTTATGG -3' and LPW12491 5'- CCACCCATCGGGTACTT -3') designed by multiple alignments of available L gene sequences of morbilliviruses. Reverse transcription, PCR and sequencing were performed according to our previous publications (13,14).

6.3 EXAMPLE 3

Real-time quantitative RT-PCR

Real-time quantitative RT-PCR to detect L gene of FmoPV was performed on the 56 positive samples using LightCycler FastStart DNA Master SYBR Green I Mix reagent kit (Roche), with primers LPW12490 and LPW12491. Complementary DNA (cDNA) was amplified by LightCycler 2.0 (Roche) with 20-μl reaction mixtures containing FastStart DNA Master SYBR Green I Mix reagent kit (Roche), 2 μl of cDNA, 4 mmol/L MgCl₂, and 0.5 mmol/l primers at

95°C for 10 min, followed by 50 cycles of 95°C for 10 s, 60°C for 5 s and 72°C for 8 s. A plasmid containing the target sequence was used for generating the standard curves.

RT-PCR for a 155-bp fragment in the L gene of morbilliviruses was positive in samples from 56 (12.3%) cats from Hong Kong, including 53 urine, 4 rectal swabs and 1 blood specimens. For the 16 diseased cats from mainland China, one (6.25%) cat was RT-PCR positive in both its oral and rectal swabs. Real-time quantitative RT-PCR showed a median viral load of 3.9×10^3 (range 0.037 to 1.4×10^6) copies/ml. Sequencing results suggested the presence of a novel paramyxovirus of the genus *Morbillivirus*, with <80% nt identities to known paramyxoviruses (Fig. 8). This novel paramyxovirus was named FmoPV.

6.4 EXAMPLE 4

Analysis of P mRNA editing

To examine the number of G insertions at the P mRNA editing site, mRNA from original specimens was extracted using the Oligotex mRNA Mini kit (QIAGEN). First strand cDNA synthesis was performed using SuperScript III kit (Invitrogen) with random hexamer primers. Primers (5'-TTCATCTCTTAGTTCAGGAA-3' and 5'-TTTCAGACTCACCTCGATATCT-3') were used to amplify a 442-bp product of FmoPV covering the putative editing site. PCR, cloning and sequencing were performed as described in our previous publication (13).

6.5 EXAMPLE 5

Cloning and purification of (His)₆-tagged recombinant nucleoprotein (N) from *Escherichia coli*

Primers (5'-ACGCGGATCCGATGTCTAGTCTA-3' and 5'-CGGAATTCGGTTTTAGAAGGTCAGTA-3') were used to amplify the N gene (519 amino acids) of FmoPV strain 761U by RT-PCR. Cloning, expression and purification of (His)₆-tagged recombinant N protein was performed as described in (1) Lau SK, et al. (2010) *Virology* 404:106-116; (2) Woo PC, et al. (2005) *J Virol* 79:884-895; and (3) Woo PC, et al. (2004) *Lancet* 363:841-845.

6.6 EXAMPLE 6

Guinea pig sera

Guinea pig antiserum against the N protein of FmoPV was produced by injecting 100 µg purified N protein of FmoPV, with an equal volume of complete Freund's adjuvant (Sigma), subcutaneously to three guinea pigs. Incomplete Freund's adjuvant (Sigma) was used in subsequent immunizations. Three inoculations at once every two weeks per guinea pig were administered. Two weeks after the last immunization, 1 ml of blood was taken via the lateral saphenous vein of the guinea pigs to obtain the sera.

Such hyperimmune guinea pig antibody can be used for diagnostic purposes or as a vaccine in the following ways:

1. The recombinant nucleoprotein, N protein, can be used as the target antigen for detecting specific antibody against this virus from cats' sera.
2. The hyperimmune antibody can be used for immunohistochemical detection of viral protein in tissues or infected cell culture to confirm the specific presence of this virus.
3. The recombinant nucleoprotein can be used as a vaccine to induce antibody production.

6.7 EXAMPLE 7

Western blot analysis

Antibodies against the N protein of FmoPV were detected in plasma samples of the 56 cats that were RT-PCR positive and 401 cats that were RT-PCR negative for FmoPV by Western blot. Western blot analysis was performed as described in our previous publications (24,25,27), using 1000 ng purified (His)₆-tagged recombinant N protein, 1:1000 dilutions of cat plasma samples, 1:4000 dilution of horse radish peroxidase conjugated goat anti-cat IgG antibody and 1:10000 dilution of goat anti-cat IgM antibody (Bethyl laboratories).

Among tested sera from the 56 cats that were RT-PCR positive and 401 cats that were RT-PCR negative for FmoPV, 49 (76.7%) and 78 (19.4%) were positive for IgG against N protein of FmoPV by western blot analysis respectively ($P < 0.0001$) (Fig. 9, Table 6). Among tested sera from the 56 cats that were RT-PCR positive for FmoPV, only 5 (8.9%) were positive for IgM against N protein of FmoPV.

Prominent immunoreactive protein bands of about 69 kDa, consistent with the expected size of 68.7 kDa of the recombinant protein, were detected in three of the six cat serum samples

shown in Fig. 9, indicating antigen-antibody interactions between the recombinant FmoPV N protein and serum antibodies. Results of RT-PCR of the corresponding urine samples for FmoPV are also shown.

6.8 EXAMPLE 8

Viral Culture

Viral culture and electron microscopy were performed according to our previous publications (28,29). Two hundred microliters of the three samples used for complete genome sequencing were subject to virus culture. After centrifugation, they were diluted five folds with viral transport medium and filtered. 200 µl of the filtrate was inoculated to 200 µl of MEM with polybrene. 400 µl of the mixture was added to 24-well tissue culture plates, with CRFK (feline kidney), B95 (marmoset B-cell), CEF (chicken embryo fibroblast), NIH/3T3 (mouse embryo fibroblast) or Vero E6 (African green monkey kidney) cells, by adsorption inoculation. After 1 h of adsorption, excess inoculum was discarded, the wells were washed twice with phosphate buffered saline and replaced by 1 ml of serum free MEM supplemented by 0.1 µg/ml of L-1-tosylamide-2-phenylethyl chloromethyl ketone-treated trypsin (Sigma). Cultures were incubated at 37°C with 5% CO₂ and inspected daily by inverted microscopy for cytopathic effects (CPE). After two to three weeks of incubation, subculturing to fresh cell line was performed even if there were no CPE and culture lysates were collected for RT-PCR for FmoPV. Immunostaining and electron microscopy were performed on samples that were RT-PCR positive for FmoPV.

CRFK and Vero E6 cells that were positive for FmoPV by RT-PCR were fixed in chilled acetone at -20°C for 10 min. The fixed cells were incubated with 1:200 dilution of guinea pig antiserum against the N protein of FmoPV, followed by 1:50 diluted FITC-rabbit anti-guinea pig IgG (Invitrogen). Cells were then examined under fluorescence microscope. Uninoculated cells were used as negative control.

At the 8th passage, CRFK cells inoculated with a urine sample (761U) positive for FmoPV showed CPE at day 14, in the form of cell rounding, followed by cell detachment from the monolayer and cell lysis. At the 16th passage, CPE were evident at day 10 with syncytia formation (Fig. 6). RT-PCR for FmoPV using the culture supernatants and cell lysates showed positive results in CRFK cells inoculated with urine sample 761U and VeroE6 cells inoculated with supernatant of CRFK cells positive for FmoPV. Specific apple green finely granular and

diffuse cytoplasmic fluorescence was also observed using serum from guinea pig immunized with recombinant N protein of FmoPV or corresponding serum of the infected cat (Fig. 6). Electron microscopy showed an enveloped virus with the typical “herring bone” appearance of the helical N in paramyxoviruses (Fig. 6). Virions are highly variable in size, ranging approximately from 130 to 380 nm in diameter. No CPE and no viruses were detected by RT-PCR in 3T3, B95 and CEF cells inoculated with the samples.

6.9 EXAMPLE 9

Immunofluorescence antibody test

CRFK cells infected with FmoPV were fixed in chilled acetone at -20°C for 10 min. The fixed cells were incubated with 4-fold dilutions of plasma from 1:10 to 1:10240 from the 27 cats with necropsies, followed by 1:20 diluted FITC-goat anti-cat IgG (Sigma). Cells were then examined under fluorescence microscope. Uninfected cells were used as negative control. Out of the 27 cats, immunofluorescent antibody was detected from 7 cats with titer from 1:40 to 1:640 (see Table 4, supra).

6.10 EXAMPLE 10

Neutralizing antibody detection

100 TCID₅₀ FmoPV were incubated with 2-fold dilutions of plasma from 1:10 to 1:2560 from the 27 cats with necropsies at 37°C for 1 h. The mixtures were inoculated to 96-well plates of confluent CRFK cells. After 1 h of adsorption, the inoculums were removed and the plates were washed once with phosphate buffered saline and replaced by serum free MEM supplemented by 0.1 µg/ml of L-1-tosylamide-2-phenylethyl cholormethyl ketone-treated trypsin (Sigma). The plates were incubated at 37°C with 5% CO₂ for 7 days. The supernatants were removed and the cell monolayers were washed once with phosphate buffered saline and fixed in chilled methanol at -20°C for 10 min. The fixed cells were incubated with 1:200 dilution of guinea pig antiserum against the N protein of FmoPV, followed by 1:50 diluted FITC-rabbit anti-guinea pig IgG (Invitrogen). Cells were then examined under fluorescence microscope. Cells infected with neutralized FmoPV showed no fluorescence. FmoPV infected cells were used as negative control. Out of the 27 cats, neutralization antibody was detected from 6 cats with titer

from 1:20 to 1:40 (see Table 5, supra), of which all were positive for immunofluorescent antibody (see Table 4, supra).

6.11 EXAMPLE 11

Histopathological examination and immunohistochemical staining of FmoPV

N protein in tissues and cauxin protein in kidneys

To determine if FmoPV is associated with renal pathologies, such as TIN, histopathology and immunohistochemistry were performed on necropsy kidney tissues of two stray cats with positive FmoPV RT-PCR in their urine samples as described below, showing histopathological features compatible with TIN as well as detection of N protein of FmoPV in the renal tubules by immunohistochemistry.

Fixed necropsy organs of the two stray cats were embedded in paraffin. Tissue sections of 5 μ m were stained with hematoxylin and eosin (H&E). Histopathological changes were observed using Nikon 80i microscope and imaging system. Expression of FmoPV N protein was examined by immunohistochemical staining. Tissue sections were deparaffinized and rehydrated, followed by blocking endogenous peroxidase with 3% H₂O₂ for 20 min, and then with 10% normal rabbit serum/PBS at room temperature for 1 h to minimize non-specific staining. The sections were incubated at 4°C overnight with 1:250 dilution of guinea pig anti-N protein anti-serum, followed by incubation of 30 min at room temperature with 1:500 dilution of biotin-conjugated rabbit anti-guinea pig IgG, H & L chain (Abcam) (30). Streptavidin/peroxidase complex reagent (Vector Laboratories) was then added and incubated at room temperature for 30 min. Color development was performed using 3,3'-diaminobenzidine and images captured with Nikon 80i imaging system and Spot-advance computer software. Double staining of lymph node was performed using mouse anti-human myeloid/histiocyte antigen antiserum MAC387 (DakoCyomation) and labeled with Texas-red conjugated goat anti-mouse IgG (Jackson ImmunoResearch) (31). Cauxin protein expression was detected according to a published protocol (32).

Histological examination of various organs of two stray cats with FmoPV detected in urine revealed interstitial inflammatory infiltrate and renal tubular degeneration or necrosis in their kidneys (Fig. 10). In addition, there was also marked decrease in cauxin expression in the degenerated tubular epithelial cells, compatible with tubulointerstitial nephritis in cats with

histological evidence of TIN (Fig. 11A), compared to cats without histological evidence of TIN where cauxin-positive proximal straight renal tubules were observed between the inner cortex and outer medulla (Fig. 11B). Immunohistochemical staining of their organs using guinea pig serum positive for anti-FmoPV N protein antibody revealed positive renal tubular cells in kidney sections and positive mononuclear cells in lymph node sections (Fig. 10). Using mouse anti-human myeloid/histiocyte antigen antiserum MAC387, the targets of FmoPV in lymph node sections were shown to be macrophages (Fig. 12).

6.12 EXAMPLE 12

Case control study

Among 27 stray cats, TIN was observed in 7 of 12 cats with evidence of FmoPV infection, but only in 2 of 15 cats without evidence of FmoPV infection ($P < 0.05$ by Fisher's exact test) (Table 4). These results support a positive association between FmoPV infection (RT-PCR and/or antibody positivity) and TIN in cats.

* * * * *

The invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

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CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising the nucleotide sequence of Figure 2.
2. An isolated nucleic acid comprising the nucleotide sequence of Figure 3.
3. An isolated nucleic acid comprising the nucleotide sequence of Figure 4.
4. An isolated polypeptide from a Feline morbillivirus comprising the sequence designated FmoPV 761U of Figure 5.
5. An isolated polypeptide from a Feline morbillivirus comprising the sequence designated FmoPV 776U in Figure 5.
6. An isolated polypeptide from a Feline morbillivirus comprising the sequence designated FmoPV M252A in Figure 5.
7. A vector comprising the isolated nucleic acid of claims 1, 2 or 3.
8. The vector of claim 7, comprising a promoter operably linked to the isolated nucleic acid.
9. A host cell comprising the vector of claim 7 or 8.
10. An immunogenic composition comprising the isolated polypeptide of claims 4, 5 or 6 and a pharmaceutically acceptable carrier.
11. An immunogenic composition of claim 10 further comprising an adjuvant.
12. A method of producing a polypeptide of claim 4, 5 or 6 comprising the steps of culturing a host cell which expresses the polypeptide and recovering the polypeptide so produced.
13. A method of inducing immunity in a mammal comprising the step of administering the mammal the immunogenic composition of claim 10 or 11.
14. A method of enhancing an immune response in a mammal comprising the step of administering to the feline the immunogenic composition of claim 10 or 11.

15. An antibody molecule having specificity for Feline morbillivirus.
16. An antibody molecule having specificity for the isolated polypeptide of claim 4.
17. An antibody molecule having specificity for the isolated polypeptide of claim 5.
18. An antibody molecule having specificity for the isolated polypeptide of claim 6.
19. A method of treating feline tubulointerstitial nephritis comprising administering a therapeutically effective amount of the antibody molecule of claims 15, 16, 17 or 18.
20. A method of diagnosing kidney disease in a feline comprising the steps of obtaining a sample from a feline, incubating the sample with an antibody specific for Feline morbillivirus or a protein fragment from the Feline morbillivirus; and measuring the amount of binding of antibody to the sample.
21. A primer having the nucleotide sequence CAGAGACTTAATGAAATTTATGG .
22. A primer having the nucleotide sequence CCACCCATCGGGTACTT.
23. A primer having the nucleotide sequence ACGCGGATCCGATGTCTAGTCTA.
24. A primer having the nucleotide sequence CGGAATTCGGTTTTAGAAGGTCAGTA.
25. A kit used in a method for diagnosing kidney disease in a feline comprising a primer of any one of claims 21-24.

FIGURE 1

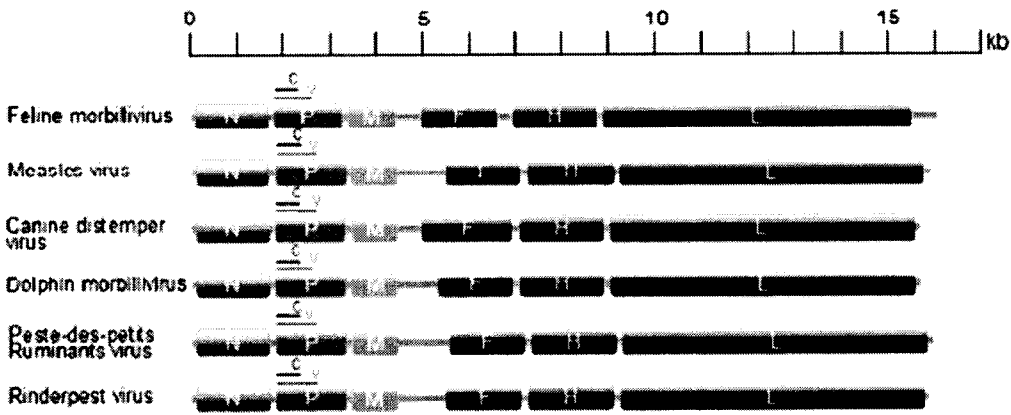


FIGURE 2

FmoPV 761U Cats/Hong Kong/2009 16050 bp

ACCAGACAAAGATGTTTGTGACCTATTCTAACGACAAGACTATTATTAATATTTAGGAA
TAACGATTCCATTAGTGAGGTGAGGGGGAGGAATCAGGTATTCCACAATGTCTAGTCTAT
TGAGGTCACTTGCTGCATTTAAGAGACATAGGGAGCAACCAACAGCACCGTCAGGTTCCG
GTGGTGCAATTAAAGGATTGAAAAATACAATTATTGTTCCAGTTCAGGGGATACAGTAA
TTACTACAAGGTCTAATTTGTTATTTAGATTAGTTTATATAATAGGCAATCCGGATACAC
CTTTAAGCACCTCGACGGGAGCAATAATATCATTGTTGACCTTATTTGTGCAATCTCCAG
GTCAATTAATTCAAAGAATTGCTGATGACCCTGATGCAGTTTTTAAATTGGTAGAGGTCA
TTCCTGAAGCTGGTAATCCTGGAGAATTAACTTTGCATCTCGAGGGATTAATTTAGACA
AGCAAGCTCAACAATACTTTAAATTGGCTGAGAAAAATGATCAGGGGTATTATGTTAGCT
TAGGATTTGAGAACCCACCAATGATGACGATATAACATCTAGTCCTGAGATATTCAATT
ATATCCTGGCATCTGTACTTGCACAAGTTTGGATICTTCTGGCAAAAGCTGTGACTGCTC
CAGATACGGCTGCTGAAGCCGAAAATCGTAGATCGATTAAATTAATGCAACAACGTAGGG
TGGATGGTGAAGTGAAGATTGAGCAAGGGATGGCTAGATTTGGTGAGAAACAAGATTGCGT
CAGATATTACAATAAGGCGATTTCATGGTAGCATTAGTTCTTGACATCAAACGTTCTCCTG
GGACAAGACCCAGGATAGCTGAAATGATTTGTGATATTGATAATTATTTGTAGAGGCAG
GGCTTGCAAGTTTCTTGTTAACTATTAAATTTGGCATAGAGACACGTTATCCAGCACTGG
CACTACATGAGTTTTCTGGAGAACTAGCCACTATTGAGGGGCTTATGAAATTGTACCAAT
CTATGGGGGAAATGGCACCATACATGGTAATTCTGGAAAATTCATCCAAACCAGGTTTA
GTGCAGGGTCTTATCCTCTGCTATGGAGTTATGCCATGGGTGTGGGGTGGAGCTTGAAA
GATCAATGGGTGGACTCAATTTACTAGAAGCTTCTTTGACCCTACATATTTAGACTTG
GTCAAGAGATGGTGAGGAGATCTTCAGGGATGGTTAATAGTTTATTGCGAGAGAACTTG
GCCTATCTGATCATGAAACACAACCTGGTCAGCCAGATTGTCAATTCGGGAGGTGAATCTG
GGATACCTAAATTTGATGGATTTCAGAGCAAAATCCAACAACCTTTCTAGGAACCAAGATA
ACATAAATGATAGAGGTGAAGATCAGTCAAAATTCGATATCAGGGTTACCTGGTCCACTAT
TACCCAGCCGTGACCTAAATCTTTCAGGTGATTCTATGGAATTAATAGTGGTGTGAAAA

