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(54) **CORONAVIRUS ISOLATED FROM HUMANS**

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(57) ABSTRACT

Disclosed herein is a newly isolated human coronavirus (SARS-CoV), the causative agent of severe acute respiratory syndrome (SARS). Also provided are the nucleic acid sequence of the SARS-CoV genome and the amino acid sequences of the SARS-CoV open reading frames, as well as methods of using these molecules to detect a SARS-CoV and detect infections therewith. Immune stimulatory compositions are also provided, along with methods of their use.

Related U.S. Application Data

(60) Provisional application No. 60/465,927, filed on Apr. 25, 2003.

(51) Int. Cl.

C12N 15/50 (2006.01)

C12N 7/00 (2006.01)

(52) **U.S. Cl.** **536/23.72**; 435/235.1

(58) **Field of Classification Search** 536/23.72;
514/44; 435/235.1, 320.1

See application file for complete search history.

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1 Claim, 7 Drawing Sheets

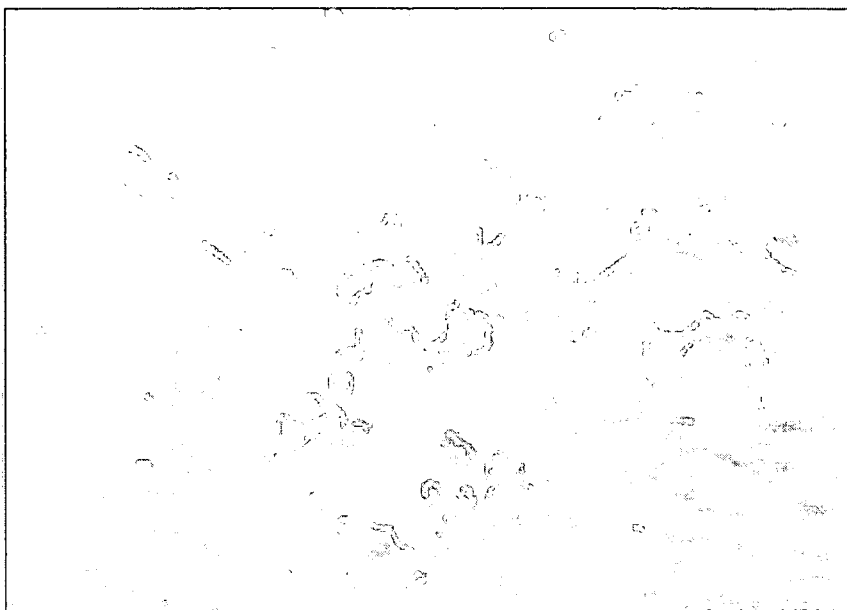


FIG. 1A

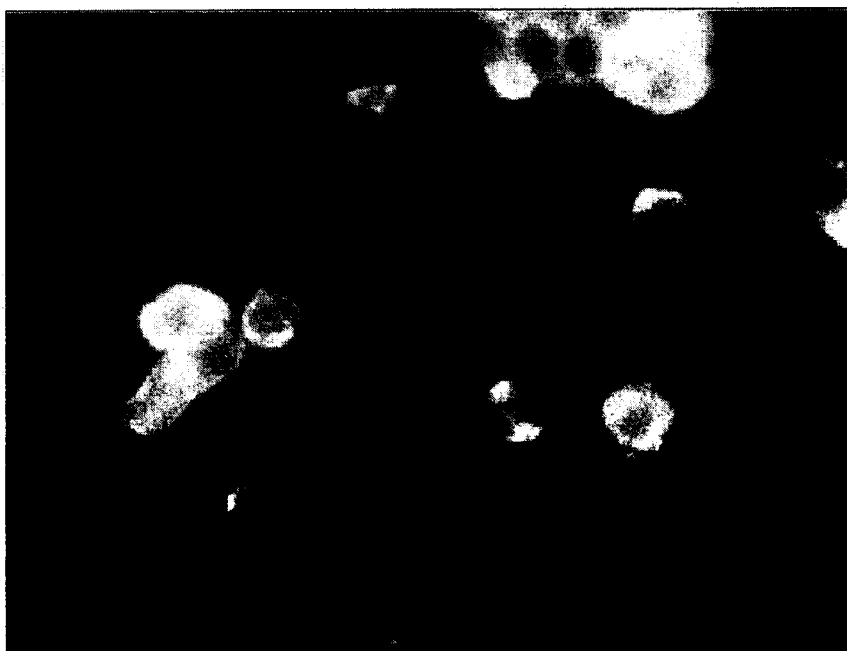


FIG. 1B

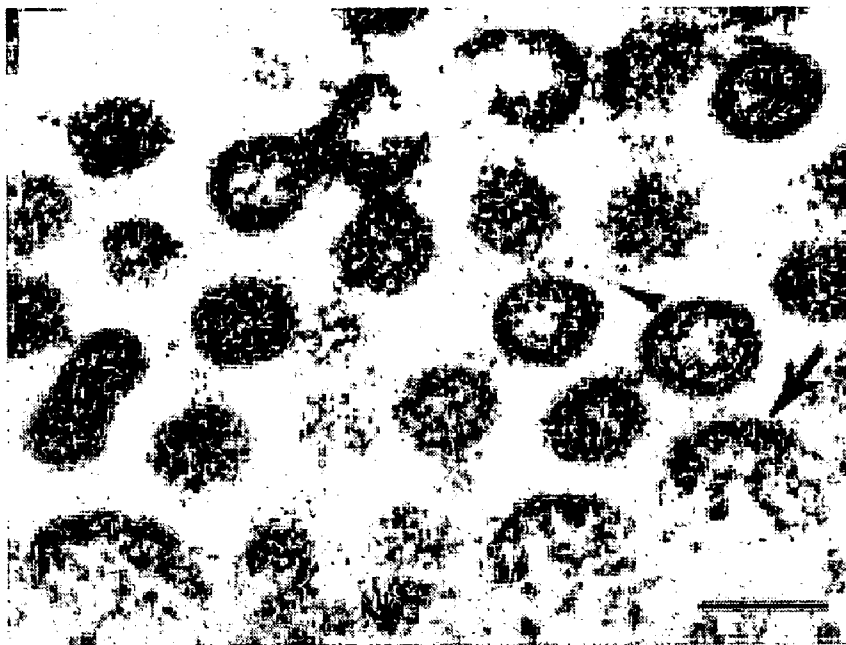


FIG. 2A

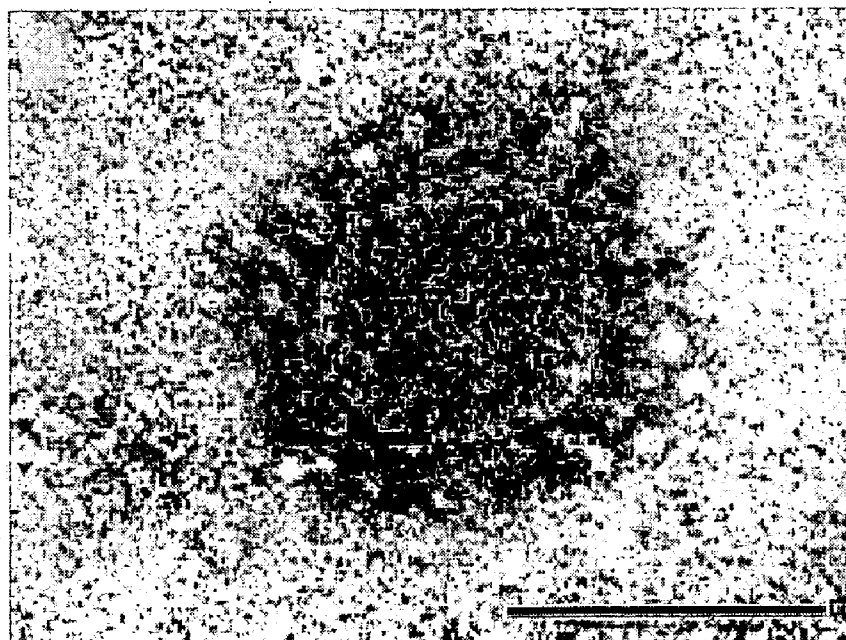


FIG. 2B

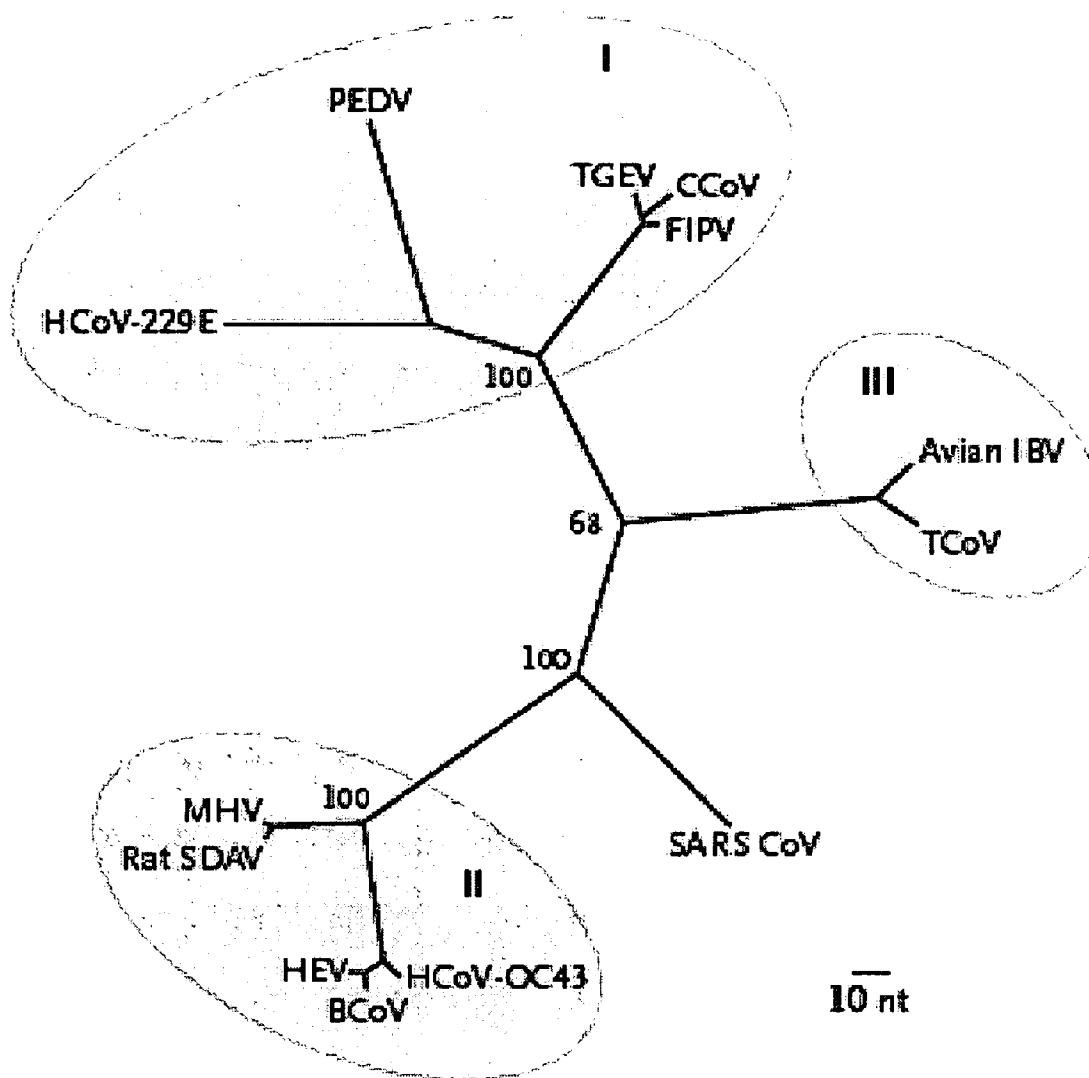


FIG. 3

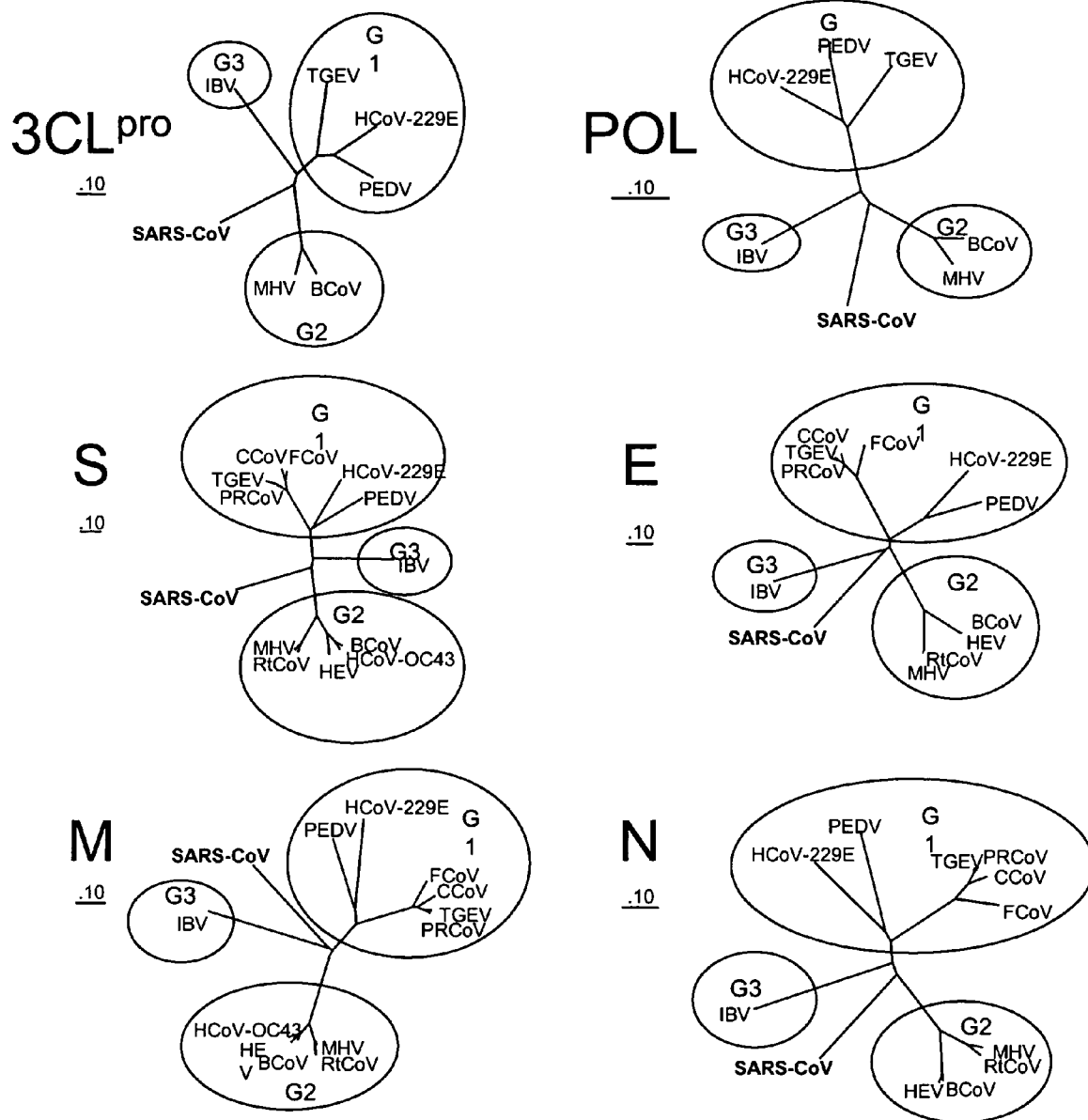


FIG. 4

FIG. 5A

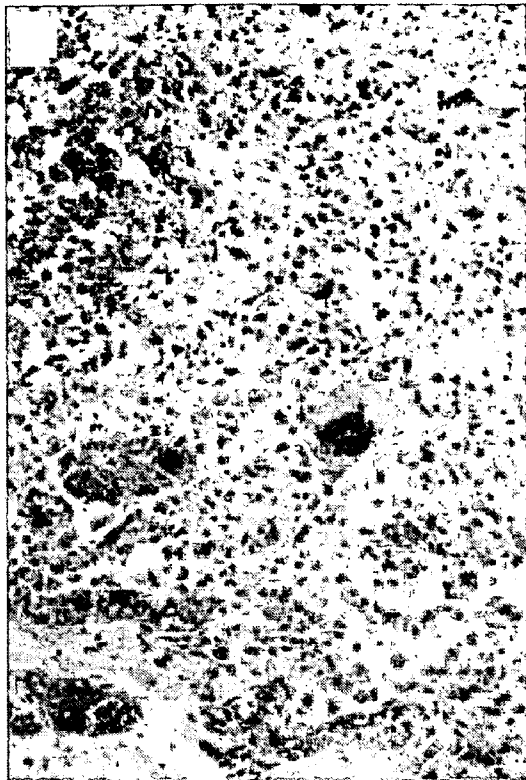


FIG. 5B

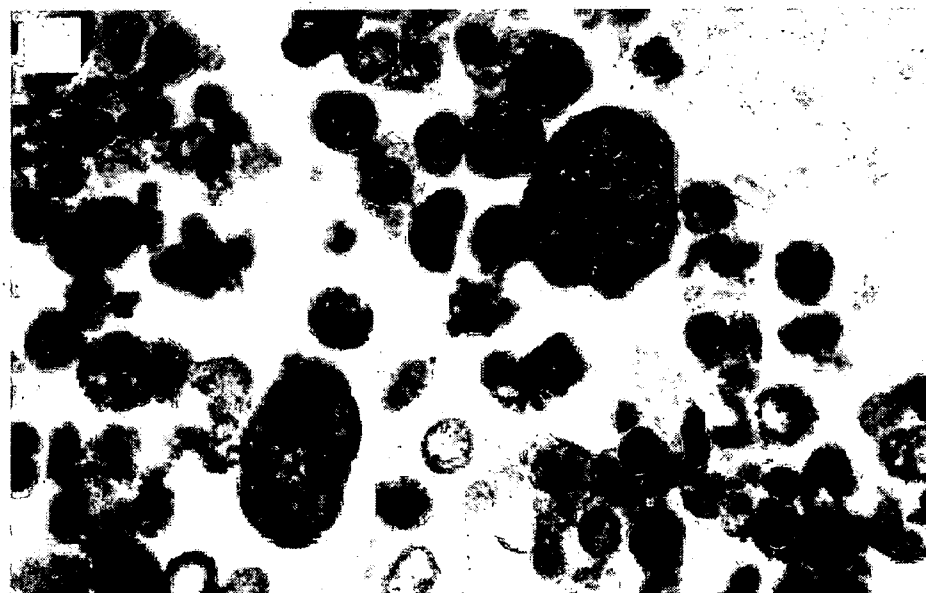
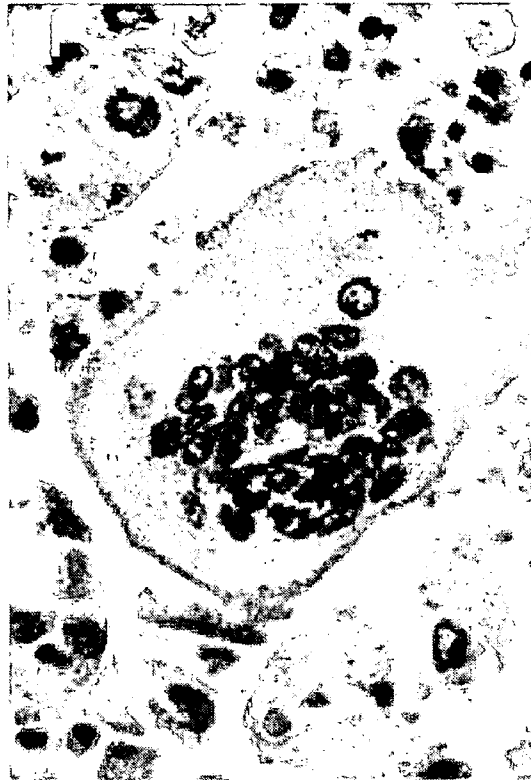