Figure 2-1

ATGTCAGTGACAACTGAATGAAGGAGTAGGTCCAGACCATGATGTGTCCAGTTCTGCCA
TGGAAGAATTGAGAAGATTGGTTGAGTCCACCAACAGAATAGACACCAAACAGCCAGAAG
CTTCAGGTGTCACCAACCATTATAATGATACTGACCTTCTAAAATAATATGAGCATACCC
TAATTGCTTATTATGCAACTCAAATTAAGAAAACTTAGGACCTCAAGGTTCACAACTGT
TGGCATATCACTAAAATACAGTCAGCTCTTCACCCACCACATGTCCTCTCACCAAATCCA
GCAAGTCAAACATGGCCTCGAATCTTTACAAGAGATCAAAAACAACCCTCCGTCTTCCCA
AGATGTCAATCTTGCCAGGGAGATTTACGAATCCATTAGACAAACAGGAACATCTTCAGT
GCAAGGAGGAGCCATTGCGGGAGATAATATTACGTCAGGGGGTAACAATGACTCAATGTA
TAGCCAAGGACCAAGTCCTCCTATTTCAAGTGTTAACAAGAATATCGAAGGACCTACTGG
ATTGATCATTACAGGACTATGGGATCCAGAGGGTAACCTCTGCATGCTATTGAAAGCGA
TGATGATGAAAACCATTATTACAGAGATTAATGGCCGGTCTTCCGCTATCGAAGGACTGGA
TGAACAGGATAATGAGAACTCAATTATTAACAACCAGGAAATCAGTGTACTGAGGGAGT
GTCTAAGACTGATTCTCTTAGTTCCCAGGAACTACACTATCTGTTGGGGGATCTGA
TATACCTGGGGCAGGAATATCAACCTGTGCCTCTTTGGATATAACTGTAAATGAACTCGA
AGATGCAACTGTAAGAAATAGCAACAATATGAAAGGGAACTGGCCAATTCCTAAATTACT
TGTTAAGCCGCCACCTAGGGTAAAAACAAGCGTTGATCACAGTAATCCATTAAAAGGGGC
CACAGGAGGGGAAATTAGCCTCACCTGGGATGGAGACTACATTATTCGAGAGGAGTGGTGC
AACCCCATCTGTACACCCATATACTCAACCTGCAAGCGACTTCAATGTAGGTGCAAGCAA
TGTCATCAACCTGCCCTAAATGTGAATAATAATTGCAATGATGGTAGGGTAACAGCGCC
TAACTCACATAAAGATATCGAGGGTGAGTCTGAAATATCTATTCAAGATATATATACTT
GATTCTTGGATTTAAGGATGATTACAGGAAATTATCAAACAAATTAGATATGGTATTAGA
GATGAAACAAGACATTGACAATCTAAAAAGAATAGTGCTAAAGTGCAATTGGCTCTATC
AACTATTGAGGGACATCTATCCAGTGTTATGATTGCCATCCCTGGTTCAGGTATTGATTG
CACAGGGGATGAGGAAAAGGATCAGATAAATTCTGACTTAAAACCACTGCTAGGAAGGGA
TCATTGTAGAGCATTCGAGAAGTTACCAATCCTCTAGATGAGTCTTCACTAGCCAATTC
TCCAACAAAACATGTTGCCAAGGTAAACAAAAACTGCACTCTCAGAAGATCAACAAGAA
CGAAACATCTGCAATCAAATTTGTTCTAGTGACAGTCATGCAAGCACATCAACCATCAG
ATCAATTATCAGGTCATCTAATCTCGATCAGGATTTGAAAACAAAATTGCTCACAATTCT

Figure 2-2

ATCCCAGATTAGAGGGGCAGACAATATTAGAGAATTCTATGAAAAGGTTATGATATTAAT
AAAGAATAAGAATTAAATATTACAAATCTACATTCATTATAGGTTGTAATTGTCTTCAAT
AAGATTTGGTCAGTTTCATATATATGGTTATTGATTTGTGATAATTATAAAAACTTAGG
AGCTAAAGGTTACTCAGTCATATACAGCATGACTGAGATATTCAACCTTGATGAGAGCTC
ATGGTCAGTCAAAGGGATACTAGATCCGTTAACACCTGATACCTATCCTGATGGTCGACT
AGTGCCTAAAGTTCGAGTTATCGATCCGGGTCTAGGAGATCGCAAGAGTGGGGGGTATAT
GTACCTACTTCTTCATGGTGTATAGAAGATAGTGAGACTATAATTAGCCCCGAAAGGAAG
AGCATTTGGTGCATTCCCATTAGGAGTGGGTCAATCAACTGAAAACCCGGAAGACTTGTT
TAAGGAAATATTAACCTCTCAACATCGTGACTCGTAGGACTGCTGGATTTAATGAGAAATT
GGTTTATTATAATACCACACCTCTACATTTACTGACCCCTGGAAAAAAGTGTTGGCATA
TGGAGGCATTTTAAATGCTAATCAGGTCTGCAGTGATACAAGTTCATACCAATAGACAT
TCCACAAAAATTTAGGCCAGTATATTTGACTGTTACAAAATTATCTGATGATGGCTATTA
TCAGATCCCCAAGATGATTCAAGATTTCAAATCGTCAAATTCTGTTGCATTCAACATCCT
TGTGCATCTGTCAATGGGCATAAATTTACTTGACCAATCCAAGGACCCTAGATTAAGAAA
TGCTGCAGAACTGTGATCACATTTATGATTCATATTGGAACTTTAAACGGAAGAGTAA
TAAGTCTTACTCACCTGAATATTGCAAGAGGAAAATAATGAGGCTGGGTTTAAATATTCTC
ATTAGGTGCAATTGGTGGCACAAGCTTGCATATTAGATGTACAGGTAAGATGAGCAAACG
ACTACAGGCTTATTTAGGATTCAAAGGACTTTATGTTACCCTTTGATGTATGTTAATGA
AGGGCTGAACAAGACCCTGTGGAGAAGTGAATGCAGAATAGAGAAGGTTCAAGCAGTCTT
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TGGTCTCTTCAAGATTAAATAGACTATAACAATAATAACAGCTACTAAATAGTATTATG
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TAAGATCTAATTGATATGTGGGTTGGTACTCGATCATTTATTAGCTCTACTGATTATCTA
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ACACTTAATTCTCAGAACTAGAATACCCAGATTGTCAAACCTATAACCTTGTTAGATTCA
TTAAAGTTAGATTCTTGTAATGTTGATCAATTATCACTTGAGCAATTATAAAAACTAAG
GACCTAATGTAATAGGAACCCAACTCCATCCAGTGAGCTCTAAATCGCCATGCTTGAAT
ATTAATTTATCTAGGGCCTGTCTAACTCAGAACAAAGATCACAACCTAGAGTCTAAAGGAG

Figure 2-3

TGGGTCAAGTCTGAACAATTATCAAGAGCCGAGATTCAAACTGATTCCTCCTTAACTC
AGAACCCTAACAAATATATCATCCACTCAACATCATGAACAGAATTAAGGTTATGATAATT
AGTTCTTTATTATTATCAGATATTACGATTGCACAAATAGGTTGGGATAATTTGACTTCG
ATTGGAGTTATAAGTACTAAGCAATACGACTATAAAATACTACTCTGAACACTGACCAG
TTAATGGTTATAAAGATGGTTCCTAATATATCATCAATCATTAAATTGCACTAACTCGAA
TTAACAAAATATAGAGAGTTAGTCTCAGGGATCATTAGACCAATAAATGAGTCATTAGAA
TTAATGAATTCATACATTAACATGAGAGCAGGTTTCAGAGAGATTTATAGGGGCTGTAATA
GCTGGTGTAGCCTTAGGAGTGGCAACTGCAGCACAAATAACATCAGGGATTGCCCTACAT
AATTCAATTATGAACAAAAAACAAATACAAGAATTGAGGAAGGCTCTTAGTACTACCAAC
AAAGCAATTGATGAAATAAGGATTGCAGGTGAAAGAACATTAATAGCAATTCAAGGTGTA
CAGGATTATATTAATAATATAATTATCCCTATGCAGGACAACTCCAATGTGATATTTTA
TCATCACAACTTTCTGTTGCTTTACTCAGATATTATACAAATATACTAACAGTTTTTGGG
CCAAGTATACGGGATCCTATTACTAGTACAATTTCACTACAAGCACTCAGTCAAGCATT
AATGGTAATCTTCAGGCATTGCTTGATGGACTGGGGTATACTGGGAGAGACTTACGTGAT
CTTCTAGAGAGTAAATCTATCACTGGCCAGATAATTCATGCAGATATGACTGATTTGTTC
CTTGTTTTGAGAATAAATTATCCTTCCATAACTGAGATGCAGGGAGTAACAATATATGGG
CTCAATTCAATTACATATCATATTGGGCCTGAAGAGTGGTATACCATTATGCCTGATTTT
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GAGTGCAAGGAAGTTGGTGTGATGGTATTCGTATTATGGTAGGACCTAGAAAATTACCA
GATATTACCTTTAATGCTAGGTTGGAAATTGGTGTACCAATATCATTGAGCAAATTGGAT
GTCGGGACTGATTTAGCGATTGCTTCAGCTAACTTAATAATTCTAAGGCATTGTTAGAG
CAATCAGATAAGATTTTAGATTCAATGTCTAAATTGGATTCTATGAATTCAAGAATAATA
GGATTAATCTTAGCAATTATGATAATCTTTATAATCATTATTACTATTATCTGGATCATA
TATAAAAAATGTAGGAATAAAGATAATAAATTCAGTACTTCAATTGAACCGCTCTACATA

Figure 2-4

CCCCCTTCTTATAACTCACCTCATAGTGTGGTTAAGTCTATTTGAGCACTGACCATATGA
TCCACTGTAATAAGTCCAATGAAAGTATCAATTAATAATATTGGTAGTGCAATGAGTATT
GATTGTATAATATACTCCTTTAACTAGATAGTGATAAAGGGTTATAGATGATTCAGTT
ATTTTAATATAATCATATATTGATTTTATTATCTTACATGACTATTATGTAATTGAATTA
TGTGTCATCAATTAATAGCTTAATAATATCGTTTAATGTACTTATATTGATGGATAGATG
TGTTATATTGTAATCAAGGATTTAGTATTTAGAAAAGGAAAGAGTTTAATTTGTTGTAA
TTAGTTATTGTGTATTCAATTAGAAAAAACTTAGGAATCCATGTTAATAAAAAATTTATTA
TCATGGAGTCCAACAATATTAAGTATTACAAAGATTCTAGCCGGTACTTTGGTAAAATAT
TAGATGAACACAAAAAATTAATAGTCAATTGTACAGTTTGAGTATCAAGGTAATTACCA
TTATTGCTATTATTGTAAGCCTGATTGCAACAATAATACTATTATCAATGCCACTAGTG
GGAGAACTACCCTAAATAGTAATACAGACATACTACTCAGCCAACGAGATGAGATTCATA
ACATCCAAGAAATGATATTTGATCGTATTTATCCTTTGATAAATGCTATGAGTACAGAGC
TAGGACTTCATATTCCTACCTTATTGGATGAACTTACTAAAGCGATTGACCAGAAAATTA
AAATAATGCATCCTCCTGTGGACACTGTGACTTCTGACCTTAATTGGTGCATCAAACCCC
CTAATGGAATTATCATAGACCCAAAAAGTTATTGTGAGAGTATGGAATTGTCTAAAACTT
ATGAACTGTTACTTGACCAGTTAGATGTCTCAAGAAAGAAATCACTTATTATAAATAGAA
AGAATATCAACCAGTGCCAATTAGTTGATAATTCAAAGATCATTTTGTCTACTGTCAACA
TACAATCTACACCGAGGTTTTTAACTTTGGTCACACGGTCAGCAATCAACGTATAACAT
TTGGTCAAGGAACATATAGTAGTACTTATGTTATAACTATCCAAGAAGATGGAGTAACTG
ATGTTCAATATCGAGTGTTTGAGATCGGATATATTTCTGATCAGTTTGGTGTATTCCCTT
CCTTAATAGTATCGAGAGTGTTGCCGATACGTATGCTATTAGGAATGGAATCCTGTACCT
TGACAAGTGATAGACTAGGCGGGTATTTTTTATGTATGAATACACTGACACGATCTATAT
ATGATTATGTTAGCATAAGGGATTTGAAATCACTTTATATAACAATCCCTCATTATGGTA
AAGTTAATTATACTTACTTTAATTTTGGTAAGATCAGGAGCCCACATGAGATTGATAAAA
TTTGGTTAACATCTGATAGAGGCCAAATTATCTCTGGTTATTTTGCAGCATTTGTTACCA
TTACAATTCGGAACATAATAATTATCCCTACAAATGCTTAAATAACCCATGTTTTGACA
ACTCTGAGAATTACTGTAGAGGATGGTATAAAAAACATAACAGGAACTGATGATGTTCCGA
TATTAGCATACTTATTGGTTGAAATGTATGATGAGGAGGGACCTTTAATTACACTTGTGG

Figure 2-5

CAATACCACCTTACAATTATACAGCTCCATCTCATAATTCTCTTTACTATGATGACAAAA
TTAATAAATTAATAATGACTACATCTCACATAGGTTATATTCAAATCAACGAGGTGCATG
AGGTAATTGTTGGCGATAATTTGAAGGCTATCCTCTTAAACAGATTGTCTGATGAACATC
CTAACCTGACTGCCTGTAGACTCAATCAGGGTATTAAGGAGCAATACAAGTCTGACGGAA
CAATAATTTCAAATTCTGCACTTATTGATATACAAGAACGAATGTACATTACAGTTAAAG
CTATTCCACCAGCAGGTAACATAACTTTACAGTTGAGTTGCATTCTAGATCAAACACAT
CCTATGTATCGTTACCAAAACAGTTTAATGCTAAGTATGACAAATTACATCTTGAGTGCT
TTAGCTGGGACAAATCCTGGTGGTGTGCTCTGATACCCCAGTTTTCATTAAGTTGGAATG
AATCCCTTTCTGTTGATACTGCCATTTTCAATTTAATAAGCTGTAAATGAACACATCAAT
CTATAGTTGATAGTTGTCAAAACATTAGCTAATTTGGGTTTAAGAAATAGGAAAATGAAA
TTACCAATATCTAATTAGATGTATGTTCAAGCTAAATTACAAAAAACTTAGGAGTCAGAG
ACTTCGTTGCAATGGAGCAGTCAGACTACCAAGATATTCTATACCCGGAAGTACATCTTA
ACAGTCCTATAGTAATTTCCAAATTAGTAGGTATTTTAGAATACGCCCAAATTGCTCATA
ATCAACAATTATCAGACCGTACAATTATCAAGAATATTCAATTTAGATTAAGGAACGGAT
TTAATAGTTCAAGGGTACAGGTACTATCAGCTATGGGTGAAATTATCAACAAAATTAGAA
ATAAATATCCTAATTATTTACACATACCTTACCCTGAATGCAACCAAAAACCTATTTGAA
TAGTAGATCCAGAACTAACATCAAAATTAGAATCTCTTCTAAACAAAGGTGACACACTGT
ATCTCAAGATTTCGATCAGATATCATAAAATGTTTTGATAGATTGAAAATGAAAATGAATA
TAAAGAATGATCTTCTTAATGACAATAGTCAATTGATTCTAGATCTTCCTTTAATTATCA
AAGGATCTCAGTGGTTCTTCCCTTTTTTATTCTGGTTTTCTATCAAACTGAACTAGAA
GCTGTATTCGCCAAAATCAAAAGACTCGTGTTAGATCACAATATCGGCCTCACTTATCAG
AGACTAAGAGAATTACATTGGTTGTTACATCTGATCTGATTACAATATTTGATCATATTA
ATAAATGTATATTTTATCTGACTTTTGAGATGCTGTTAATGTATTGCGATGTGATAGAAG
GTCGGTTAATGACTGAAACAGCTATGAGCTTGGACTGTCGGTTTACCAATCTATTGCCAA
GAGTGCAATATATGTGGGATTTACTAGATGGAATGTTTGAAAGTTTAGGCAATCAATTAT
ATTCAGTTATTGCATTATTAGAGCCTCTTCTCTTGCTTATTTGCAATTGATAGATGCAG
ATCCACAGATTTCGGGAACATTCTTGCACTACTGCTTTTCCGAGTTAGAAGAAATTATAT
TTGACAAAACCCCTTTTGATCCTTTTGTGTATGAAAATTTAATTAATGGGCTTGATTACA

Figure 2-6

TTTATTTGACAGGTGATATTCATCTAACTGCAGAAGTTTTTCTTTTTTAGAAGTTTTG
GTCATCCTTTTTTAGAGGCACAAAATGCTGCTAATAATGTAAGGAAGTATATGAATAAGC
CTAAGGTAATATCATATCAGACTTTAATGCAAGGACATGCGATTTTTTGCGGTATTATAA
TAAATGGATTTAGAGACCGCCACGGGGGAACATGGCCTCCTGTGGAGTTACCAAATCATG
CATCTGCTGTAATTAGAAATGCCCAGTTATCTGGAGAAGGGTTAACATCTGAACAATGTG
CTCAACACTGGAGATCCTTTTGTGGATTTAGATTTAAATGTTTTATGCCATTGAGTCTAG
ATAGTGACCTTACAATGTACCTTAGAGACAAGGCGCTGTCACCTGTCAGAAATGAGTGGG
ATTCAGTTTATGCTAAGGAGTATTTAAGGTATAATCCAGGATTACCCACAAGTTCAGAA
GATTGGTAAATGTATTCTTAGAAGATGATAAGTTTGACCCATATGAAATGATCATGTACG
TGATAAATGGTGATTACTTAAGAGACAAAGAGTTTAACTTTTCATACAGCCTTAAAGAGA
AAGAAATTAAAGAGGTAGGTCGATTGTTTGCTAAAATGACCTATAAGATGAGGGCTTGTC
AAGTAATAGCTGAAAACCTGATTGCCAATGGAGTAGGGAAGTTTTTCAAAGATAATGGAA
TGGCAAAAGATGAACATAAATTAATAAGACGTTACACAAATTAGCCATTTTCAGGTGTAC
CTAAAGATAATTCTAACTTTATTTAGATGAATGTTGGGAGCAAGTAATTCGACAATGTT
CAAGTAGTACACAGATAAGGGAACAGACTATGAATTCACAATCAAATAGGGAAATTGAAT
CAAAGTCTTCTAGGGCACGTCTTAATAATAGAGATATCTTAAAGGGCAAGAGAGATTCTGA
ACAAACAAGTAAAGTATCCTTCAAACACCGAGTATTATGAGACTATCAGTAGTTTCATAA
CTACTGACCTTAAAAAGTATTGTCTTAAGTGGCGATATGAATCAAGTAGTATGTTTGCAG
AGAGACTTAATGAAATTTATGGACTGCCTGGATTTTTCCAGTGGCTTCACAAGATTTTGG
AGAAATCTGTTCTATACGTTAGTGATCCATCTAGTCCACCTGACTTTGATCAACATGTCG
ATATAGAATCAGTCCCAAATGACCATATCTTTATCAAGTACCCGATGGGTGGAATAGAGG
GGTCTGTCAAAAATTATGGACCATTAGTACAATTCCGTTCTATATTTAGCAGCTTTTG
ATACAGGGGTTAGAATCTCATCATTGGTTCAAGGCGATAACCAGGCAATTGCAGTAACCA
AAAGAGTTCGTCATCTTGGAGTTACTCAAAGAAAAAGGAAGAATCAACTAAAATAACAA
CACAATATTTTCTTAATTTAAGACAACGCTTACACGATATAGGTCATGAATTGAAAGCAA
ATGAGACTATTATATCCTCTCATTTCTTTGTTTACTCTAAAGGTATTTATTATGATGGAA
TACTTCTCTCCAGGCACTTAAAAGTATTGCAAGATGTGTCTTTTGGTCTGAAACGATTG
TTGATGAGACTAGGTCAGCTTGCAGTAATATATCTACGACACTCGCAAAGGCAATTGAAA