FIG. 5C

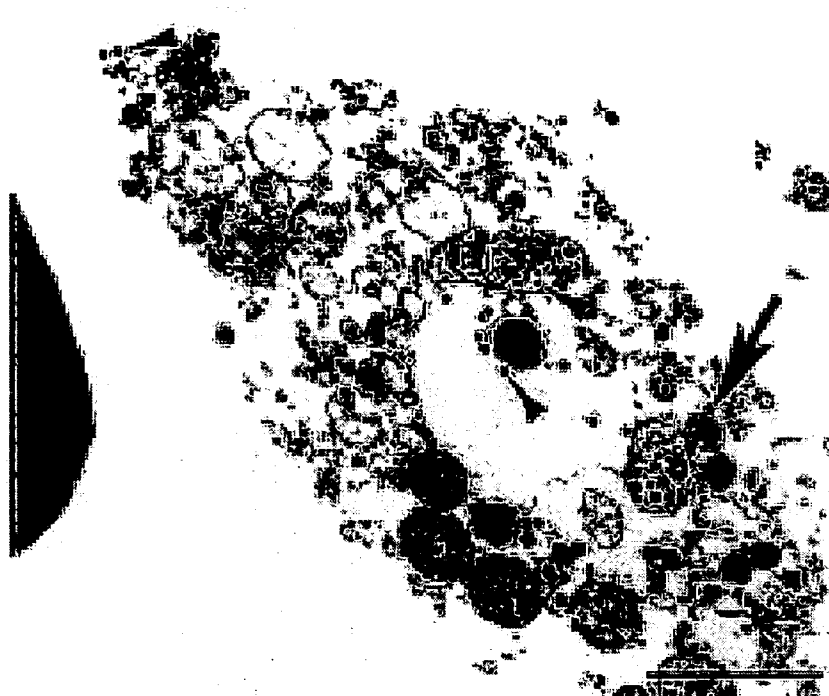


FIG. 6A

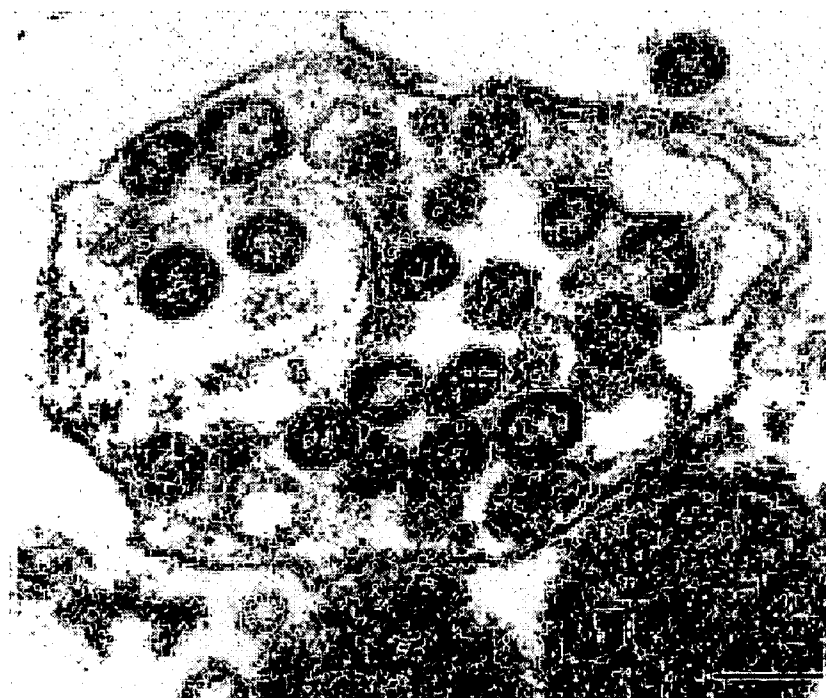
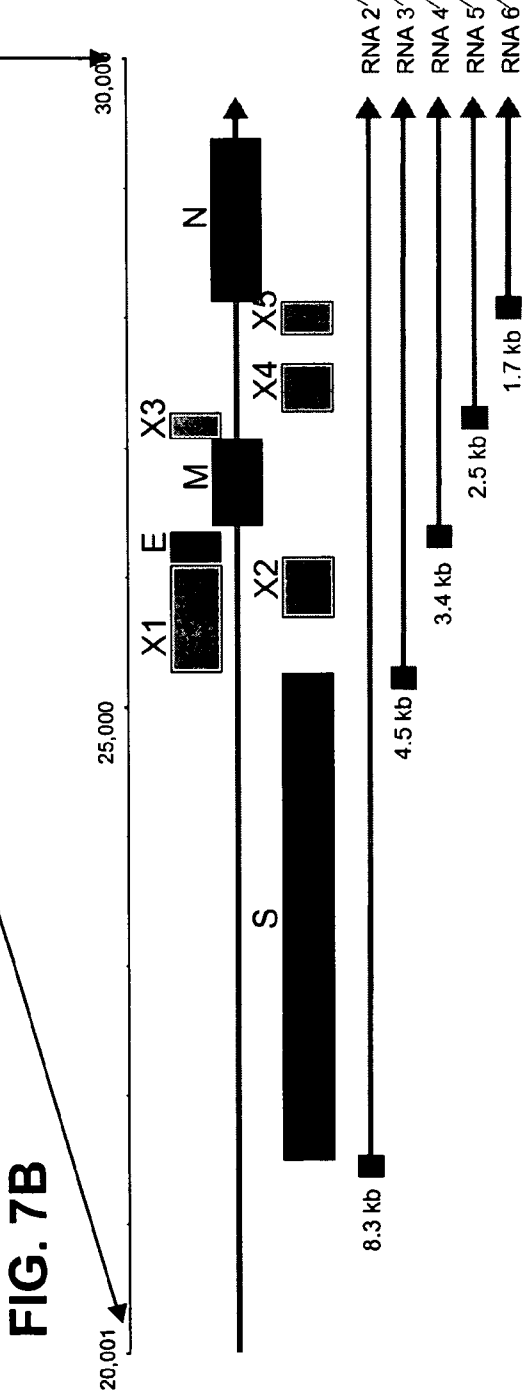
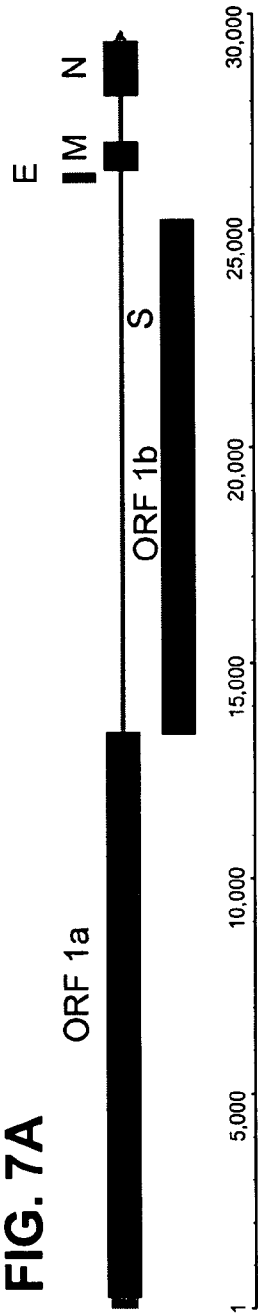


FIG. 6B



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CORONAVIRUS ISOLATED FROM HUMANS

PRIORITY CLAIM

This application claims the benefit of U.S. Provisional Patent Application No. 60/465,927 filed Apr. 25, 2003, which is incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

This invention was made by the Centers for Disease Control and Prevention, an agency of the United States Government. Therefore, the U.S. Government has certain rights in this invention.

FIELD OF THE DISCLOSURE

This invention relates to a newly isolated human coronavirus. More particularly, it relates to an isolated coronavirus genome, isolated coronavirus proteins, and isolated nucleic acid molecules encoding the same. The disclosure further relates to methods of detecting a severe acute respiratory syndrome-associated coronavirus and compositions comprising immunogenic coronavirus compounds.

BACKGROUND

The coronaviruses (order Nidovirales, family Coronaviridae, genus Coronavirus) are a diverse group of large, enveloped, positive-stranded RNA viruses that cause respiratory and enteric diseases in humans and other animals. At approximately 30,000 nucleotides (nt), their genome is the largest found in any of the RNA viruses. Coronaviruses are spherical, 100–160 nm in diameter with 20–40 nm complex club shaped surface projections surrounding the periphery. Coronaviruses share common structural proteins including a spike protein (S), membrane protein (M), envelope protein (E), and, in a subset of coronaviruses, a hemagglutinin-esterase protein (HE). The S protein, a glycoprotein which protrudes from the virus membrane, is involved in host cell receptor binding and is a target for neutralizing antibodies. The E and M proteins are involved in virion formation and release from the host cell. Coronavirus particles are found within the cisternae of the rough endoplasmic reticulum and in vesicles of infected host cells where virions are assembled. The coronavirus genome consists of two open reading frames (ORF1a and ORF1b) yielding an RNA polymerase and a nested set of subgenomic mRNAs encoding structural and nonstructural proteins, including the S, E, M, and nucleocapsid (N) proteins. The genus Coronavirus includes at least 13 species which have been subdivided into at least three groups (groups I, II, and III) on the basis of serological and genetic properties (deVries et al., *Sem. Virol.* 8:33–47, 1997; Fields et al. eds. *Fields Virology*, 3rd edition, Raven Press, Philadelphia, 1323–1341, 1996; Mahey and Collier eds. *Microbiology and Microbial Infections*, Volume 1 Virology, 9th edition, Oxford University Press, 463–479, 1998).

The three known groups of coronavirus are associated with a variety of diseases of humans and domestic animals (for example, cattle, pigs, cats, dogs, rodents, and birds), including gastroenteritis and upper and lower respiratory tract disease. Known coronaviruses include human Coronavirus 229E (HCoV-229E), canine coronavirus (CCoV), feline infectious peritonitis virus (FIPV), porcine transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV), human coronavirus OC43 (HCoV-

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OC43), bovine coronavirus (BCoV), porcine hemagglutinating encephalomyelitis virus (HEV), rat sialodacryoadenitis virus (SDAV), mouse hepatitis virus (MHV), turkey coronavirus (TCoV), and avian infectious bronchitis virus (IBV-Avian) (Fields et al. eds. *Fields Virology*, 3rd edition, Raven Press, Philadelphia, 1323–1341, 1996; Mahey and Collier eds. *Microbiology and Microbial Infections*, Volume 1 Virology, 9th edition, Oxford University Press, 463–479, 1998).

Coronavirus infections are generally host specific with respect to infectivity and clinical symptoms. Coronaviruses further exhibit marked tissue tropism; infection in the incorrect host species or tissue type may result in an abortive infection, mutant virus production and altered virulence. Coronaviruses generally do not grow well in cell culture, and animal models for human coronavirus infection are lacking. Therefore, little is known about them (Fields et al. eds. *Fields Virology*, 3rd edition, Raven Press, Philadelphia, 1323–1341, 1996). The known human coronaviruses are notably fastidious in cell culture, preferring select cell lines, organ culture, or suckling mice for propagation. Coronaviruses grown in cell culture exhibit varying degrees of virulence and/or cytopathic effect (CPE) depending on the host cell type and culture conditions. The only human or animal coronavirus which has been shown to grow in Vero E6 cells is PEDV, and it requires the addition of trypsin to culture medium for growth in Vero E6 cells. Moreover, PEDV adapted to Vero E6 cell culture results in a strikingly different CPE, with cytoplasmic vacuoles and the formation of large syncytia (Hofmann and Wyler, *J. Clin. Micro.* 26:2235–39, 1988; Kusanagi et al., *J. Vet. Med. Sci.* 554: 313–18, 1991).