Figure 2-7

GGGGTTATGATAAATTTGTGGCGTACGCTATCAATATTTATAAAACAATACATCAGGTGT
TGATTGCATTGTCCTTTACGATTAATCCTACTATGACACCAGACATTACAGAACCTTTCT
ACAAGAGTTTAGATCTACTTAAGAATCTAGTTCTGATTCTGCACCATTAGGGGGCATGA
ACTATATGAACATGAGCAGGTTATTTGTTAGGAATATAGGAGATCCCATTACTGCTTCAT
TTGCTGATATAAAGCGCATGATTGAATGTGGGTTGTTAGGATGTAGTATTCTGTCACAAA
TAATGTACCAAAAATGTGGTTCCTCCAAATACTTAGACTGGGCTAGTGATCCTTATTCAA
TAAACCTTCCTTATAGCCAAAGTATGACCAAGGTTTTAAAAATGTAACGGCAAGATATG
TACTTATGCATAGTCCCAACCCTATGCTCAAAGATTTGTTCCATGAAAAGTCTCAGGAAG
AAGATGAAATCCTTGCTGAGTTTCTGTTAGACCGACACTTAATAATCCCTAGAGCAGCAC
ACGAGATTTTATCAAATTCAGTAACAGGTGCTAGAGAATCTATAGCAGGTATGCTTGACA
CTACTAAGGGTTTAATCCGTGCTAGTATGTCAAGAGGTGGGTTGACCTCATCACTTGTTT
TAAAATTATCAACATATGATTACCAACAGTTTAGAACATGTCTTGAATGGCTTTATGCTC
CTACTACGGGAATTGCTGTAAGCGTTGATTCTTGCTCTGTATTCTTAGCTAAGACCATCC
GGAAGAGAATGTGGGTTACCTAACTAAAGGAAGGGAGATTTATGGGTTAGAAGTACCTG
ACATTTTGGAATGTATGCAAAACAATATTATTGTTGATCACGAAGATTGTTACTCATGTA
TTCAAGGATCAAGATATTATACATGGTTTTTTGTACCTTCAAATTGTCAACTCGATCAAA
TAAATAAGTCAACAAATTCTCTCCGAGTACCTTATGTTGGATCAACAACTGAAGAAAGGA
GTGATATGAAGTTGTCATATGTGAGGTCACCTAGTCGGCCACTTAAAGCAGCAGTTCGAA
TTGCAGCAGTATATACATGGGCTTATGGTGATGATAATTTGTCTTGGCATGAAGCTTGGT
ATTTAGCAAGGACTAGAGCAAATATTACTTTTGACGAACTCAAATTAATAACACCTATAG
CTACATCTACAAATTTAGCACATAGATTGAGAGATAGAAGCACTCAAGTTAAATATTCAG
GAACTTCTTTAGTAAGAGTGGCAGCTATACAACAATATCTAATGATAATATGTCGTTCA
TTATTAATAACAAAAAAGTCGATACTAATTTTGTCTACCAGCAAGGAATGTTATTAGGTT
TGAGTATATTAGAATATATATTAGATACTGTACAAGTACTGGACAGTCAAACACTGTAA
TTCATTACATGCAGATGTTAATTGTTGTATAGTACAGATGACTGATCAGCCTTATACAC
CAAGCTTAACAAAAAAGCTACCTGATATTAGGCCCATTAATAATAAACTGATATATGATC
CGGCTCCTATAATCGATACCGATGCAGCTAGGCTATATTCCCAAAAATACCTGTCACATT
TAATAGATTTCCAAGTTGGTCAACTACTCAGCTTAACACAGTGTTGGCGAAAGTGGTGG

Figure 2-8

CGGTATCCATTGTAGAATTAATTACAAAAGCTAGTAAAGACCATCTCAATGAGATAATAG
CAGTTGTTGGTGATGATGATATCAATAGCTTTATTACAGAATTTCTACTTGTTGATCCAC
GTCTGTTTACACTATATTTAGGCCAATACACATCATTACAATGGGCATATGAAGTCCATT
ATCATAGACCAGTGGGTAAATACCAGATGGCTGAAGTGTTGCATAATTTGCTGTCAAGAG
CTAGTAGAGGTATATTCAGCATATTGACCAATGCCTTTAGCCACCCCAGAGTCTACAAAA
GATTCTGGGAGTGTGGTTTATTGGAGCCTATTTATGGGCCCTATATAGGAAGTCAAAATC
TACATAATGCAATGATTGATTATATCTATAATGCATACATTACTTATTTGGATGCTTATT
TATCTGATCAAGTAGATGATACTGATATTATAATATGTGAAACAGAGGAGACATGTTTGG
CGAATCGAATTGACAATTATCAAAGCAGACACTTAGCTGTGCTTATAGATCTGTATTGTG
ATTCCACTAGATGTCCCAATATAAAAGGGGCAGATACAATTATGAGAACTCAATTCTTA
GATCTTTTCATTGATAATGAGAGGAGAACAAATCCACTCGGTTTGACATGGAACCTTGACC
CGTTACTCGTGGATCATTTTAGCTGTTCTATTACGTATCTGAGGAGAGGTATTATTAAC
AGATGAGGTAAAGATTTGATCCAAGTGTATCGTTGGAACCTATCTAGGATGATTAAGCCTG
ATGCGGTTTATCAAGCACCTAAAATTCCGTCTTCATGGGCTCTTATAGATATCAACCCTG
AAGTAAATGACCTTAATGTAATTTTTGGAGAGCTGAATAGCAAATGGAAAGACATTCCTA
TTGGACAGATTAGGATACAGAATTATGAAATACATGCATATAGGAGGATCGGAGTTAATT
CAACTGCATGTTATAAAGCTCTAGAGCTATTGTCTGTTCTAAATCGGTTTATGTCTAATC
CATCAGGTGCATTGTTTTTAGGTGAAGGAGCAGGATCAATGCTGGTCACATACCGTGCTT
TTGTCCCATTAAAGACAATTTATTATAATAGTGGTATTTTCAGTTCAAATGTTTCAGGGCC
AGAGAGAATTGAGTCTATATCCATCTGAAGTGCCACTAGTTGACAACAAAAATCGCTTGG
CTAATGACCCCAATATCAAAGTCTTGTTCAATGGTAAACCAGAGTCTACGTGGGTTGGAA
ACATCGACTGTTTTGCTTATATTCTTAGCCACATTGAGACCTCAAGCTTGACATTGATAC
ATAGTGATATTGAGTCCAGCTTAAGCAAGACGAAGAATAAAATCTTGAGGAGCTGTGCC
ACATTCTGTCAATGGCACTCATTTTGGGGAAAATCGGATCTTTATTAGTTGTCAAGTTAT
TACCAAGGGTCGGTGACTATACGTATTCATTTTGCAGGTATGCATCGGAATTCTATCAAC
AAAGCCTCCTTGTTTTACCTAGGTTTAGTAACATGTCATCATCTGAGGTTTACTATATAG
GGATTCACCTCAATACAAATCGATTGATTGATCCTGATAGAATAGTACAATACATAGTTA
GAAATTTACAACCAACTCCAGTTACATTTTGTCTATATTTTGAACCTAAGTATAGAA

Figure 2-9

ATAATATGGTTACAAATTATGGACTGTGCTTGTCAGACGGACACAAAAGTGATTACCTGT
CATCAATTACAAAAATAGAGAACGTTCTTCTGTCATGTGGGTTAGAATTGAATGGACCTA
AGATTATACAGCAATTATCAGGACATGACTATGCTAATGGGGAGACTAGTCTAGAATCAA
GTATAATGATATTAGTTAGAGAATATCTTAATGCAACTATACAAGGCCGGGAAACATTAG
GCTTGTTTTACCTTACCCAGTCTTACATGAGAGTCAGTTAAGAGAAATTAATAAGTGTA
TTGCATTGAAATATGTTGTATATCTACTCTTTTATTCAAGCTCTACATTATCTAGTAAAC
AAATAATGAGTAATCTTAGAAAGGGAATATTGATGTATGATTTGAGAGATGAATTTTICA
TATCAAGATTGTCAGCAAATTACAAGAAAAAGGTGATGTCACAAGAAGTCAAACTACCT
GGATCTTTAATCTTGATACTCCGACACGAAAAGCATTATATAAGTTAGTAGGTTATTCAT
TAATAATTAATCATGTATGATGATAGAGTATGATTATCCATCTTTAAAAGAGTAAGATAA
TATCAGATGTATGATAACCAATTAAGTATTACTTTTGAATTGAAAGGTTGCTCAATTACA
CGCTTTTTTAGTAATCGGGTTTTTATTCCAATTAGGGCAATTAGAAAAAACTTCAACGGT
TAGTCGAGCCCGAATTCATTCATATAAGTTATATTTATAATCTTGGATAAGACTTTTGT
TTAGAATTATAACAGTAATACTAATTTATGAATGGAAGACAATTGATATCTAGTGTGAAT
TTTATGTTTATGTGTCTTAAACCTTATACTCACTATAATTGTTCTTTATTTGAGAATTTA
ATTATAGGTGTTTATGTGTTATGTGATGGGAACCATCAGTGCTGACATTATTAATAACCA
TAGGTATTGTATGGGATAGTGTTTATTTACTACCAATGTACAATCTCATATGTCCGACCC
CTCAACCTCCTCCTTATAGTTGAGTTTTCTGGAAAAACACAAAAGATGATCTTGAGTAAT
TGTACGGACCTATAGCTTTCTTTGTCTGGT

FIGURE 3

FmoPV 776U Cats/Hong Kong/2009 16050 bp

ACCAGACAAAGATGTCTGTGACCTATTCTAACGACAAGATTATTACTAAATATTTAGGAA
TAACGATTCCATTAGTGAGGTGAGGAGGAGGAATCAGGTATTCCACAATGTCTAGTCTAT
TGAGGTCACTTGCTGCATTTAAGAGACATAGGGAGCAACCAACAGCACCGTCAGGTTTCAG
GTGGTACAATTAAGGATTGAAAAATACAATTATTGTTCCGGTTCAGGGGATACAGTAA
TTACTACAAGGTCTAATTTGTTATTTAGATTAGTTTATATAATAGGCAATCCGGATACAC
CTTTAAGCACCTCGACGGGAGCAATAATCATTGTTGACCCTATTTGTCGAATCTCCAG
GTCAATTAATTCAAAGAATTGCTGATGACCCTGATGCAGTTTTTAAATTGGTAGAGGTCA
TTCCTGAAGCTGGTAATCCTGGAGAATTAACTTTTGCATCTCGAGGGATTAATTTAGACA
AGCAAGCTCAACAATACTTTAAATTGGCTGAGAAAAATGATCAGGGGTATTATGTTAGCT
TAGGATTTGAGAACCCTCCAAATGATGACGATATAACATCTAGTCCTGAGATATTCAATT
ATATCCTGGCATCTGTACTTGCACAAGTTTGGATTCTTCTGGCAAAAGCTGTGACTGCTC
CAGATACGGCTGCTGAAGCCGAAAATCGTAGATGGATTAAATTAATGCAACAACGTAGGG
TGGATGGTGAAGTGAAGATTGAGCAAAGGATGGCTAGATTTAGTGAGAAACAAGATTGCGT
CAGATATTACAATAAGGCGATTTCATGGTAGCATTAGTTCTTGACATCAAACGTTCTCCTG
GGACAAGACCCAGGATAGCTGAAATGATTTGTGATATTGATAATTATATTGTAGAAGCAG
GGCTTGCAAGTTTCTTATTAACCATTAATTTGGCATAGAAACACGTTATCCAGCACTGG
CACTACATGAGTTTTCTGGAGAACTAGCCACTATTGAGGGGCTTATGAAATTGTACCAAT
CTATGGGGGAAATGGCACCATACATGGTAATTCTGGAAAACCAATCCAAACCAGGTTTA
GTGCAGGGTCTTATCCTCTGCTATGGAGTTATGCAATGGGTGTGGGGTGGAGCTTGAAA
GATCAATGGGTGGACTCAATTTCACTAGAAGCTTCTTTGACCCGACATATTTCAGACTTG
GTCAAGAGATGGTGAGGAGATCTTCAGGGATGGTTAATAGTTCATTTGCGAGAGAATTG
GCCTATCTGAGCATGAAACACAACCTGGTCAGCCAGATTGTCAATTCGGGAGGTGAATCCG
GGATACCTAAATTTGATGGATTTCAGAGCAAATCCAACAACCTTTCTAGGAACCAAGGATA
ACATAGATGATAGAGGTGAAGATCAGTCAAATTCGATATCAGGGTTACCTGGTCCACTAT
TACCCAGCCGTGACCTAGATCTTTCCGGTGATTCATATGGAATTAATAGTGGTGTGAAAA
ATGTCAGTGACAACTGAATGAAGGAGTAGGTCCAGACCATGATGTGTCCAGTTCTGCCA

Figure 3-1

TGGAAGAATTGAGAAGATTGGTTGAGTCTACCAACAGAATTGACACCAAACAGCCGGAAG
CTTCAGGTGTACCAACCATTATAATGATACTGACCTTCTAAAATAATATGAGCATACCC
TAATTGATTATGATACAACCTCAAATTAAGAAAACTTAGGACCTCAAGGTTCACTCACTGT
TGGCATATCACCAAAACACAGTCAGCTCTTACCCACCCCATGTCCTCTCACCAAATCCA
ACAAGTCAAACATGGCCTCGAATCTTTACAAGAGATCAAAAGCAACCCTCCGCCTTCCCA
AGATGTCAATCTTGCCAGGGAGATTTACGAATCCATTAGACAAACAGGAACATCTTCAGT
GCAAGGAGGAGCCATTGCGGGAATAATATTACGTCAGGGGGTAACAATGACTCAATGTA
TAGCCAAGGACCAAGTCTCCTATTTCAAGTATTAACAAGAATATCGAAGGACCTACTGG
ATTCGATCATTGAGGACTATGGGATCCAGAGGGTAACCTCTGCATGCTATTGAAAGCGA
TGATGATGAAAACCATTATTCAGAGATTAATGGCCGGTCTTCCACTATCGAAGGACTGGA
TGAACAGGATAATGAGAACTCAATTATTAACAACCAGGAAATCAGTGTACTGAGGGAGT
GTCTAAGACTGATTCATCTCCTAGTTCCAGGAACTACACTATCTGTTGGGGGATCTGA
TATACCTGGGACAGGAATATCAACCTGTGCCTCTTTGGATATAACTGTAAATGAACTCGA
AGATGCAACTGTAAGAAATAGCAACAATATGAAAGGGAACTGGCCAATTCCTAAATTACT
AGTTAAGCCGCCACCTAGGGTAAAATCAAGTGTTGATCACAGTAATCCATTAAGGGGGC
CACAGAAGGGAAATTAGCCTCACCTGGGATGGAGACTACATTATTCGAGAAGAGTGGTGC
AACCCCATCTGTACACCCATATACTCAACCTGCAAGCGACTTCAATGTAGGTGCAAGCAG
TGTCATCAACCTGCCCTAAATGTGAATAATAATTGCAATGACGGTAGGGTAACAGCGCC
TAACTCACATAAAGATATCGAGGGTAAGTCTGAAATATCTATTCAAGATATATATAACTT
GATTCITGGATTTAAGGATGATTACAGGAAATTATCAAACAAATTAGATATGGTATTAGA
GATGAAACAAGACATTGACAATCTTAAAAAGAATAGTGCTAAAGTGCAATTAGCTCTATC
AACTATTGAGGGACATCTATCCAGTGTTATGATTGCTATCCCTGGTTCAGGTATTGATTC
CACAGGGGATGAGGAAAAGGACCAGATAAATTCTGACTTAAACCACTGCTAGGAAGGGA
TCATTGTAGAGCATTTGAGAAGTTACCAATCCTCTAGATGAGTCTTCACTAGCCAATTC
TCCAACAAAACATGTTGCCAAGGTAAACAAGAACTGCACTCTTCAGAAGATCAACAAGAA
CGAAACATCTGCAATCAAATTTGTTCTAGTGACAGTCATGCAAGCACATCAACCATCAG
GTCAATTATCAGGTCATCTAATCTCGATCAGGATTTGAAAACAAAATTGCTCACAATTTT
ATCCCAGATAAGAGGTGTAGACAATATTAGAGAATTCTATGAAAAGGTTATGATATTAAT