Coronavirus have not previously been known to cause severe disease in humans, but have been identified as a major cause of upper respiratory tract illness, including the common cold. Repeat infections in humans are common within and across serotype, suggesting that immune response to coronavirus infection in humans is either incomplete or short lived. Coronavirus infection in animals can cause severe enteric or respiratory disease. Vaccination has been used successfully to prevent and control some coronavirus infections in animals. The ability of animal-specific coronaviruses to cause severe disease raises the possibility that coronavirus could also cause more severe disease in humans (Fields et al. eds. *Fields Virology*, 3rd edition, Raven Press, Philadelphia, 1323–1341, 1996; Mahey and Collier eds. *Microbiology and Microbial Infections*, Volume 1 Virology, 9th edition, Oxford University Press, 463–479, 1998).

In late 2002, cases of life-threatening respiratory disease with no identifiable etiology were reported from Guangdong Province, China, followed by reports from Vietnam, Canada, and Hong Kong of severe febrile respiratory illness that spread to household members and health care workers. The syndrome was designated “severe acute respiratory syndrome” (SARS) in February 2003 by the Centers for Disease Control and Prevention (*MMWR*, 52:241–48, 2003).

Past efforts to develop rapid diagnostics and vaccines for coronavirus infection in humans have been hampered by a lack of appropriate research models and the moderate course of disease in humans. Therefore, a need for rapid diagnostic tests and vaccines exists.

SUMMARY OF THE DISCLOSURE

A newly isolated human coronavirus has been identified as the causative agent of SARS, and is termed SARS-CoV.

The nucleic acid sequence of the SARS-CoV genome and the amino acid sequences of the SARS-CoV open reading frames are provided herein.

This disclosure provides methods and compositions useful in detecting the presence of a SARS-CoV nucleic acid in a sample and/or diagnosing a SARS-CoV infection in a subject. Also provided are methods and compositions useful in detecting the presence of a SARS-CoV antigen or antibody in a sample and/or diagnosing a SARS-CoV infection in a subject.

The foregoing and other features and advantages will become more apparent from the following detailed description of several embodiments, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A–B are photomicrographs illustrating typical early cytopathic effects seen with coronavirus isolates and serum from SARS patients. FIG. 1A is a photomicrograph of Vero E6 cells inoculated with an oropharyngeal specimen from a SARS patient (×40). FIG. 1B is a photomicrograph of infected Vero E6 cells reacting with the serum of a convalescent SARS patient in an indirect fluorescent antibody (IFA) assay (×400).

FIGS. 2A–B are electronmicrographs illustrating ultrastructural characteristics of the SARS-associated coronavirus (SARS-CoV). FIG. 2A is a thin-section electron-microscopical view of viral nucleocapsids aligned along the membrane of the rough endoplasmic reticulum (arrow) as particles bud into the cisternae. Enveloped virions have surface projections (arrowhead) and an electron-lucent center. Directly under the viral envelope lies a characteristic ring formed by the helical nucleocapsid, often seen in cross-section. FIG. 2B is a negative stain (methylamine tungstate) electronmicrograph showing stain-penetrated coronavirus particle with the typical internal helical nucleocapsid-like structure and club-shaped surface projections surrounding the periphery of the particle. Bars: 100 nm.

FIG. 3 is an estimated maximum parsimony tree illustrating putative phylogenetic relationships between SARS-CoV and other human and animal coronaviruses. Phylogenetic relationships are based on sequence alignment of 405 nucleotides of the coronavirus polymerase gene ORF1b (nucleic acid 15,173 to 15,578 of SEQ ID NO: 1). The three major coronavirus antigenic groups (I, II and III), represented by HCoV-229E, CCoV, FIPV, TGEV, PEDV, HCoV-OC43, BCoV, HEV, SDAV, MHV, TCoV, and IBV-Avian, are shown shaded. Bootstrap values (100 replicates) obtained from a 50% majority rule consensus tree are plotted at the main internal branches of the phylogram. Branch lengths are proportionate to nucleotide differences.

FIG. 4 is a pictorial representation of neighbor joining trees illustrating putative phylogenetic relationships between SARS-CoV and other human and animal coronaviruses. Amino acid sequences of the indicated SARS-CoV proteins were compared with those from reference viruses representing each species in the three groups of coronaviruses for which complete genomic sequence information was available [group 1: HCoV-229E (AF304460); PEDV (AF353511); TGEV (AJ271965); group 2: BCoV (AF220295); MHV (AF201929); group 3: infectious bronchitis virus (M95169)]. Sequences for representative strains of other coronavirus species, for which partial sequence information was available, were included for some of the structural protein comparisons [group 1: CCoV (D13096); FCoV (AY204704); porcine respiratory coronavirus (Z24675); group 2: HCoV-OC43 (M76373, L14643, M93390); HEV (AY078417); rat coronavirus (AF207551)]. Sequence alignments and neighbor-joining trees were gen-

erated by using Clustalx 1.83 with the Gonnet protein comparison matrix. The resulting trees were adjusted for final output using tree2ool 2.0.1.

FIGS. 5A–C are photomicrographs illustrating diffuse alveolar damage in a patient with SARS (FIGS. 5A–B), and immunohistochemical staining of SARS-CoV-infected Vero E6 cells (FIG. 5C). FIG. 5A is a photomicrograph of lung tissue from a SARS patient (×50). Diffuse alveolar damage, abundant foamy macrophages and multinucleated syncytial cells are present; hematoxylin and eosin stain was used. FIG. 5B is a higher magnification photomicrograph of lung tissue from the same SARS patient (×250). Syncytial cells show no conspicuous viral inclusions. FIG. 5C is a photomicrograph of immunohistochemically stained SARS-CoV-infected cells (×250). Membranous and cytoplasmic immunostaining of individual and syncytial Vero E6 cells was achieved using feline anti-FIPV-1 ascitic fluid. Immunoalkaline phosphatase with naphthol-fast red substrate and hematoxylin counter stain was used.

FIG. 6A–B are electronmicrographs illustrating ultrastructural characteristics of a coronavirus-infected cell in bronchoalveolar lavage (BAL) from a SARS patient. FIG. 6A is an electronmicrograph of a coronavirus-infected cell. Numerous intracellular and extracellular particles are present; virions are indicated by the arrowheads. FIG. 6B is a higher magnification electronmicrograph of the area seen at the arrow in FIG. 6A (rotated clockwise approximately 90°). Bars: FIG. 6A, 1 µm; FIG. 6B, 100 nm.

FIGS. 7A–C illustrate the organization of the SARS-CoV genome. FIG. 7A is a diagram of the overall organization of the 29,727-nt SARS-CoV genomic RNA. The 72-nt leader sequence is represented as a small rectangle at the left-most end. ORFs1a and 1b, encoding the nonstructural polyproteins, and those ORFs encoding the S, E, M, and N structural proteins, are indicated. Vertical position of the boxes indicates the phase of the reading frame (phase 1 for proteins above the line, phase two for proteins on the line and phase three for proteins below the line). FIG. 7B is an expanded view of the structural protein encoding region and predicted mRNA transcripts. Known structural protein encoding regions (dark grey boxes) and regions and reading frames for potential products X1–X5 (light gray boxes) are indicated. Lengths and map locations of the 3'-coterminal mRNAs expressed by the SARS-CoV are indicated, as predicted by identification of conserved transcriptional regulatory sequences. FIG. 7C is a digitized image of a nylon membrane showing Northern blot analysis of SARS-CoV mRNAs. Poly(A)+ RNA from infected Vero E6 cells was separated on a formaldehyde-agarose gel, transferred to a nylon membrane, and hybridized with a digoxigenin-labeled riboprobe overlapping the 3'-untranslated region. Signals were visualized by chemiluminescence. Sizes of the SARS-CoV mRNAs were calculated by extrapolation from a log-linear fit of the molecular mass marker. Lane 1, SARS-CoV mRNA; lane 2, Vero E6 cell mRNA; lane 3, molecular mass marker, sizes in kB.

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. In the accompanying sequence listing:

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SEQ ID NO: 1 shows the nucleic acid sequence of the SARS-CoV genome.

SEQ ID NO: 2 shows the amino acid sequence of the SARS-CoV polypeptide 1a (encoded by nucleic acid 265 to nucleic acid 13,398 of SEQ ID NO: 1).

SEQ ID NO: 3 shows the amino acid sequence of the SARS-CoV polypeptide 1b (encoded by nucleic acid 13,398 to 21,482 of SEQ ID NO: 1).

SEQ ID NO: 4 shows the amino acid sequence of the SARS-CoV S protein (encoded by nucleic acid 21,492 to 25,256 of SEQ ID NO: 1).

SEQ ID NO: 5 shows the amino acid sequence of the SARS-CoV X1 protein (encoded by nucleic acid 25,268 to 26,089 of SEQ ID NO: 1).

SEQ ID NO: 6 shows the amino acid sequence of the SARS-CoV X2 protein (encoded by nucleic acid 25,689 to 26,150 of SEQ ID NO: 1).

SEQ ID NO: 7 shows the amino acid sequence of the SARS-CoV E protein (encoded by nucleic acid 26,117 to 26,344 of SEQ ID NO: 1).

SEQ ID NO: 8 shows the amino acid sequence of the SARS-CoV M protein (encoded by nucleic acid 26,398 to 27,060 of SEQ ID NO: 1).

SEQ ID NO: 9 shows the amino acid sequence of the SARS-CoV X3 protein (encoded by nucleic acid 27,074 to 27,262 of SEQ ID NO: 1).

SEQ ID NO: 10 shows the amino acid sequence of the SARS-CoV X4 protein (encoded by nucleic acid 27,273 to 27,638 of SEQ ID NO: 1).

SEQ ID NO: 11 shows the amino acid sequence of the SARS-CoV X5 protein (encoded by nucleic acid 27,864 to 28,115 of SEQ ID NO: 1).

SEQ ID NO: 12 shows the amino acid sequence of the SARS-CoV N protein (encoded by nucleic acid 28,120 to 29,385 of SEQ ID NO: 1).

SEQ ID NOS: 13–15 show the nucleic acid sequence of several SARS-CoV-specific oligonucleotide primers.

SEQ ID NOS: 16–33 show the nucleic acid sequence of several oligonucleotide primers/probes used for real-time reverse transcription-polymerase chain reaction (RT-PCR) SARS-CoV assays.

SEQ ID NOS: 34–35 show the nucleic acid sequence of two degenerate primers designed to anneal to sites encoding conserved coronavirus amino acid motifs.

SEQ ID NOS: 36–38 show the nucleic acid sequence of several oligonucleotide primers/probe used as controls in real-time RT-PCR assays.

DETAILED DESCRIPTION OF SEVERAL EMBODIMENTS

I. Abbreviations

M:	coronavirus membrane protein
N:	coronavirus nucleoprotein
ORF:	open reading frame
PCR:	polymerase chain reaction
RACE:	5' rapid amplification of cDNA ends
RT-PCR:	reverse transcription-polymerase chain reaction
S:	coronavirus spike protein
SARS:	severe acute respiratory syndrome
SARS-CoV:	severe acute respiratory syndrome-associated coronavirus
TRS:	transcriptional regulatory sequence

II. Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes*

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VII, published by Oxford University Press, 2000 (ISBN 019879276X); Kendrew et al. (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Publishers, 1994 (ISBN 0632021829); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by Wiley, John & Sons, Inc., 1995 (ISBN 0471186341); and other similar references.

As used herein, the singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. Also, as used herein, the term “comprises” means “includes.” Hence “comprising A or B” means including A, B, or A and B. It is further to be understood that all nucleotide sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

In order to facilitate review of the various embodiments of this disclosure, the following explanations of specific terms are provided:

Adjuvant: A substance that non-specifically enhances the immune response to an antigen. Development of vaccine adjuvants for use in humans is reviewed in Singh et al. (*Nat. Biotechnol.* 17:1075–1081, 1999), which discloses that, at the time of its publication, aluminum salts and the MF59 microemulsion are the only vaccine adjuvants approved for human use.