Figure 3-2

AAAGAATAAGAATTAATATTACAAATCTACATGCATTATAGGTTGTAATTGTCTTCAAT
AAGATTTGGTCAGTTTCATATATATGGTTATTGATTTGTGATAATTATAAAAACTTAGG
AGCTAAAGATTACTCAGTCATATACAGCATGACTGAGATATTCAACCTTGATGAGAGCTC
ATGGTCAGTCAAAGGGACACTAGATCCGCTAACACCTGATACCTATCCTGATGGTCGACT
AGTGCCTAAAGTTCGAGTTATCGATCCGGGTCTAGGAGATCGCAAGAGTGGGGGGTATAT
GTATCTACTTCTTCATGGTGTATAGAAAGATAGTGAGACTATAATTAGTCCGAAAGGAAG
AGCATTTGGTGCATTCCCATAGGAGTGGGTCAATCAACTGAAAACCCGGAAGACTTGTT
TAAGGAAATATTAACCTCTCAACATCGTGACTCGTAGGACTGCTGGATTTAATGAGAAATT
GGTTTATTATAATACCACACCTCTACATTTACTGACCCCTGGAAGAAAGTGTTGGCATA
TGGAGGCATTTTAAATGCTAATCAGGTCTGCAGTGATACAAGTTCATACCAATAGACAT
TCCACAAAATTTAGGCCAGTATATTTGACTGTTACAAAATTATCTGATGATGGCTATTA
TCAGATCCCAAAGATGATTCAAGATTTCAAATCGTCAAATTCTGTTGCATTTAACATCCT
TGTGCATCTGTCAATGGGCACAAATTTACTTGACCAATCCAAGGACCCTAGATTAAGAAG
TGCTGCAGAACTGTGATCACATTTATGATTCATATTGGAACTTTAAACGGAAGAGTAA
TAAGTCTTACTCACCTGAATATTGCAAGAGGAAAAATAATGAGGCTTGGTTAATATTCTC
ATTAGGTGCAATTGGTGGCACAAGCTTGTCATATTAGATGTACAGGTAAGATGAGCAAACG
ACTACAGGCTTATTTGGGATTCAAAGGACTTTATGTTACCCTTTGATGTATGTTAATGA
AGGGCTGAACAAGACCCTGTGGAGAAATGAATGCAGAATAGAGAAGGTTCAAGCAGTCCT
ACAGCCATCAGTCCCGAATGAGTTTAAGATATATGATGATGTTATTATTGATAATACCAA
TGGTCTCTTCAAGATTAAATAGACTATAACAATAATAAACCGCCACCAAATGGTACCATG
TATTCAAGTGTACACTGACAATTGCGAATAAAATATACCAGATTAACAACAGTATAGAGT
TAAGATCTAATTGATATGTGGGTTGGTACTCGATCATTTATTAGCTCTACTGATTATCTA
TATCCTAAATCACCAAATATAAGAGCATCAACAGGTAATAAGTTTGGGATTGCTAGATTA
ATACTTAATTCTCAGAACTAGAAATACACAGATTGTCAAACCTATAATCTTGTTAGATTCA
TTAAAGTTAGATTCTTGTAATGTTGATCAATTATCACTCGAGCAATTATAAAAACTAAG
GACCTAATGTAATAGGAGCCCAAATCCATCCAGTGAGCTTTAAATCGCCATGCTTAAAC
ATTAATTTGTCCAGGGCCTATCTAACTCAGAACAAAGATCACAACCTAGAGTCTGAAGGAG
TGGGTAAAGTCTGAATAATTATTAAGAGTTGAGATTTAAACTGATTCCTTCTTAAATTT

Figure 3-3

AGAATTTTAATAATATATCATCCATTCAATATCATGAACAGGATTAAGGTTATAATAATT
AGTTCTTTATTACTATCAGATATTACGATTGCACAAATAGGTTGGGATAATTTGACTTCG
ATTGGAGTTATAAGTACTAAGCAATACGACTATAAAATAACTACTCTGAACACTGACCAG
TTAATGGTTATAAAGATGGTTCCTAATATATCATCAATCATTAAATTGCACTAAACTCGAA
TTAACAAAATACAGAGAGTTAGTCTCAGGGATCATTAGACCAATAAATGAGTCATTAGAA
TTAATGAATTCATACATTAACATGAGAGCAGGTTGAGAGAGATTCATAGGGGCTGTAATA
GCTGGTGTAGCCTTAGGAGTGGCAACTGCAGCACAAATAACATCAGGGATTGCCCTACAT
AATTCAATTATGAACAAAAACAGATACAAGAATTGAGGAAGGCTCTTAGTACTACTAAC
AAAGCAATTGATGAAATAAGGATTGCAGGTGAAAGAACATTAATAGCAATTCAAGGTGTA
CAGGATTATATTAATAATATAATTATCCCTATGCAGGACAAACTCCAATGTGATATTTTA
TCATCACAACTTTCTGTTGCTTTACTCAGATATTATACAAATATATTAACAGTTTTTGGG
CCAAGTATACGGGATCCTATTACTAGTACAGTTTCAGTACAGGCACTCAGTCAAGCATTC
AATGGTAATCTTCAGGCATTGCTTGATGGATTGGGATATACTGGGAAAGACTTACGTGAT
CTTCTAGAGAGTAAATCTATCACTGGCCAGATAATTCATGCAGATATGACTGATTTGTTT
CTTGTTCTGAGAATAAATTATCCTTCTATAACTGAGATGCAGGGAGTAACAATATATGGG
CTCAATTCAATTACATATCATATTGGGCCTGAAGAGTGGTATACCATTATGCCTGATTTT
ATTGCTGTTTCAGGGTTTTTTAATATCTAATTTTGATGAGAGAAAGTGTTCAATAACTAAA
TCAAGTATATTGTGCCAACAAAATTCAATTTACCCAATGTCAACAGAGATGCAAAGATGT
ATTAAGGGCGAAATAAGATTCTGTCCAAGATCCAAGGCAATTGGGACATTAGTCAATCGG
TTTATATTGACCAAAGGTAATTTGATGGCTAATTGTTTAGGGATTATATGCAGATGTTAT
ACTTCAGGCCAAGTTATAACACAAGACCCTAGTAAATTGATCACGATAATATCGCAAGAG
GAGTGCAAGGAAGTTGGTGTGATGGTATCCGTATTATGGTAGGACCTAGAAAATTACCA
GATATTACCTTTAACGCTAGGTTGGAAATTGGTGTACCGATATCATTAAAGCAAATTAGAT
GTCGGGACTGATTTAGCGATTGCTTCAGCTAAACTTAATAATTCTAAGGCATTGTTAGAG
CAATCAGATAAGATTTTGGATTCAATGTCTAAATTGGATTCTATGAACTCAAGAATAATA
GGGTAAATCTTAGCAATTATGATAATCTTTATAATCATTATTACTATTATCTGGATCATG
TATAAGAAATGTAAGAATAAAGATAATAAATTCAGTACTTCAATTGAACCGCTCTACATA
CCCCCTTCTTATAACTCACCTCATAGTGTGGTTAAATCTATTTGAGTACTGACTATATGA

Figure 3-4

TCCACTGTAATAAGTCCAATGAAAGTATCAATTAATAATATTGGTAGTGCAATAAGTATT
GATTGTATAATATACTCCTTTAACTAGATAGTGATAAAGGGTTATAGATGATTTTCAGTC
ACTTTAATATAATCATATATTGGTTTTATTATCTTGCATAACTATTATGTAATTGAATTA
TGTATCATCAATTAATAGCTTAATAATATGTTTTAATACTTATATTGATAGATAAATG
TGTTATATTGTAATCAAGGAGTTGGTATTTAGAAGAGGAAAGAGTTAAATTTGTTGTTAA
TTAGTTATTGTGTATTCAATTAGAAAAAAGTTAGGAATCCATGTTAATAGAAATTTATTA
TCATGGAGTCCAACAATATTAAGTACTACAAAGATTCTAGCCGGTACTTTGGTAAAATAT
TAGATGAACACAAAACAATTAATAGTCAATTATACAGTTTGAGTATCAAGGTAATTACCA
TTATTGCTATTATTGTAAGCCTGATTGCAACAATAATACTATTATCAATGCCACTAGTG
GGAGAACTACCCTAAATAGTAATACAGACATACTACTCAGCCAACGAGATGAGATTCATA
ACATCCAAGAAATGATATTTGATCGTATTTATCCTTTGATAAATGCTATGAGTACAGAGC
TAGGACTTCATATTCCTACCTTATTGGATGAACTTACTAAAGCGATTGACCAGAAAAATTA
AAATAATGCATCCTCCTGTGGACACTGTGACTTCTGACCTTAATTGGTGCATCAAACCCC
CTAATGGAATTATCATAGACCCAAAAAGTTATTGTGAGAGTATGGAATTGTCTAAAAGTT
ATGAATTGTTACTTGACCAGTTAGATGTCTCAAGAAAGAAATCACTTATTATAAATAGAA
AGAATATTAACCAATGCCAATTAGTTGATAATTCAAAGATCATTTTTGCCACTGTCAACA
TACAATCTACACCGAGGTTTTTAACTTTGGTCACACGGTCAGCAATCAACGTATAACAT
TTGGTCAAGGAACATATAGTAGTACTTATGTTATAACTATCCAAGAAGATGGAGTAACTG
ATGTTCAATATCGAGTGTTTGAGATCGGATATATTTGTGATCAGTTTGGTGTATCCCCCT
CCTTAATAGTATCGAGAGTGTTGCCGATACGCATGCTATTAGAAATGGAATCCTGTACCT
TGACAAGTGATAGACTAGGCGGGTATTTTTATGTATGAATACACTGACACGATCTATAT
ACGATTATGTTAGCATAAGGGATTTGAAATCACTTTATATAACAATCCCTCATTATGGTA
AAGTTAATTATACTTACTTTAATTTTGGTAAGATCAGGAGCCCACATGAGATTGATAAAA
TTTGGTTAACATCTGATAGAGGCCAAATTATCTCTGGTTATTTTGCAGCATTGTACCA
TTACAATTCGGAAGTATAATAATTATCCCTACAAATGCTTAAATAACCCATGTTTTGACA
ACTCTGAGAATTACTGTAGAGGATGGTATAAAAACATAACAGGAACTGATGATGTTCCGA
TATTAGCATACTTATTGGTTGAAATGTATGATGAAGAGGGACCTTTAATTACACTTGTGG
CAATACCACCTTACAATTATACAGCTCCATCTCATAATTCTCTTTACTATGATGACAAAG

Figure 3-5

TTAATAAATTAATAATGACTACATCTCACATAGGTTATATTCAAATCAATGAGGTGCATG
AGGTAATTGTTGGCGATAATTTGAAGGCTATCCTCTTAAACAGATTATCTGATGAACATC
CTAACCTGACTGCCTGTAGACTCAATCAGGGTATTAAGGAGCAATACAAGTCTGACGGAA
CAATAATTTCAAATTCTGTACTTATTGATATACAAGAACGAATGTACATTACAGTTAAAG
CTATTCCACCAGCAGGTAACATAACTTTACAGTTGAGTTGCATTCTAGATCAAACACAT
CTTATGTATCGTTGCCAAGACAGTTTAATGCTAAGTATGACAAATTACATCTTGAGTGCT
TTAGCTGGGACAAATCCTGGTGGTGTGCTCTGATACCTCAGTTTTCATTAAGTTGGAATG
AATCCCTTTCTGTTGATACTGCCATTTTCAATTTAATAAGCTGTCAATGAACACATCAAT
CTATAGTTGATAGTTGTCAAAACATTAGCCAATTTGGGTAAAGAAATAGGAAAATGAAA
TTATCAATATCTAATTAGATGTATGTTCAAGCTAAATTACAAAAAACTTAGGAGTCAGAG
ATTTCTGTTGCAATGGAGCAGTCAGACTACCAAGATATTCTATACCCGGAAGTACATCTTA
ACAGTCCTATAGTAATTTCCAAATTAGTAGGTATTTTAGAATATGCCCAAATTGGTCATA
ATCAACAATTATCAGACCGTACAATTATCAAGAATATTCAATTTAGATTAAGGAACGGAT
TTAATAGTTCAAGGGTACAGGTACTATCAACTATGGGTGAAATTATCAACAAAATTAGAA
ATAAATATCCTAATTATTTACACATACCTTACCCTGAATGCAACCAAAAACTATTTGAA
TAGTAGATCCAGAACTAACATCAAAATTAGAATCTCTTCTAAACAAAGGTGACACACTGT
ATCTCAAGATTGATCAGATATCATAAAGTGTTTTGATAGATTGAAAATGAAAATGAACA
TAAAGAATGATCTTCTCAATGACAATAGTCAATTGATTCTAGATCTTCCTTTAATTATCA
AAGGATCTCAGTGGTTCTTCCCTTTTTTATTTTGGTTTTCTATCAAACTGAACTAGAA
GCTGTATTCGCCAAAATCAAAAGACTCGTGTTAGATCACAATATCGGCCTCACTTATCAG
AGACTAAGAGAATTACATTGGTTGTTACATCTGATCTGATTACAATATTTGATCATATTA
ATAAATGTATATTTTATTTGACTTTTGAGATGCTGTTAATGTATTGCGATGTGATAGAAG
GTCGGTTAATGACTGAAACAGCTATGAGCTTGGACTGTCGGTTTACCAATCTATTGCCAA
GAGTGCAATATATGTGGGATTTACTAGATGGAATGTTTGAAAGTTTAGGCAATCAATTAT
ATTCAGTTATTGCATTATTAGAGCCTCTTTCTCTTGCTTATTTGCAATTGATAGATGCAG
ATCCACAGATTCGGGGAACATTCCTGCATCACTGCTTTTCCGAGTTAGAAGAAATTATAT
TTGACAAAACCCCTTTTGATCCTTTCGTATATGAAAATTTAATTAATGGACTTGATTACA
TTTATTTGACAGATGATATTCATCTAACTGCAGAAGTTTTTCTTTTTTTAGAAGTTTTG

Figure 3-6

GTCATCCTTTTTTAGAAGCACAAAATGCTGCCAATAATGTAAGGAAGTATATGAATAAAC
CTAAGGTAATCTCATATCAGACTTTAATGCAAGGACATGCGATTTTTTGCGGTATTATAA
TAAATGGATTTAGAGATCGCCACGGGGGAACATGGCCTCCTGTAGAGTTACCAAATCATG
CATCTGCTGTAATTAGAAATGCCCAGTTATCTGGAGAAGGGTTAACATCTGAACAATGTG
CTCAACACTGGAGATCCTTCTGTGGATTTAGATTTAAATGTTTTATGCCATTGAGTCTAG
ACAGTGACCTTACAATGTACCTTAGAGACAAGGCGTTATCACCTGTCAGAAATGAGTGGG
ATTCAGTTTATGCTAAGGAGTATTTAAGATATAATCCAGGATTACCCACAAGTTCAGAA
GATTGGTAAATGTATTCTTAGAAGATGATAAGTTTGATCCATATGAAATGATCATGTACG
TGATAAATGGTGATTACTTAAGAGACAAAGAGTTTAACTTTTCATACAGCCTTAAAGAGA
AAGAAATTAAAGAGGTAGGTCGATTGTTTCGCTAAAATGACCTATAAAATGAGGGCTTGTC
AAGTAATAGCTGAAAACCTGATTGCCAATGGAGTAGGGAAGTTTTTCAAAGATAATGGAA
TGGCAAAAGATGAACATAAAATTAATAAAACGTTACACAAATTAGCCATTTTCAGGTGTAC
CTAAAGATAATTCTCAACTTTATTTAGATGAATGTTGGGAGCAAGTAATTCGACAATGTT
CAAGTAGTACACAGATAAGGGAACAGGCTATGAATTCACAATCAAATAGGGAAATTGAAT
CAAAGTCTTCTAGGGCACGTCTTAATAATAGAGATATCTTAAAGGGCAAGAGAGATTCTGA
ACAAACAAATAAAGTATCCTTCAAACACCGAGTATTATGAGACTATCAGTAGTTTCATAA
CTACTGACCTTAAAAAGTATTGTCTTAATACTGGCGATATGAATCAAGTAGTGTATTTGCAG
AGAGACTTAATGAGATTTATGGACTGCCTGGATTTTTCCAGTGGCTTCACAAGATTTTGG
AGAAATCTGTTCTATACGTTAGTGATCCATATAGTCCACCTGACTTTGATCAACATATCG
ATATAGAATCAGTCCCAAACGACCATATCTTTATCAAGTACCCGATGGGTGGAATAGAGG
GGTTCTGTCAAAAATTATGGACCATTAGTACAATTCCGTTCCCTATATTTAGCAGCTTTTG
ATACAGGGGTTAGAATCTCATCATTAGTTCAAGGCGATAACCAGGCAATTGCAGTGACCA
AAAGAGTTCCGTCATCTTGGAGTTATTCAAAGAAAAAGGAAGAATCACTAAAATAACAA
CACAGTATTTTCTTAATTTAAGACAACGCTTACACGACATAGGTCATGAATTGAAAGCAA
ATGAGACTATTATATCCTCTCATTTCCTTTGTTTACTCTAAAGGTATTTATTATGATGGAA
TACTTCTCTCCAGGCACTTAAAAGTATTGCAAGATGTGTCTTCTGGTCTGAAACGATTG
TTGATGAGACTAGGTCAGCTTGCAAGTAACATATCTACGACACTCGCAAAGGCAATTGAAA
GGGGTTATGATAAATTTGTGGCGTACGCTATCAATATTTATAAAACAATACATCAGGTGT

Figure 3-7

TGATTGCATTGTCCTTTACGATTAATCCTACTATGACACCAGACATCACAGAACCTTTCT
ACAAGAGTTTAGATCTACTTAAGAATCTAGTCCTGATTCCCTGCACCATTAGGGGGCATGA
ACTATATGAACATGAGCAGGTTATTTGTTAGGAATATAGGAGATCCCATTACTGCTTCAT
TTGCTGATATAAAGCGCATGATTGAATGTGGGTGTTAGGATGTAGTATTCTGTCACAAA
TAATGTACCAAAAATGTGGTTCCTCTAAATACTTAGACTGGGCTAGTGATCCTTATTCAA
TAAACCTTCCTTATAGCCAAAGTATGACCAAGGTTTTAAAAAATGTAACGGCAAGATATG
TACTTATGCATAGTCCCAACCCTATGCTCAAAGATTTGTTCCATGAAAAGTCTCAGGAAG
AAGATGAAATCCTTGCTGAGTTTCTGTTAGACCGACACTTAATAATCCCTAGAGCAGCAC
ACGAAATTTTATCAAATTCAGTAACAGGTGCTAGAGAATCTATAGCAGGTATGCTTGACA
CTACTAAGGGTTTAATCCGTGCTAGTATGTCAAGAGGTGGGTGACCTCATCACTTGTTT
TAAAATTATCAACATATGATTACCAACAGTTTAGAACATGTCTTGAATGGCTTTATGCTC
CTACTACGGGAATTGCTGTAAGCGTTGATTCTTGCTCTGTATTCTTAGCTAAGACCATCC
GGAAGAGAATGTGGGTTCACCTAACTAAAGGAAGGGAGATTTATGGGTTAGAAGTACCTG
ACATTTTGAATGTATGCAAAACAATATTATTGTTGATCACGAAGATTGTTACTCATGTA
TTCAAGGATCAAGATATTATACATGGTTTTTTGTACCTTCAAATTGTCAACTCGATCAAA
TAAATAAGTCAACAAATTCTCTCCGAGTACCTTATGTTGGATCAACAACCTGAAGAAAGGA
GTGATATGAAGTTGTCATATGTAAGGTCACCTAGTCGGCCACTTAAAGCAGCAGTTAGGA
TTGCAGCAGTATATACATGGGCTTATGGTGATGATAATTTGTCTTGGCATGAAGCTTGGT
ATTTAGCAAGGACTAGAGCAAATATTACTTTTGACGAACTCAAATTAATAACACCTATAG
CTACATCTACAACTTAGCACATAGATTGAGGGATAGAAGCACTCAAGTTAAATATTAG
GAACTTCTTTAGTAAGAGTGGCACGCTATACAACAATATCTAATGATAATATGTCGTTCA
TTATTAATAACAAGAAAGTCGATACTAATTTTGTCTACCAGCAAGGAATGTTATTAGGTT
TGAGTATATTGGAATACATATTCAGATACTGTACAAGTACTGGACAGTCAAACACTGTAA
TTCACCTACATGCAGATGTTAATTGTTGTATAGTACAGATGACTGATCAGCCTTATACAC
CAAGTTTAACAAAAAGCTACCTGATATTAAGCCATTAATAATAAACTGATATATGATC
CGGCTCCTATAATCGATACTGATGCAGCTAGGCTATATCCCAAAAGTACCTGTCACATT
TAATAGATTTCCCAAGTTGGTCAACTACTCAGCTTAACACAGTATTGGCGAAAGTAGTGG
CGGTATCTATTGTGGAATTAATTACAAAAGCGAGTAAAGACCATCTCAATGAGATAATAG

Figure 3-8

CAGTTGTTGGTGATGATGATATCAATAGCTTTATTACAGAATTTCTACTTGTTGATCCAC
GTCTGTTTACACTATATTTAGGCCAATACACATCATTACAATGGGCATATGAAGTCCATT
ATCATAGACCAGTGGGTAAATACCAGATGGCTGAAGTGTGCATAATTTGCTGTCAAGAG
CTAGTAGAGGTATATTCAGTATATTGACCAATGCCTTTAGCCACCCCAGAGTCTACAAAA
GATTCTGGGAGTGTGGTTTATTGGAGCCTATTTATGGGCCCTATATAGGAAGTCAAAATC
TACATAATGCAATGATTGATTATATCTATAATGCATACATTACTTATTTGGATGCTTATT
TATCTGATCAAGTAGATGATACTGATATTATAATATGTGAAACAGAGGAGACATGTTTGG
CGAATCGAATTGACAATTATCAAAGCAGACACTTAGCTGTGCTTATAGATCTGTATTGTG
ATTCCACTAGATGTCCAATATAAAAGGGGCAGATACAATTATGAGAAATTCAATTCTTA
GATCTTTCATTGATAATGAGAGGAGAAACAAATCCACTTGGTTTGACATGGAACCTTGACC
CGTTACTTGTGGATCACTTTAGCTGTTCTATTACGTATCTGAGGAGAGGTATTATTAAAC
AGATGAGGTAAAGATTTGATCCAAGTGTATCGCTGGAACCTATCTAGGATGATTAAACCTG
ATGCGGTTTATCAAGCACCTAAAATTCCGTCTTCATGGGCTCTTATAGATATCAACCCTG
AAGTAAATGACCTTAATGTAATTTTTGGAGAGCTGAATAGCAAGTGGAAAGATATCCCTA
TTGGACAGATTAGAATACAGAATTATGAAATACATGCATATAGGAGGATTGGAGTTAATT
CAACTGCCTGTTATAAAGCTCTAGAGCTATTATCTGTTCTAAATCGGTTTATGCCTAATC
CATCAGGTGCATTGTTTTTAGGTGAAGGAGCAGGATCAATGCTGGTCACATACCGTGCTT
TTGTCCCATTAAAGACAATTTATTACAATAGTGGTATTTTCAAGTTCAAAATGTTTCAAGGCC
AGAGAGAATTGAGTCTATATCCATCTGAAGTGGCACTAGTTGACAACAAAAATCGCTTGG
CTAATGACCCTAATATCAAAGTCTTGTTCAATGGTAAGCCAGAGTCTACGTGGGTTGGAA
ACATCGACTGTTTTGCTTATATTCTTAGCCACATTGAGACCTCAAGCTTGACATTGATAC
ATAGTGATATTGAGTCCAGCTTAAGCAAGACGAAGAATAAAATTTCTTGAGGAGCTGTGCC
ACATTCTGTCAATGGCACTCATTTTGGGGAAAAATCGGATCTTTATTAGTTGTTAAGTTAT
TACCAAGGGTCGGTGACTATACGTATTCATTTTGCAGGTATGCATCGGAATTCTATCAAC
AAAGCCTCCTTGTTTTACCTAGGTTTAGTAACATGTCATCATCTGAGGTTTACTATATAG
GAATTCACCTCAATACAAATCGATTGATTGATCCTGATAGAATAGTACAATACATAATTA
GAAATTTACAACCAACTCCAGTTACATTTTTGTCTTATATTTTGAACCTAAGTATAGGA
ATAATATGGTTACAAATTATGGACTGTGCTTGTGACGACGACACAAAAGTGATTACCTGT

Figure 3-9

CATCAATTACAAAAATAGAGAATGTTCTCCTGTCATGTGGGTTAGAATTGAATGGACCTA
AGATTATACAGCAATTATCAGGACATGACTATGCTAATGGGGAGACTAGTCTAGAATCAA
GTATAATGATATTAGTTAGGGAATATCTTAATGCAACTATACAGGGCCGGGAAACATTAG
GCTTGTTTTACCTTACCCAGTCTTACATGAGAGTCAGTTAAGAGAGATTAATAAGTGTA
TTGCATTGAAATATGTTGTATATCTACTCTTTTATTCAAACCTCTACATTATCTAGTAAAC
AAATAATGAGTAATCTCAGAAAGGGAATATTGATGTATGATTTGAGAGATGAATTTTTCA
TATCAAGATTGTCAGCAAATTACAAGAAAAAGGTGATGTCACAGGAAGTCAAGACTACCT
GGATCTTTAATATTGATACTCCGACACGAAAAGCATTATATAAGTTAGTAGGTTATTCAT
TAATAATTAATCATGTATGATGATAGAGTGTGATTATCCATCTTTTAGAGAGTAAGATAA
TATCAGATGTATGATAACCAATTAAGTATTGCTTTTGAATTGAAAGGTTGCTCAATTACA
CGCTTCTTTAGTAATCGGGTTTTTATTCCAATTAAGGCAATTAGAAAAAACTTCAACAGT
TAGTCGAGCCCGAATTCATTTTATATAAGTTATATTATAATCTTGGATAAGACTTTTGT
TTAGAATTATAACAGTAATACTAATTTATGAATGGAAGACAATTGATATCTAGTGTGAAT
TTCATGCTTATGTGTCCTTAACCTTATACTCACGATCATTATTCTTTATTTGAGAATTTA
ATTATAGGTGTTTATGTGTTATGTGATGGGAACCATCAATGCTGACATTATTAATAACCA
TAGGTATTGTATGAGATAATGTTTATTTACTACCAATGTACAATCTCATATGTCGGACCC
CTTAACCTCCTCCTTATAGTTGAGTTTTCTGGAAAAACACAAAAGATGATCTTGAGTAAT
TGTAACGGACCTATAGCTTTCTTTGTCTGGT

FIGURE 4

FmoPV M252A Cats/China/2010 16050 bp

ACCAGACAAAGATGTCTGTGACCTATTCTAACGACAAGACTATTATTAATATTTAGGAA
TAACGATTCCATTAGTGGGGTGAGGGGAAGGAATCAGGTATTCCAGAATGTCGAGTCTAC
TGAAGTCACTTGCCGCATTTAAAAGACATAGAGAGCAACCAACTACACCGTCAGGTTCAG
GTGGTACAATTAAGGATTGAAAAACACAATTATTGTTCCAGTACCAGGGGATACAGTAA
TTACCACGAGGTCTAATTTGTTATTTAGATTAGTTTATATAATAGGCAATCCAGATACGC
CTCTAAGCACCTCGACGGGAGCAATAATATCATTATTGACCCTATTCGTCGAATCCCCAG
GTCAATTAATTCAAAGAATTGCCGATGACCCTGATGCAGTTTTTAAATTAGTAGAGGTCA
TTCCTGAAGCTGGTAATCCTGGAGAATTGACTTTTGCATCTCGAGGGATTAATTTAGATA
AGCAAGCCCCACAATACTTTAACTGGCTGAGAGAAATGATCAGGGGTATTATGTTAGCT
TAGGATTTGAGAACCCACCAAACGATGATGATATAACATCTAGTCCTGAGATATTTAATT
ATATTTGGCATCTGTACTTGCGCAAGTTTGGATTCTTCTGGCAAAAGCTGTGACTGCTC
CGGATACAGCTGCTGAAGCTGAAAACCGTAGATGGATTAAATTGATGCAACAACGTCGGG
TGGATGGTGAATTAAGATTGAGTAAAGGATGGCTAGATTTGGTGAGAAATAAAATTGCGT
CAGATATTACAATAAGACGATTTATGGTGGCATTAGTCCTTGACATCAAACGTTCTCCTG
GGACAAGACCCAGAATAGCTGAAATGATTTGTGATATTGATAATTATATTGTAGAGGCAG
GGCTTGCAAGTTTCTTGTTAACTATTAATTTGGCATAGAGACACGTTATCCAGCATTGG
CATTGCATGAGTTCTCTGGAGAATTAGCTACTATTGAGGGACTTATGAAATTGTACCAAT
CTATGGGAGAAATGGCACCATATATGGTAATTCTGGAAAATTCAATTCAAACCAGGTTTA
GTGCCGGGTCTTATCCTTTGCTATGGAGTTATGCCATGGGCGTTGGTGTGGAGCTTGAAA
GATCGATGGGTGGACTTAATTTTACTAGGAGCTTCTTTGACCCTACGTACTTCAGACTTG
GTCAAGAGATGGTGAGAAGATCTTCAGGGATGGTTAATAGTTCATTTGCGCGAGAACTTG
GGCTATCTGAACATGAGACACAACCTTGTCAGCCAAATTGTTAATTCGGGAGGTGAATCTG
GGATACCTAAATTTGATGGATTCAGAGCAAATCCAACAACCTTTCTAGGAACCAAAGATA
ATATTAATGATAAAGGTGAGGATCAGTCAAGTTCAGTATCAGGGTTACCTGGTCCATTAT
TACCCAGTCGTGACCTAACTCATCCAGGTGATTCATATGGAGCAGATGATGGTGTGAAAA
ATGTCAGTAATAAATTGAGTGAAGGAATAAGTCCAGATCATGATGTGTCTAGCTCTGCCA

Figure 4-1

TGGAAGAATTGAGGAGGTTAGTTGAGTCTACCAACAGAATTGACACCAAAAAGCCGGAAG
CTCCAGGTGTACCAACCATTATAATGACACCGACCTTTTAAGATAATATGAGTATATCT
TATTTGATCATCATACAATTCAAATTAAGAAAACTTAGGACCTCAAGGTTCACTCACTGT
TGGCACATCACTGAGATATAGTCAATTCTTTACCCACCACATGTCCTCTCACCAGATTCA
ACAAGTCAAACATGGCCTCGAATCTTTACAAGAGATCAAAAACAACCCTCCGTCTTCCAA
AGATGTCGATCTTGCCAGGGAGATTTACGAATCCATTAGACAAACAGGAACATCTTCAGT
GCAAGGAGGAGCCATTGCGGGAGATAATATTACGTCAGGGGGTAACAATCACTCAATGCA
TAGCCAAGGACCAAGTTCTCCTATTTCAAGTGTTAACAAGAATATCGAAGGATCTACTGG
ATTCGATCATTACAGGACTATGGGATTGAGAGGGTAACCTCTGCATGTTATTCGAAAGCGA
TGATGATGAAAACCAATTATTCAGAGATTAATGGCCGGTCTCCCGCTATCGAAGGATTGGA
TGAACAGGATACTGAGAACTCAATTATTAACAACCAGGAAATCAGTGTACTGAGGGAGT
GTCTAAGACTAATTCACCTTCTAGTCCCCAGGAACTACACTATCTGTTGGGGGATCTAA
TATACCTGGGACAGGAATATCAACCTGTGCCTCTTTGGATATAACTGTAAATGAACTTGA
GGATGCAACTATAAGAAACAGCGACAATATGAAGGGAACTGGCCAATTCCGAAATTACT
TGTTAAGCCGCCACCTAGGGCAAGATCAAGCATTGATCATAGCAATCCATTAAGGGGGC
CACAGGAGGGAAATTAGTCTCACCTGGGATGGAGACTACATTATTCGAGAAGAGTGGTGC
AACCTATCTGTACACCCATCTACTCAACCTGCAAGCGACTTCAATGTAAATGTAAGCAA
TGTCCATCAACCTGCCCCAAGTGTGAATAATGATTACAGAGACAGTGAGGTAACAGTGCT
TAACTTACATAAAGATATTGAGGATAAGTCTGAAATATCTATACAGGATATATATACTT
GATTCTTGGATTTAAGGATGATTATAGGAAATTATTAACAAATTAGATATGGTATTAGA
GATGAAACAAGACATTGACAATCTAAAAAAGAGTAGTGCTAAGGTACAATTGGCATTGTC
AACTATTGAAGGACATCTATCTAGTGTTATGATTGCCATCCCTGGTTCAGGTATTGATT
CACTGGGGAAGAGAAAAAGGATCAGATGAATTCTGACTTAAACCATTTAGGGAGGGA
TCATTGTAGAGCATTTGAGAAGTTACTAATCCTCTAGATGAGTCGTTACTGGCCAATTC
TCCAACAAAACATGTTGCCAAAATAGACAAGAATTGCACTCTTCAGAAAATCAACAAGAA
TGAAACATCTGCAATCAAGTTTGTTCCTCAATGATAGTCATGCAAGCACATCGACCATCAA
ATCAATTATCAGGTCATCTAATCTCGATCAGGATTTGAAGACAAAATTGCTCACAATTCT
ATCCCAAATTAGAGGGACAGAGAATGTTAAAGAATTTTATGAGAAGGTCATGATATTGAT

Figure 4-2

AAAGAATAAGAACTAAATATCACCAATCTACATGCACTATGAGTTGTAATTGTCTTCAGT
AGAATTTAGTTGATTTAATACATACTGTTGTTGATTTGTAATAATTATAAAAACTTAGG
AGCTAAAGGCTACTCAGTCATATACAACATGACTGAGATATTCACCTCTTGATGAGAGCTC
ATGGTCAATCAAAGGAACACTTGATCCGCTAACACCTGATATCTATCCTGATGGGAGACT
CGTGCCCAAAGTTCGGGTTATCGATCCGGGCCTAGGAGATCGCAAGAGTGGGGGATATAT
GTATCTACTTCTCCATGGTGTATAGAAGACAGCGAGAACATGATTAGTCCAAAGGGGAG
AGCATTTGGGGCATTCCCATTAGGAGTGGGTCAATCAACTGAAAACCCAGAAGATTTGTT
TAAGGAAATATTAACCTCTCAATATCGTGACTCGTAGAACTGCTGGATTTAATGAGAAGTT
AGTTTATTATAATACCACACCTATACATTTACTGACCCCCTGGAAAAAGGTGTTGGCATA
TGGAAGCATCTTTAATGCTAATCAGGTCTGCAGTGATACAAGCTCTATACCAATAGATAT
TCCACAAAAGTTTAGACCTGTATATTTGACTGTTACAAAATTATCTGATGATGGCTATTA
TCAGATACCAAAGATGATTCAAGATTTCAAATCGTCAAATTCTGTTGCATTCAACATCCT
TGTGCATCTATCAATGGGTACAAATTTACTTGACCAATCCAAAGACTCTCGATTAAGAAA
TGCTGGGGAACTGTGATTACATTTATGATTCATATTGGGAACTTCAAACGGAAGAGTAA
TAAATCTTATTCAGCGGAATACTGCAAGAGGAAAATAATGAGGCTTGGTTTGATATTCTC
ATTAGGTGCAATTGGTGGCACAAGCTTACATATTAGATGCACAGGTAAGATGAGCAAACG
ACTACAGGCCTACTTAGGATTCAAAGGACTTTATGTTACCCTCTGATGTATGTAAATGA
AGGGCTAAATAAAACACTGTGGAGAAATGAATGTAGAATAGAGAAGGTTCAAGCAGTCTT
ACAGCCATCTGTTCCAAATGAATTTAAGGTATATGATGATGTCATTATTGACAATACCAA
TGGTCTCTTCAAGATTAAATAGGTTATAACCGTAACAAACAGCTAATAAATGGTATTATG
TATTTAAGTGTACACTGATAATTGTGAATAAAATACATTGGGTAAATAACGGTATAGAGT
TAAAATCTAATTGATATGTGGGTAAATGCTTAAACACTTATTAGCTCTATTGATTATCTA
TATCTTGAGTTATCTAATATCAGAGTATCAACATGTAATCAGTTTAAACTTGTTGGATTA
ACGTTCAATTATTATAACCAGAATACACAAATTGTTAAACTTATAATTCTGTTAGATTCA
TTCAAGTTGAACTTATGTAGGGTTAACCAATTATCATTCGAGCAATTATAAAAACTAAG
GATCTAATGTAGTAGGAACCTAAACTCCATCCAGTGAGCTCAAAATCACCACACTCAAAT
ATCAATTTGTCTAGGGCCTGTCTAACTCAAAACAAAGCTCATAACCAGGATCCAGACGAG
TGGGTAAATCTGAATAACTATTAGGAATTGAGATTTTAAATTGATTCTCTCTTAACTCT

Figure 4-3

AAAGTTT TAGTAATATAGCATCAATTCAGCACCATGAACAGAATTAAAGTTATAATAATT
AGTTCTTTGTTATTATCAGATATTACGATTGCACAAATAGGCTGGGATAATTTAACTTCG
ATTGGGGTTATAAGTACTAAGCAGTACAACATAAAATAACTACTCTAAATACTAATCAG
TTGATGGTTATAAAGATGGTTCCCAATATATCGTCAATCATTAAATTGCACTAAACTTGAA
TTGATAAAATATAGAGAGTTAGTCTCAGGGATCATTAGACCAATAAATGAGTCATTAGAA
TTAATGAACTCATACTAATATGAGAGTAGGTTTCAGAGAGATTTATAGGGGCTGTAATA
GCTGGAGTAGCATTAGGAGTGGCAACTGCAGCACAAATAACATCAGGGATTGCCCTACAT
AATTCAATTATGAACAAAAACAGATACAAGAGTTGAGGAAGGCTCTTAGTACTACCAAC
AAAGCAATTGATGAAATAAGGATTGCAGGTGAACGAACATTAATGGCAGTACAAGGTGTA
CAGGATTATATCAATAATATAATTGTCCCTATGCAGGACAACTCCAATGTGATATTTTA
TCATCACAGCTTTCTGTTCATTACTCAGATATTATACAAATATATTAACAGTCTTTGGA
CCAAGTATACGAGATCCTATCACTAGCACGATTTCCGTACAAGCACTTAGTCAAGCATTC
AATGGTAATCTTCAGGCACTACTTGACGGACTAGGATATACTGGGAGAGACTTACATGAC
CTTCTAGAGAGTAAATCTATCACTGGTCAGATAATTCATGCAGATATGACTGATTTGTTT
CTTGTTCTGAGAATTAATTACCCCTCCATAACTGAGATGCAGGGAGTAACAATATATGAA
CTGAATTCAATTACATATCATATTGGGCCTGAAGAGTGGTATACTATTATGCCTGATTTT
ATAGCTGTTCAAGGGTTTTTAATATCTAATTTTGATGAAAGAAAGTGTTCAATAACTAAA
TCGAGTGTAATATGCCAACAAATTCAATTTACCCGATGTCAGCAGAGATGCAAAGATGT
ATTAAGGGCGAAATAAGATTCTGTCCAAGATCTAAGGCAATTGGGACGTTAGTTAATCGG
TTCATATTGACCAAAGGTAATTTAATGGCTAATTGTCTGGGAATTATATGCAGATGTTAT
ACCTCAGGCCAAGTTATAACACAGGACCCAGTAAGTTAATTACAATAATATCACAAGAG
GAGTGCAAAGAAGTCGGTGTTGATGGTATCCGTATTATGGTAGGACCTAGAAAATTACCA
GATATTACCTTTAATGCTAGGTTAGAAATTGGTGTACCGATATCATTAAAGCAAATTAGAT
GTCGGAAATGATTTAGCAATTGCTTCAGCTAAGCTTAATAATTCCAAAGCATTGTTAGAG
CAATCAGATAAGATTCTGGGTTCTATGTCTAAGTTGGATTCTATTAATTCAAGAATTATA
GGATTAATCTTAGCAATCATGATAATCTTTATAATTATTGTTACCATTATCTGGATCATA
TATAAAAATTGTAGAAATAAAGATACTAAATTCAGTACTTCAATTGAACCGCTCTACATA
CCCCCTTCTTATAACTCACCTCATAGTGTGGTCAAGTCTATTTGAGTACTGACCATATGA

Figure 4-4

TTTACTGTAATAAGTCCAGTGGAAGTATCAATTGACAATACTGGTAGTATAATGAATATT
GAATATATAATATACTCTCTTAAATTGGATAGTGATAAAGAGTTATAGATGATTGCAATC
ATTTTAATATAATTATATATTGATTTGATTACCTGGTATAATTCTTATGCAATTGAATTA
TGTGTCATCAATTAATAGCTTAATAGCACTGTTTTATACACTTATGTTGATAGATAGATG
TGTTATATTGTAATCAAGGATTTAGTATCTAGAAGAGGAAAGAGTTCAATTGGTTGTAA
TTGGTTATTGTGTATTCAATTAGAAAAAACTTAGGAATCCATGTTAATAAAAACTCATT
TCATGGAGTCCAATAATGTTAAATATTACAAGGATTCTAACCGATACTTTGGTAAAATAT
TAGATGAACACAAAACAATTAATAGTCAATTGTACAGCTTAAGTATTAAAGTAATTACCA
TTATTGCCATAATTGTAAGCCTAATTGCAACAATAATGACTATTATTAATGCCACAAGTG
GGAGGACTGCCCTAAACAGTAATACAGACATACTGCTTAGCCAAAGAGATGAGATTCATA
ATATCCAAGAAATGATATTTGATCGTATTTATCCTTTGATAAATGCTATGAGTACAGAGT
TAGGACTTCATATTCCTACCTTATTGGATGAACTTACTAAAGCGATTGACCAAAAGATTA
AAATAATGAATCCCCCTATTGACACTGTGACGTCTGATCTTAATTGGTGCATCAAACCCC
CTAACGGAATTATTATAGACCCGAAGGGTTATTGTGAGAGTATGGAATTGTCCAAAACCT
ATAAATTACTACTTGACCAATTAGATGTCTTAAGAAAGAAATCACTCATTATAAATAGAA
AGAATATTAACCAGTGTCAATTAGTTGATGATTCAAAGATCATTTTTGCTACTGTCAACA
TACAATCTACACCGAGGTTTTTGAATTTTGGTCACACAGTCAGCAATCAACGTATAACAT
TTGGTCAAGGAACATATAGTAGTACTTATGTTATAACTATCCAAGAAGATGGGATAACTG
ATGTTCAATATCGAGTTTTTGAATTCGGGTATATCTCTGATCAGTTTGGTGTTCCTT
CCTTAATAGTATCCAGAGTGTTGCCTATACGCATGCTATTAGGAATGGAATCCTGTACCT
TGACAAGTGACAGACTAGGTGGGTATTTCTTGTGTATGAATACACTGACACGATCTATAT
ATGATTATGTTAGCATAAGGGATTTGAAATCATTATATATAACACTCCCTCATTATGGTA
AGGTTAATTATACTTACTTTGATTTTGGTAAGATCAGAAGCCCACATGAAATAGATAAAA
TTTGGTTAACATCTGAGAGGGGCCAAATTATTTCTGGTTATTTTGCAGCATTGTGTACCA
TTACAATTCGGAATTATAATAATTATCCCTACAAATGTTTAAATAATCCATGCTTTGACA
ACTCTGAGAATTACTGTAGAGGGTGGTATAAAAACATAACAGGTAAGTACGATGTTCCGA
TATTAGCATACCTATTAGTTGAAATGTATGATGAAGAAGGACCTTTAATTACACTTGTAG
CAATCCCGCCTTACAATTATACAGCTCCATCTCATAATTCTCTTTACTATGATGATAAAA

Figure 4-5

TCAATAAATTGATAATGACTACATCTCACATAGGTCATATTCAAGTTAATGAGGTGCATG
AGGTGATTGTTGGCGATAATTTAAAGGCTATCCTCCTAAACAGATTATCTGATGAACATC
CTAATCTTACTGCCTGTAGACTCAATCAGGGCATTAAAGGAGCAGTACAGGTCTGACGGAA
CAATAATTTCAAATTCTGCACTTATTGATATACAAGAACGGATGTATATTACAATTAAAG
CTGTTCCACCAGTGGGTAACTATAACTTTACAGTTGAATTGCATTCTAGATCAAACACAT
CTTATCTATTGTTACCAAAAACAGTTTAATGCTAAATACGACAAATTACATCTTGAGTGCT
TTAGCTGGGACAAATCTTGGTGGTGCGCCTTGATACCTCAGTTTTTCATTAAGTTGGAATG
AATCCCTTTCTGTTGATACTGCTATTTTTAATTTAATAAGTTGTAAATGAATATGTCAAC
TGATAGTTGATAGTTGTCAAAACATCAGCTAATTGAGATTAAAGAAATAAAAAAATGAAA
TTATCAAGATTTGACTAGATGTATACTCAAGCTAAATTACAAAAAAGTTAGGAGTCAGAG
ACTTCGTTGCAATGGAGCAGTCAGACTACCAAGATATTCTATATCCTGAGGTACATCTTA
ACAGTCCTATAGTAATCTCTAAATTAGTAGGTATTTTAGAATATGCCCCGAATTGCTCACA
ATCAACAACATCAGACCATAACAATTATCAAGAATATTCAATTTAGATTAAAGAAATGGCT
TTAATAGTCCAAGGATACAGACACTATCAACTATGGGTGAAATCATCAACAAAATTTAAAA
GCAAACACCCCAATTATTTACACATACCTTACCCCGAATGTAACCAAAAGCTATTTTCGAA
TAGTAGATCCAGAACTGACATCAAAATTGGAATCTCTTCTGAACAAAGGTGATACACTGT
ATCTCAAAATTCGGTCAGATATCATAAAATGCTTTGATAGATTGAAAATGAAGATGAACA
TAAGGAATGATCTTCTTAATGACAATAGTCAATTAATTCTGGATCTTCCTTTAATTCTCA
AAGGATCTCAGTGGTTCTTCCCGTTTTTATTTTGGTTTTTCGATTAAAACTGAGACTAGAA
GCTGTATCCGACAAAATCAAAAAGCTCGTGTTAGATCACAATATCGGCCTCACTTATCAG
AGACTAAGAGAATTACATTGGTTGTTACATCTGATCTAATTACGATATTTGATCATATTA
ATAAATGTATATTTTATCTGACTTTTGAGATGTTGTTAATGTATTGCGATGTGGTAGAAG
GTAGATTAAATGACTGAAACAGCTATGAGCTTGGATTGTCGATTTATCAATCTATTGCCAA
GAGTGCAATATATGTGGGATTTGCTAGATGGAATGTTTGAAAGTTTAGGTAATCAATTAT
ATTCAGTTATTGCATTGTTAGAGCCTCTTCTCTTGCTTATTTGCAATTAATAGATGCAG
ATCCACAGATTCGGGGAACATTCTTGCACTACTGTTTTTCAGAGTTAGAAGAAATTATAT
TTGACAAGTCTCCTTTTGATCCTTTTGTGTATGAAAATTTAATTAATGGACTAGATTATA
TTTATTTGACAGATGATATTCATCTAACTGCAGAAGTTTTTTCTTTTTTTAGGAGCTTTG

Figure 4-6

GTCATCCTTTTTTAGAAGCACAAAATGCTGCTAATAATGTGAGGAAGTATATGAATAAGC
CTAAAGTGATCTCATACCAGACTCTAATGCAAGGACATGCGATTTTCTGTGGTATTATAA
TAAATGGATTTAGAGATCGCCATGGGGGAACATGGCCTCCTGTAGAGTTACCAAATCATG
CATCTGCTGTAATTAGAAATGCCCAGCTATCTGGAGAAGGGTTAACATCTGAACAATGTG
CTCAACACTGGAGATCCTTTTGTGGATTTAAATTTAAATGTTTTATGCCACTGAGTCTAG
ATAGTGACCTTACAATGTACCTTCGGGACAAGGCGTTGTCACCTGTCAAAAGTGAGTGGG
ATTCTGTTTATGCGAAAGAGTATTTAAGATACAATCCAGGATTACCTACAAGCTCTAGAA
GACTAGTGAATGTATTCTTAGAAGATGATAAGTTTGATCCATATGAAATGATCATGTACG
TGATAAATGGTGATTACTTAAGAGACAAAGAGTTTAATCTTTCATACAGTCTTAAAGAGA
AAGAGATCAAAGAGGTAGGTGCGATTGTTCCGCAAAAATGACTTATAAAATGAGGGCTTGCC
AAGTAATAGCTGAAAACCTGATTGCCAATGGAGTAGGGAAGTTCTTCAAAGATAATGGAA
TGGCAAAAGATGAACATAAACTAACTAAAACGTTACACAAATTAGCCATTTTCAGGTGTAC
CTAAAGATAATTCTCAACTTTATTTAGATGAATGCTGGGAGCAAGTAGTTCGACAATGCT
CAAGTAGTACACAGATAGGAGAACAGACTATGAATTCACAATCGAAGAGGGCAATTGAAT
CAAAGTCTTCTAGATCACATCGAAATAATAGGGATATCTTAAGGGGCAGGAGAGATTTGA
ATAAACAGATAAAGTACCCTTCCAACACCGAGTATTATGAGACTATTAGTAGTTTCATAA
CTACTGACCTTAAAAAGTACTGTCTTAATTGGCGATATGAATCAAGTAGTGTGTTTGCAG
AGAGACTTAATGAAATTTATGGATTGCCCTGGGTTTTTTCAGTGGCTTCACAAAATATTGG
AGAAATCTGTTTTATACGTTAGCGATCCGTCTAGTCCACCTGATTTTGATCGACATATCG
ATATAGAATCAGTTCCGAATGACCATATCTTTATTAAGTACCCGATGGGTGGAATAGAGG
GGTTCTGTCAAAAATTATGGACTATTAGTACGATTCCATTCCTATATTTAGCAGCTTTTG
ATACAGGAGTTAGAATCTCATCATTGGTTTCAGGGCGATAATCAGGCAATTGCAGTGACCA
AAAGAGTTCCATCATCTTGGAGTTACTCAAAGAAAAAGGAAGAATCAACTAAAATAACAA
CACAATATTTCTTAATTTAAGACAACGCTTACACGACATAGGTCATGAATTTAAAGCAA
ATGAGACTATTATATCCTCTCATTTCTTTGTTTACTCTAAAGGTATTTATTACGATGGAA
TACTTCTCTCTCAAGCACTTAAAAGTATTGCAAGATGTGTTTTTTGGTCTGAAACAATTG
TTGATGAAACTAGATCAGCTTGCAGTAATATATCTACGACACTTGCAAAGGCAATTGAAA
GGGGTTATGATAAATTTGTGGCATATGCTATTAATATTTATAAAACAATACATCAAGTTT

Figure 4-7

TGATTGCATTATCTTTTACGATTAATCCTACTATGACACCAGACATTACAGAACCTTTCT
ACAAAAGTTTGGATCTACTTAAAAATCTAGTTCTAATCCCTGCACCATTGGGAGGCATGA
ATTATATGAACATGAGCAGGTTATTTGTTAGGAACATAGGTGACCCCATTACTGCTTCAT
TTGCTGATATAAAGCGCATGATCGAATGTGGGTATTAGGATGTAGCATTCTGTACACAGA
TAATGTACCAAAAATGTGGTTCCTCTAAATACTTAGACTGGGCTAGTGATCCTTACTCAA
TAAACCTTCCTTATAGCCAAAGTATGACCAAGGTCTTAAAAAATGTAACAGCAAGATATG
TACTTATGCATAGCCCCAATCCTATGCTCAAAGATTTGTTCCATGAAAAGTCACAAGAAG
AAGATGAAATCCTTGCTGAATTTCTGTTAGACCGACACTTAATAATCCCTAGAGCAGCAC
ACGAAATTTTATCAAATTCAGTGACAGGTGCTAGGGAATCTATAGCAGGTATGCTTGACA
CTACTAAGGGTTTAATCCGTGCTAGTATGTCAAGAGGTGGGCTGACATCATCACTAGTTT
TAAAATTATCAACATATGACTACCAACAGTTTAGAACGTGTCTTGAATGGCTTTATGCTC
CTATCACGGGAATTGCTGTAAGCGTTGATTCTTGTTCTGTATTCTTAGCTAAGACCATCC
GAAAGAGAATGTGGGTTCATCTAACTAAGGGAAGGGAGATTTACGGGTGGAGGTACCTG
ACATTTTGAATGCATGCAAAACAATATAATTATTGATCATGAAGATTGTTACTCATGTA
TTCAAGGATCAAAATATTATACATGGTTTTTTGTACCTTCAAATGTCAACTCGATCAGA
TAAATAAGTCAACAAATTCTCTCCGAGTACCTTATGTTGGATCAACAACCTGAAGAAAGGA
GTGATATGAAGTTGTCATATGTGAGGTCACCAAGTAGACCACTTAAAGCAGCAGTCCGAA
TTGCAGCAGTATATACATGGGCTTATGGTGATGATGATTTATCCTGGCATGAGGCTTGGT
ATTTGGCAAGGACTAGGGCAAATATTACATTTGATGAACTCAAATTAATAACACCTATAG
CTACATCTACTAATTTGGCACATAGGTTGAGAGATAGAAGTACTCAAGTTAAATATTCAG
GGACTTCCTTAGTAAGAGTGGCACGCTATACAACAATATCTAATGATAACATGTCGTTCA
CTATTAACAACAGGAAAGTCGATACTAATTTGTCTACCAGCAAGGGATGTTATTAGGCT
TGAGTATACTCGAATACATATTCAGATACTGTACAAGTACTGGACAATCAAACACTGTAA
TTCACCTACATGCAGATGTTAATTGTTGTATAGTACAGATGACTGATCAGCCTTATACGC
CAAGCTTAACTAAGAAGCTACCTGATATCAAACCCATCAATAATAAATTGATATATGATC
CGGCTCCTATAATTGATACTGATGCAGCTAGGTTGTATTCTCAAAAATATCTGTACATC
TAATAGATTTTCCAAGTTGGTCAACTACTCAGCTTAACACAGTGTTGGCAAAAGTGGTAG
CAGTATCTATAGTAGAATTGATCACAAAAGCGAGTAAAGACCATCTCAATGAGATAATAG

Figure 4-8

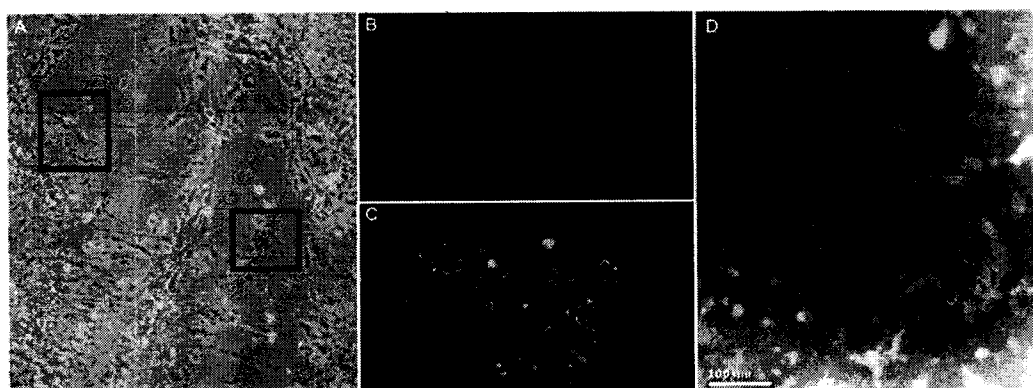
CGGTTGTTGGTGATGATGATATCAATAGCTTTATTACAGAATTTCTACTTGTTGATCCAC
GTTTGTTTACACTATACTTAGGCCAATACATGTCTTTACAATGGGCATATGAAATCCATT
ATCATAGACCAGTGGGCAAGTACCAGATGGCCGAAGTATTACATAATTTGCTGTCAAGAG
CTAGTAGAGGCATATTTAGCATATTGACCAATGCCTTTAGCCATCCCCGGGTCTATAAAA
GATTCTGGGAATGTGGTTTATTGGAGCCTATTTATGGGCCTTATATAGGAAGTCAAAATC
TACATAGTGCAGTGATTGATTATATCTATAATGCATATCTTACTTATTTGGATGCTTATT
TATCTGATCAAGTAGATGATACTGATATTATAATCTGTGAAACAGAGGAGACATGTTTAG
CAAATAGAATTGACAATTACCAAAGTAGACACCTAGCTGTACTCATAGACTTGTACTGCG
ATTCCACTAGATGCCCCAATATAAAAAGGGTCAGATACAATTATGAGAAATTCAATCCTTA
GATCCTTCATTGATAATGAGAGGAAAACAAACCCACTCGGTTTGACATGGAATCTTGATC
CATTACTTGTGGATCACTTTAGCTGTTCTATTACATATCTAAGGAGAGGTATTATTAAAC
AGATGAGATTAAGATTTGACCCAAGCGTATCTCTTGAATTATCTAGAATGATTAAACCTG
ATGTGATTTATCAAGCACCTAAAGTTCCGTCCTCATGGGCTCTTATAGATATCAACCCTG
AAGTAAATGACCTTAATACAATTTTTGGAGAGCTTAATAGCAAGTGGAAGACATCCCTA
TAGGACAAATCAGAATCCAAAATTATGAAATACATGCATATAGGAGGATTGGAGTTAATT
CAACTGCATGTTATAAGGCTTTAGAGCTATTATCTGTTCTAAATCGGTTTCATGTCTAACC
CATCAGGTGCATTGTTTTAGGTGAAGGGGCAGGATCGATGCTGGTCACATATCGTGCCT
TTATTCCATTCAAGACAATTTATTATAATAGTGGTATTTTCAGTTCAAAATGTTTCAGGGTC
AGAGAGAATTAAGTCTATATCCATCTGAAGTGGCACTAGTTGATAACAAAAATCGCTTGG
CTAATGACCCTAATATCAAAGTCTTGTTTAATGGTAAGCCAGAGTCTACATGGGTTGGAA
ATATTGACTGTTTTGCTTATATTCTTAGCCATATTGAGACTTCAAGCTTGACATTGATAC
ATAGTGATATTGAGTCCAGCTTGAGCAAGACAAAGAATAAAATTCTTGAGGAGCTGTGCC
ATATTCTGTCAATGGCACTCATTTTGGGAAAGATCGGATCTTTATTAGTTGTTAAGTTGC
TACCAAGGGTCAGTGATTATACGTATTCATTTTGCAAATATGCATCAGAGTTCTATCAAC
AAAACCTTCTTGTTCTGCCTAGATTTAGTAACATGTCATCATCTGAGGTTTACTACATAG
GAATTCACCTTAATACAAATCGATTGATTGACCCTGATAGAATAGTACAATACATAATTA
GAAATTTACAACCTACTCCAGTTACATTTTTATCCTACATTTTGAACCTAAGTATCGAA
ATAATATGGTTACAAATTATGGACTATGCTTGTCAGACGGACACAAAAGTGATTACTTGT

Figure 4-9

CATCAATTACAAAATAGAGAGTGTTCTTCTGTCATGTGGGTTAGAATTGAACGGACCTA
AGATTATACAGCAATTATCAGGACATGACTATGCCAGTGGAGAGACTAGTCTGGAATCAA
GTATAATGATATTAGTTAGAGAATATCTTAATGCAACTATACAAGGCCGGGAAACATTAG
GCTTGTTTTACCTTACCCGGTCCTTCATGAGAGTCAGTTAAGAGAAATCAATAAGTGTA
TTGTATTGAAGTATATTGTATATCTGCTCTTTTATTCAAACCTACATTATCTAGTAAAC
AAATAATGAGTAATCTTAGAAAAGGAATATTGATGTATGATTTGAGAGATGAGTTTTTCA
TATCAAGATTGTCAGCAAATTACAAGAAAAAGTAATGTCACAGGAAGTTAAGACTACCT
GGATATTTAATATTGATACTCCGACACGAAAGGCGTTATATAAGTTAGTAGGTTACTCAT
TAATAATTAATCACATATGAAGGTTGGGCATGGTTATTCATTTTTTAAGGAGTAAGATAA
GACTTGATATATGATAACTGATTAAACATTACCTCTGAATTGAAGGATTGCTCAATTACA
TGGTTTTTGAGTAATTGAGATTTTATTCCAATTAGTACAATTAGAAAAAACTTCAACAGT
TGATTGAGCCTTAATTTACTCCATACTAGCTATATTTATAAGCTCGGATAAAACTTTGGT
TTGAAATTATAACAGTCATACCAATCTATCAAGGAAACACAATTGATGTCTAGTATGAAG
TTCATATTTATATGTTTTTAATCTTATACCCACTCTAATTAGTTCCTATTTAAGAATTAA
ATTATAGATGTTAACATGTTATATAATGGGAACCATCAATGCTGCTATTGTTGGTAACTA
TAGGCATTGTATTAGATAATGTTTATTTCTTAGAAATGTGCAATCTCATACGTCGGACCC
CTCAGCCTCCCCCTTATAGTTGCGTGATTTGAAAAAACACAAAAATAATCATGAATGGG
TGTACGTACCTATAGCTTTCTTTGTCTGGT

Figure 5.

MA(S,I)L		
761U	MSCLLESLAAFKHEBEQPTAPSSGSGIAIKGLKNTIIVFVPGDTVITTRSNLLFRLVYIIGNPDTPPLSTOTGATISLLPLEVFSFGQIQTQIACDPAVFK	100
776U	T.....	100
M252A	K.....T.....	100
CdiPV	A.....K.....TL.....T.....D.....FLA.....R.....I.....HV.....V.....LI.....SS.....V.....R.....D.....RLV.....D.....KINGPK.....IL.....I.....S.....	100
DmoPV	AT.....L.....L.....NKDRTPLIA.....P.....I.....HV.....V.....LI.....SS.....V.....R.....D.....RLA.....D.....YISGPK.....VM.....I.....S.....	100
NeaPV	AT.....L.....L.....NKDKFIT.....P.....I.....HI.....I.....SS.....V.....R.....D.....RLA.....D.....YISGPK.....L.....GI.....S.....	100
PprPV	AT.....K.....L.....L.....NKDKAPTA.....R.....I.....V.....I.....SS.....I.....R.....D.....FLA.....D.....ING.....KH.....VM.....M.....S.....	100
RinPV	A.....K.....L.....L.....AFDKFLAA.....P.....I.....HV.....V.....LI.....SS.....V.....R.....D.....KRV.....D.....ISGPK.....L.....I.....S.....	100
PdiPV	A.....K.....L.....L.....KT.....FLA.....R.....I.....HV.....V.....LI.....SS.....V.....R.....D.....RMV.....D.....EVSQPK.....L.....I.....S.....	100
QxW(I,V)xxxK(A,C)XT		
761U	LVVEIPEAGNPGELTFASRSINLRKQAQYFKLAENQDQYVLSGFENFNDDDITSSPEIFPNYILACVLA.....VWILLARAVTAPDTAAEAENRRRIKLM	200
776U	R.....	200
M252A	SINACG.....AS.....SE.....DEF.....IVDEGSKAQDUGWL.....KDIV.....IEVDNA.....Q.....IL.....I.....	200
CdiPV	SINACG.....AS.....SE.....DEF.....IVDEGSKAQDUGWL.....KDIV.....IEVDNA.....Q.....IL.....I.....	200
DmoPV	QSEKESG.....A.....MEDE.....OD.....STQAGEEGDTFGTHW.....KEIVIEVQD.....E.....IL.....I.....	200
NeaPV	L.....VQSDQSGS.....T.....MEDE.....G.....SHDPPSSDQSRKQW.....KEIS.....IEVQD.....G.....M.....PTI.....I.....V.....	200
PprPV	VQSTREGSG.....AD.....NE.....OM.....STEGPSSG.....KKRINW.....KEIL.....IEVQD.....E.....ML.....I.....	200
RinPV	VQSDRTQSG.....TSM.....DE.....DK.....TYE.....F.....GEFEGYW.....KDIQ.....IEIQD.....G.....M.....TI.....I.....	200
PdiPV	SINACG.....AS.....AE.....DEF.....GTHDESSPDHNRQWL.....KDII.....IEVDNA.....Q.....IL.....I.....	200
FxxTLLR(K)G(A)LLVxT		
761U	QFVKVDELRLSKGWLHVRNKIACDITIREFMVALVLDIKESFGTRPRAEMICIDNYIVEAGLAS.....FLITRFQITETRYPALAIHEFSGELATIEGLM	300
776U		300
M252A	V.....F.....MN.....I.....I.....K.....E.....LSL.....I.....NK.....	300
CdiPV	V.....F.....D.....A.....K.....E.....LSL.....I.....T.....NK.....	300
DmoPV	V.....F.....ERR.....V.....K.....E.....LSL.....I.....T.....NK.....	300
NeaPV	I.....F.....D.....A.....K.....E.....LSL.....S.....I.....T.....NK.....	300
PprPV	I.....F.....D.....T.....K.....V.....E.....LSL.....I.....T.....NK.....	300
RinPV	I.....F.....D.....T.....K.....V.....E.....LSL.....I.....T.....NK.....	300
PdiPV	I.....F.....MN.....I.....I.....K.....E.....LSL.....I.....NK.....	300
FxxxxYTxSQAAMG		
761U	KLYQSMGEMAPYMYILENSIQTK.....FAGSYPLMSYAM.....VGVELRSMGSLNPTFSFPTTYFRQIQEMVRRSSGMVNSGFARELGLSDHETQLVSGQVNS	400
776U		400
M252A		400
CdiPV	N.....Q.....T.....V.....NK.....	400
DmoPV	N.....Q.....T.....V.....NK.....	400
NeaPV	N.....Q.....T.....V.....NK.....	400
PprPV	N.....Q.....L.....V.....NK.....	400
RinPV	N.....Q.....L.....V.....NK.....	400
PdiPV	V.....Q.....T.....V.....NK.....	400
761U	GCESGIPKFGFRANFTTFLTKFNINRGEDQSGNISGLPGL-LPSRLNLRGDSYGINS--VKNVSDKINEGVGPDHVVSSGAM--ELKRLVEST	495
776U	D.....	495
M252A	K.....S.....V.....-.....THP.....AED.....N.....S.....IS.....	495
CdiPV	TT.DRTIKAT.FKQSQI...HSEES--EVANG.PPT.NKRSNQ-GGQRYPIHFS.E--RLP.YTEAVN.SEWS.SRAYDTQIQDQGN--DD.KSM.AI	493
DmoPV	ANDRANRAI.FKQ.QIS...HDFG--ASTEG--NI.PANE-GDGETMKR.GNIATPK.TSIPQT.TT.SKDTLDIDQ.DNIDDFISQKSA.AL	493
NeaPV	TT.DK.SRAV.F.QAQS...HDKS--ENELPGLGKEERVKQ-GGGEARESYRET--SRASDARAAPPTSMPL-DITTA.E.QQPEQS..SADAL	495
PprPV	A.DERTARGET.F.QAQS...QH.TG--EGESSAPATRE.VKAAIPNG.EEFDRKOTPS.RPP.--STP.QI.L.IMPE.EVSKR.QQNER.AQ.SA.AL	495
RinPV	TSDRHNKTS.FKQQS...R.DOG--SEAQSASKKEARA.Q-VKKEITS.RSD-KHKE.TKEP..SS-AMTLIDVDTTLEAULTDPL.SKSA.AL	495
PdiPV	TT.DRTIKAT.FKQSQI...HSEP--EAFNQRLFP.TMKSEFQ-GGDEYS.QLI.D--RL..YTSVQ.SWD.SRQITQLTQEGDHD--NDQSMOGL	493
761U	NRIDT----KQPEASGVNH--YNDTLLK	519
776U		519
M252A	K.....P.....	519
CdiPV	AKMRMLTKMLS..GT.KDNQPV.S.YE..N	523
DmoPV	AKMRAMKILEDDQSPRO..A.V...K...G	523
NeaPV	L.LQAMAGILEEQGSDTL.PEV...R...D	525
PprPV	F.LQAMAGILEDDQSKNSQV...K...G	525
RinPV	L.LQAMAGILEDDTLGMSGLRA...K...N	525
PdiPV	AKMRQGTILN.SDTN.EVSPAH..R...S	523

FIGURE 6

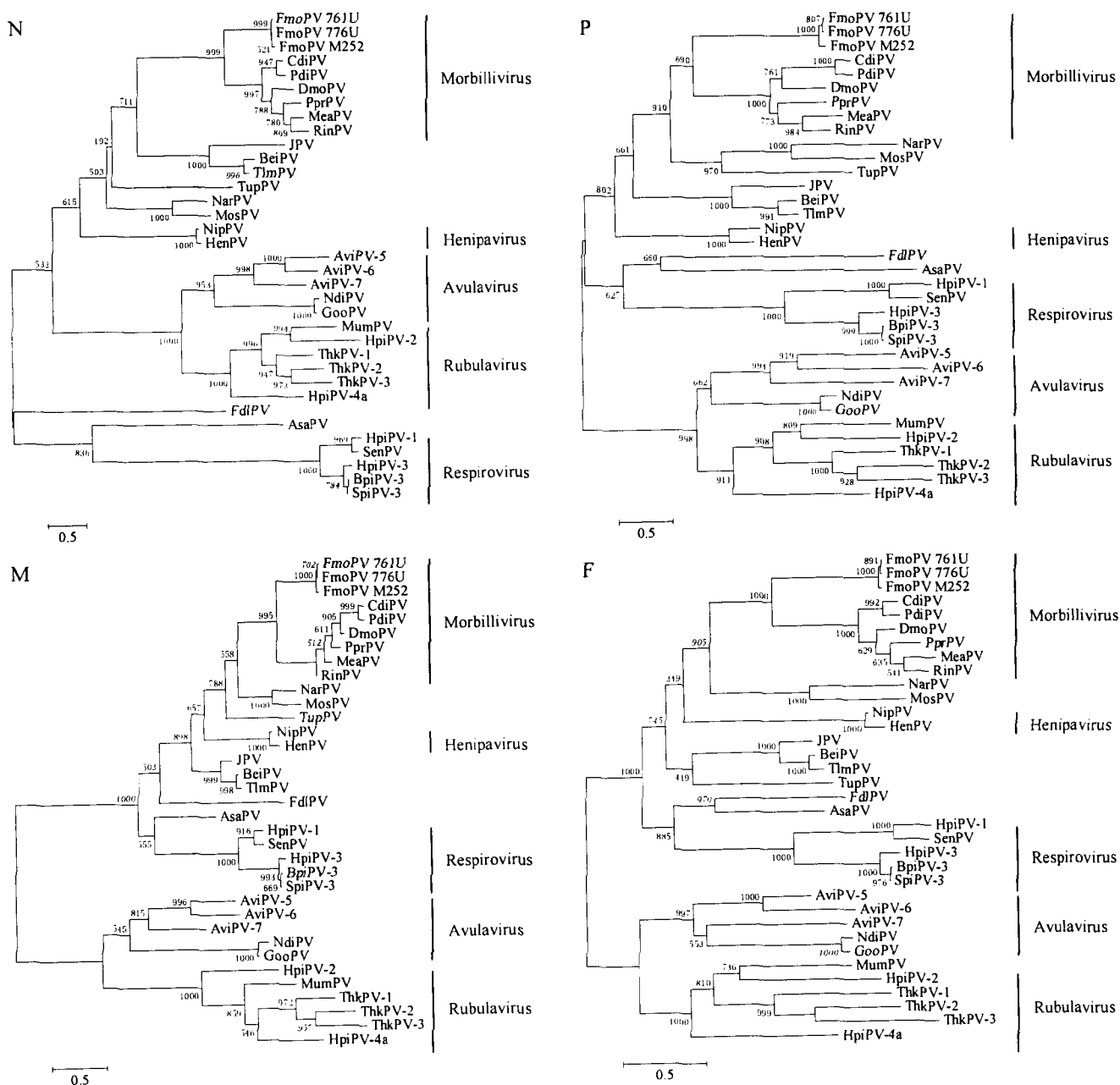


FIGURE 7

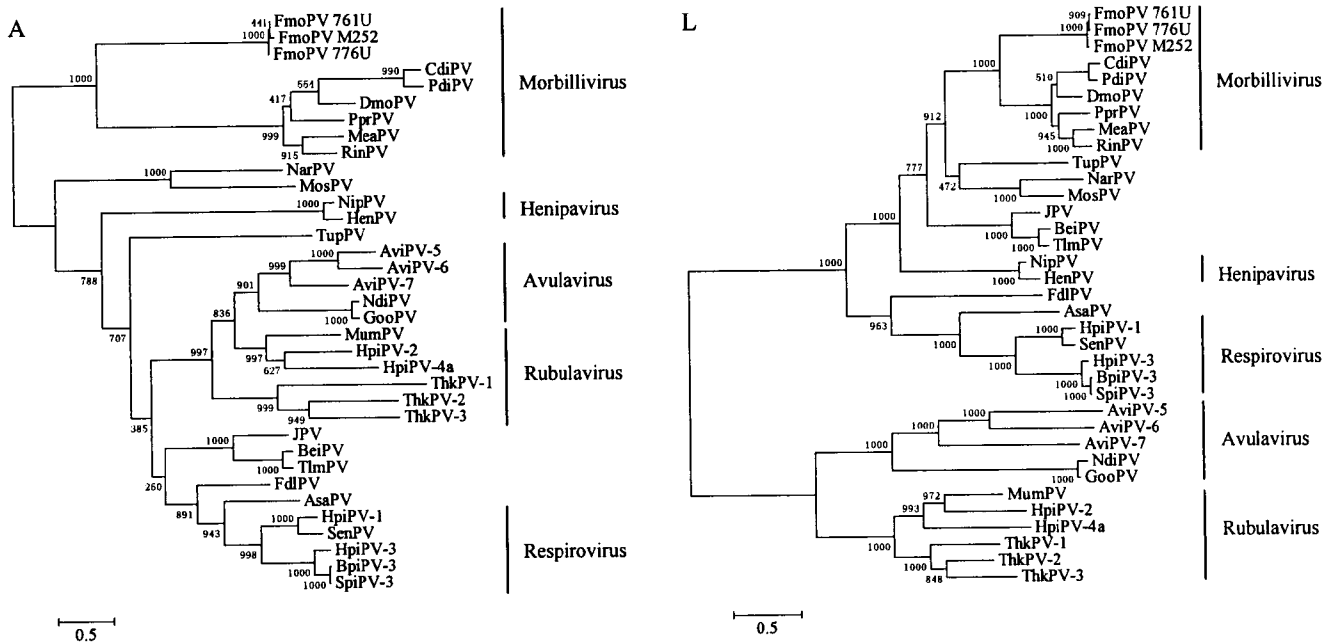


FIGURE 7-1

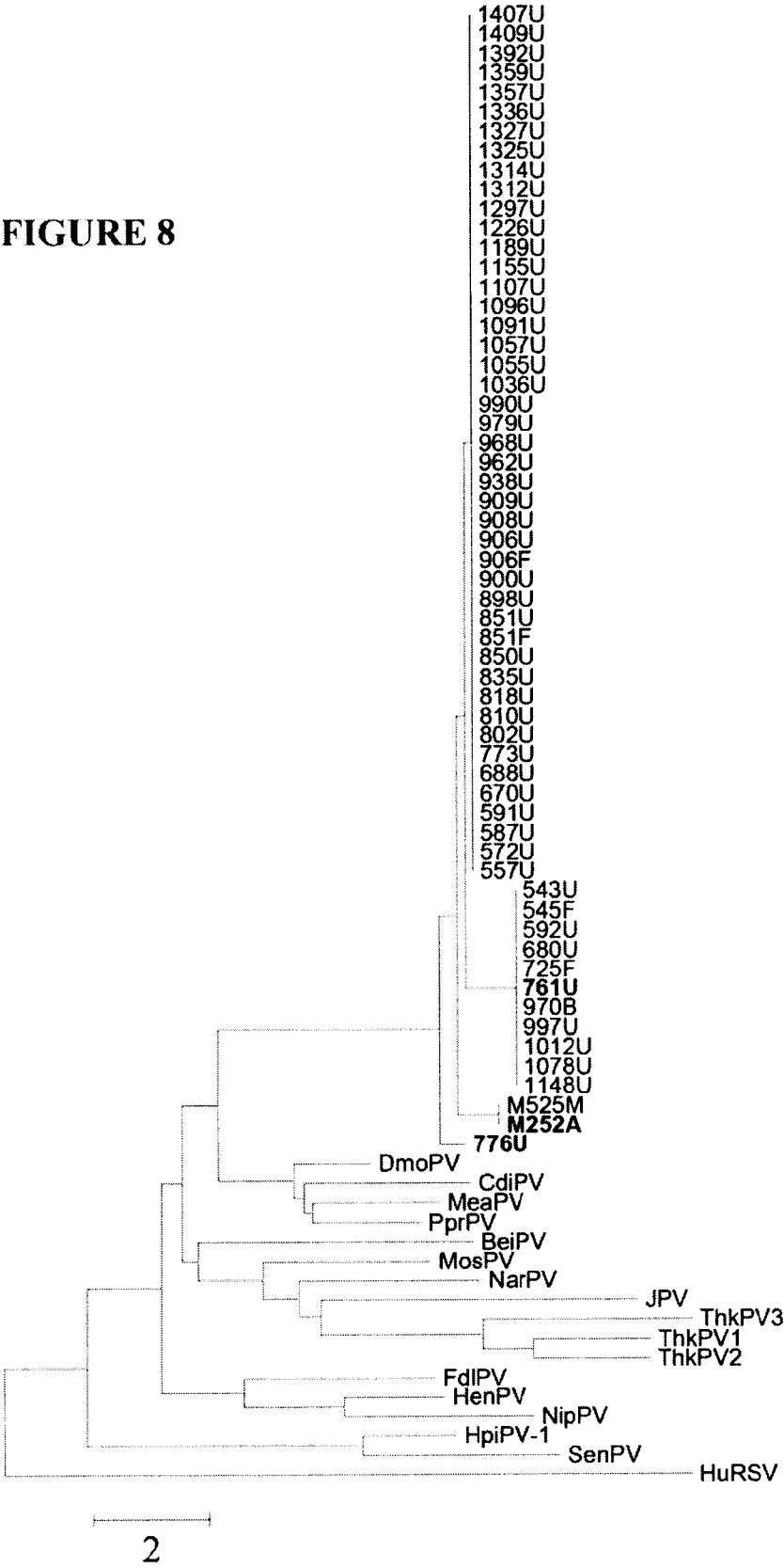


FIGURE 9

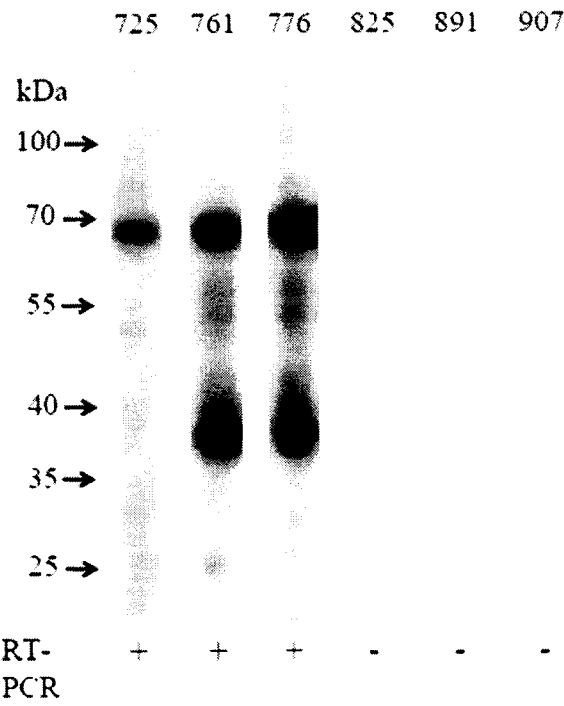


FIGURE 10

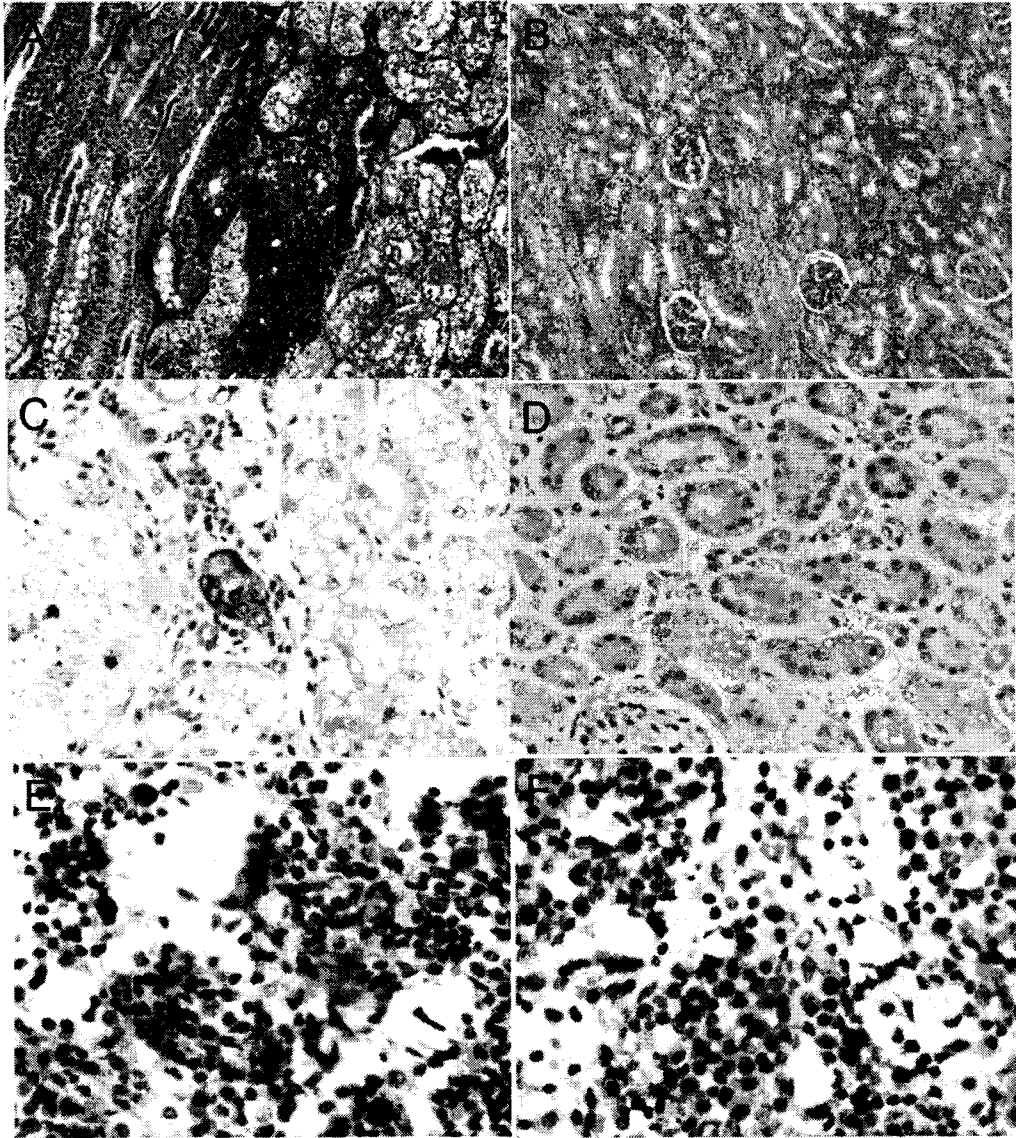


FIGURE 11

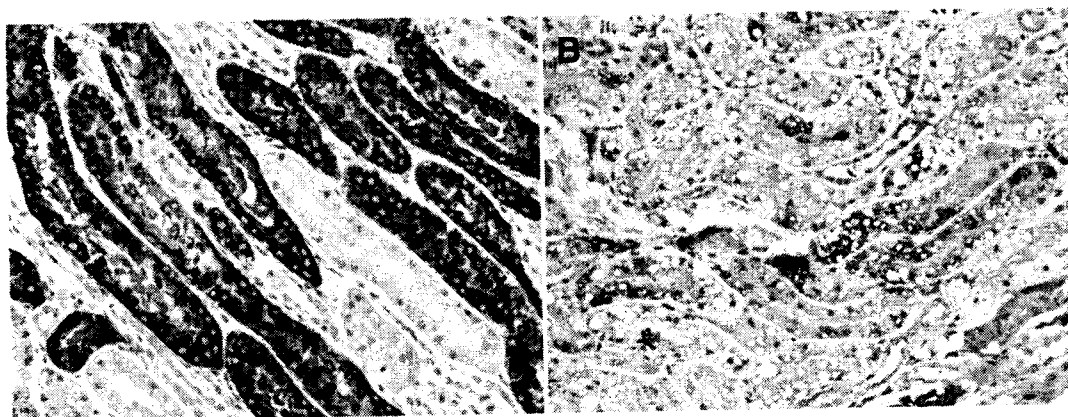
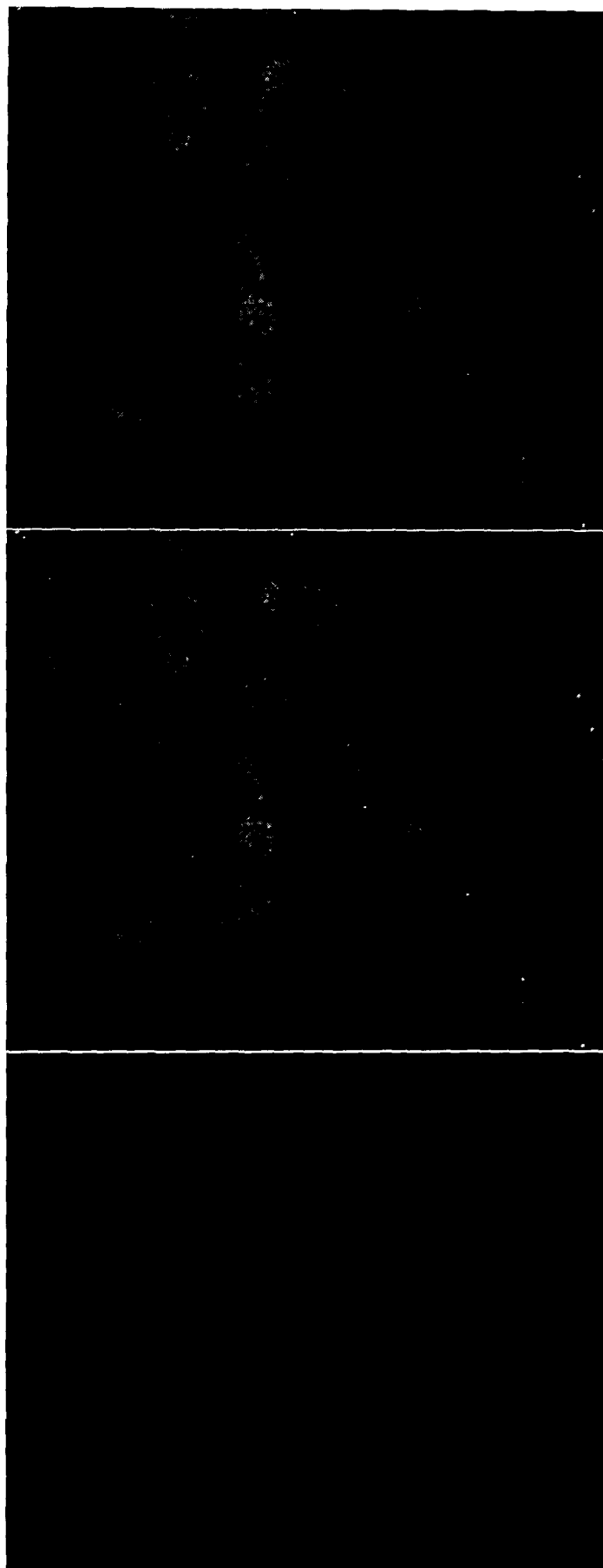


FIGURE 12



Mouse anti-Human Myeloid/Histocyte Antigen and Texas-red conjugated Goat Anti-mouse IgG (JacksonImmunoResearch, West Grove, PA, USA)	Guinea Pig anti-NP and FITC Conjugated Rabbit anti-Guinea Pig IgG (Invitrogen, Camarillo CA, USA)	Merged image
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2013/000060

A. CLASSIFICATION OF SUBJECT MATTER**See the extra sheet**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: C12N, C07K, C12Q, A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CRRSABS, CNABS, DWPI, SIPOABS, CNKI, BA, CA, PUBMED, Genbank, EMBL, DDBJ: feline morbillivirus, morbillivirus, feline, cat, cats, antibody, antibodies, sequence search on the sequence of Figure 2 (SEQ ID NO: 1), sequence search on the sequence of CAGAGACTTAATGAAATTTATGG, sequence search on the sequence of CCACCCATCGGGTACTT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	Woo PC, et al., "Feline morbillivirus, a previously undescribed paramyxovirus associated with tubulointerstitialnephritis in domestic cats", Proc Natl Acad Sci U S A., vol. 109, no. 14, 03 Apr. 2012 (03.04.2012), pages 5435-5440, see the whole document	1
PX	DATABASE Genbank [Online], Database accession no. JQ411014, 17 Apr. 2012 (17.04.2012) , "Feline morbillivirus strain 761U, completely genome" , see the whole document	1
A	CN1408851A(VIROLOGY INST CHINA ACAD PREVENTIVE MED),	1; 7-9 (partially)

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 09 Apr. 2013 (09.04.2013)	Date of mailing of the international search report 25 Apr. 2013 (25.04.2013)
Name and mailing address of the ISA/CN The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451	Authorized officer SONG Zhigang Telephone No. (86-10)62411078

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2013/000060

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	09 Apr. 2003 (09.04.2003), see the whole document CN1437613A(AMERICAN CYANAMID CO),	1; 7-9 (partially)
A	20 Aug. 2003 (20.08.2003), see the whole document CN101852806A(SYSMEX CORP),	15, 20
A	06 Oct. 2010 (06.10.2010), see the whole document CN102140547A(UNIV WUHAN),	21-22; 25 (partially)
A	03 Aug. 2011 (03.08.2011), see the whole document CN1904069A(ZHEJIANG CENT DISEASE CONTROL&PREVENTI),	21-22; 25 (partially)
	31 Jan. 2007(31.01.2007), see the whole document	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2013/000060

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item item1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of:

a. a sequence listing filed or furnished

☐ on paper

☒ in electronic form

b. time of filing or furnishing

☐ contained in the application as filed

☐ filed together with the application in electronic form

☒ furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

The nucleotide sequences of SEQ ID NOs: 4-6 listing on paper and listing in electronic form are not disclosed in the international application.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2013/000060

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: 13-14,19
because they relate to subject matter not required to be searched by this Authority, namely:
claims 13-14, 19 relate to a method for treatment of the human or animal body
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See the extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: **1, 15, 20-22 (all completely); 7-9, 25 (all partially)**
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on protest

- ☒ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2013/000060

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
CN1408851A	2003-04-09	None	
CN1437613A	2003-08-20	WO0200694A2	2002-01-03
		AU6701401A	2002-01-08
		WO0200694A3	2002-10-10
		EP1292615A2	2003-03-19
		MXPA02012256A	2003-04-01
		KR20030032971A	2003-04-26
		BR0111827A	2003-05-20
		US2003206925A1	2003-11-06
		US6664066B2	2003-12-16
		JP2004513616A	2004-05-13
		EP1292615B1	2006-10-25
		DE60124098E	2006-12-07
		CN1298738C	2007-02-07
		AU2001267014B2	2007-03-15
		DE60124098T2	2007-04-05
		ES2273852T3	2007-05-16
		MX2512772 B	2007-11-07
		KR100885325B1	2009-02-25
		CA2409432C	2010-08-03
CN101852806A	2010-10-06	FR2935384A1	2010-03-05
		US2010055675A1	2010-03-04
		JP2010122205A	2010-06-03
CN102140547A	2011-08-03	None	
CN1904069A	2007-01-31	CN1904069B	2010-07-21

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2013/000060

A. CLASSIFICATION OF SUBJECT MATTER

C12N 15/45 (2006.01) i

C07K 14/12 (2006.01) i

C12N 15/86 (2006.01) i

C12N 5/16 (2006.01) i

C07K 16/10 (2006.01) i

C12Q 1/68 (2006.01) i

A61K 39/155 (2006.01) i

A61P 31/14 (2006.01) i

Box No. III Observations where unity of invention is lacking

This International Searching Authority finds 9 groups of inventions in this international application, as follows:

Group 1: claims 1; 7-9 (partially)

relates to an isolated nucleic acid comprising the nucleotide sequence of Figure 2; a vector comprising such isolated nucleic acid; a host cell comprising such vector;

Group 2: claims 2; 7-9 (partially)

relates to an isolated nucleic acid comprising the nucleotide sequence of Figure 3; a vector comprising such isolated nucleic acid; a host cell comprising such vector;

Group 3: claims 3; 7-9 (partially)

relates to an isolated nucleic acid comprising the nucleotide sequence of Figure 4; a vector comprising such isolated nucleic acid; a host cell comprising such vector;

Group 4: claims 4, 16; 10-14, 19 (partially)

relates to an isolated polypeptide from a Feline morbillivirus comprising the sequence designated FmoPV 761U of Figure 5; an immunogenic composition comprising such isolated polypeptide; a method of producing such isolated polypeptide; a method of inducing immunity in a mammal comprising the step of administering the mammal such immunogenic composition; a method of enhancing an immune response in a mammal comprising the step of administering to the feline such immunogenic composition; an antibody molecule having specificity for such isolated polypeptide; a method of treating feline tubulointerstitial nephritis comprising administering a therapeutically effective amount of such antibody;

Group 5: claims 5, 17; 10-14, 19 (partially)

relates to an isolated polypeptide from a Feline morbillivirus comprising the sequence designated FmoPV 776U of Figure 5; an immunogenic composition comprising such isolated polypeptide; a method of producing such isolated polypeptide; a method of inducing immunity in a mammal comprising the step of administering the mammal such immunogenic composition; a method of enhancing an immune response in a mammal comprising the step of administering to the feline such immunogenic composition; an antibody molecule having specificity for such isolated polypeptide; a method of treating feline tubulointerstitial nephritis comprising administering a therapeutically effective amount of such antibody;

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2013/000060

Group 6: claims 6, 18; 10-14, 19 (partially)

relates to an isolated polypeptide from a Feline morbillivirus comprising the sequence designated FmoPV M252A of Figure 5; an immunogenic composition comprising such isolated polypeptide; a method of producing such isolated polypeptide; a method of inducing immunity in a mammal comprising the step of administering the mammal such immunogenic composition; a method of enhancing an immune response in a mammal comprising the step of administering to the feline such immunogenic composition; an antibody molecule having specificity for such isolated polypeptide; a method of treating feline tubulointerstitial nephritis comprising administering a therapeutically effective amount of such antibody;

Group 7: claims 15, 20; 19 (partially)

relates to an antibody molecule having specificity for Feline morbillivirus; a method of treating feline tubulointerstitial nephritis comprising administering a therapeutically effective amount of such antibody; a method of diagnosing kidney disease in a feline by using such antibody molecule;

Group 8: claims 21-22; 25 (partially)

relates to a primer having the nucleotide sequence CAGAGACTTAATGAAATTTATGG and a primer having the nucleotide sequence CCACCCATCGGGTACTT; a kit used in a method for diagnosing kidney disease in a feline comprising such primers;

Group 9: claims 23-24; 25 (partially)

relates to a primer having the nucleotide sequence ACGCGGATCCGATGTCTAGTCTA, and a primer having the nucleotide sequence CGGAATTCGGTTTTAGAAGGTCAGTA; a kit used in a method for diagnosing kidney disease in a feline comprising such primers;

The nucleotide sequences or amino sequences between groups 1-9 are different, and the structure units among them are also different. That is to say, groups 1-9 do not have the same or corresponding special technical feature, and are not linked by a single general inventive concept. Therefore, they do not meet the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3.