Amplification: Amplification of a nucleic acid molecule (e.g., a DNA or RNA molecule) refers to use of a laboratory technique that increases the number of copies of a nucleic acid molecule in a sample. An example of amplification is the polymerase chain reaction (PCR), in which a sample is contacted with a pair of oligonucleotide primers under conditions that allow for the hybridization of the primers to a nucleic acid template in the sample. The primers are extended under suitable conditions, dissociated from the template, re-annealed, extended, and dissociated to amplify the number of copies of the nucleic acid. The product of amplification can be characterized by such techniques as electrophoresis, restriction endonuclease cleavage patterns, oligonucleotide hybridization or ligation, and/or nucleic acid sequencing.

Other examples of amplification methods include strand displacement amplification, as disclosed in U.S. Pat. No. 5,744,311; transcription-free isothermal amplification, as disclosed in U.S. Pat. No. 6,033,881; repair chain reaction amplification, as disclosed in WO 90/01069; ligase chain reaction amplification, as disclosed in EP-A-320,308; gap filling ligase chain reaction amplification, as disclosed in U.S. Pat. No. 5,427,930; and NASBA™ RNA transcription-free amplification, as disclosed in U.S. Pat. No. 6,025,134. An amplification method can be modified, including for example by additional steps or coupling the amplification with another protocol.

Animal: Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. Similarly, the term “subject” includes both human and veterinary subjects, for example, humans, non-human primates, dogs, cats, horses, and cows.

Antibody: A protein (or protein complex) that includes one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

The basic immunoglobulin (antibody) structural unit is generally a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" (about 50–70 kDa) chain. The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms "variable light chain" (V_L) and "variable heavy chain" (V_H) refer, respectively, to these light and heavy chains.

As used herein, the term "antibodies" includes intact immunoglobulins as well as a number of well-characterized fragments. For instance, Fabs, Fvs, and single-chain Fvs (SCFvs) that bind to target protein (or epitope within a protein or fusion protein) would also be specific binding agents for that protein (or epitope). These antibody fragments are defined as follows: (1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain; (2) Fab', the fragment of an antibody molecule obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule; (3) (Fab')₂, the fragment of the antibody obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; (4) F(ab')₂, a dimer of two Fab' fragments held together by two disulfide bonds; (5) Fv, a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and (6) single chain antibody, a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. Methods of making these fragments are routine (see, for example, Harlow and Lane, *Using Antibodies: A Laboratory Manual*, CSHL, New York, 1999).

Antibodies for use in the methods and devices of this disclosure can be monoclonal or polyclonal. Merely by way of example, monoclonal antibodies can be prepared from murine hybridomas according to the classical method of Kohler and Milstein (*Nature* 256:495–97, 1975) or derivative methods thereof. Detailed procedures for monoclonal antibody production are described in Harlow and Lane, *Using Antibodies: A Laboratory Manual*, CSHL, New York, 1999.

Antigen: A compound, composition, or substance that can stimulate the production of antibodies or a T-cell response in an animal, including compositions that are injected or absorbed into an animal. An antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous immunogens. In one embodiment, an antigen is a coronavirus antigen.

Binding or Stable Binding: An oligonucleotide binds or stably binds to a target nucleic acid if a sufficient amount of the oligonucleotide forms base pairs or is hybridized to its target nucleic acid, to permit detection of that binding. Binding can be detected by either physical or functional properties of the target:oligonucleotide complex. Binding between a target and an oligonucleotide can be detected by

any procedure known to one skilled in the art, including functional or physical binding assays. Binding can be detected functionally by determining whether binding has an observable effect upon a biosynthetic process such as expression of a gene, DNA replication, transcription, translation, and the like.

Physical methods of detecting the binding of complementary strands of DNA or RNA are well known in the art, and include such methods as DNase I or chemical footprinting, gel shift and affinity cleavage assays, Northern blotting, Southern blotting, dot blotting, and light absorption detection procedures. For example, a method which is widely used, because it is so simple and reliable, involves observing a change in light absorption of a solution containing an oligonucleotide (or an analog) and a target nucleic acid at 220 to 300 nm as the temperature is slowly increased. If the oligonucleotide or analog has bound to its target, there is a sudden increase in absorption at a characteristic temperature as the oligonucleotide (or analog) and target dissociate or melt.

The binding between an oligomer and its target nucleic acid is frequently characterized by the temperature (T_m) at which 50% of the oligomer is melted from its target. A higher T_m means a stronger or more stable complex relative to a complex with a lower T_m .

cDNA (complementary DNA): A piece of DNA lacking internal, non-coding segments (introns) and regulatory sequences that determine transcription. cDNA is synthesized in the laboratory by reverse transcription from messenger RNA extracted from cells.

Electrophoresis: Electrophoresis refers to the migration of charged solutes or particles in a liquid medium under the influence of an electric field. Electrophoretic separations are widely used for analysis of macromolecules. Of particular importance is the identification of proteins and nucleic acid sequences. Such separations can be based on differences in size and/or charge. Nucleotide sequences have a uniform charge and are therefore separated based on differences in size. Electrophoresis can be performed in an unsupported liquid medium (for example, capillary electrophoresis), but more commonly the liquid medium travels through a solid supporting medium. The most widely used supporting media are gels, for example, polyacrylamide and agarose gels.

Sieving gels (for example, agarose) impede the flow of molecules. The pore size of the gel determines the size of a molecule that can flow freely through the gel. The amount of time to travel through the gel increases as the size of the molecule increases. As a result, small molecules travel through the gel more quickly than large molecules and thus progress further from the sample application area than larger molecules, in a given time period. Such gels are used for size-based separations of nucleotide sequences.

Fragments of linear DNA migrate through agarose gels with a mobility that is inversely proportional to the \log_{10} of their molecular weight. By using gels with different concentrations of agarose, different sizes of DNA fragments can be resolved. Higher concentrations of agarose facilitate separation of small DNAs, while low agarose concentrations allow resolution of larger DNAs.

Hybridization: Oligonucleotides and their analogs hybridize by hydrogen bonding, which includes Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary bases. Generally, nucleic acid consists of nitrogenous bases that are either pyrimidines (cytosine (C), uracil (U), and thymine (T)) or purines (adenine (A) and guanine (G)). These nitrogenous bases form hydrogen bonds between a pyrimidine and a purine, and the bonding of the pyrimidine to the purine is referred to as "base pairing." More specifically, A will hydrogen bond to T or U, and G will bond to C. "Complementary" refers to the

base pairing that occurs between two distinct nucleic acid sequences or two distinct regions of the same nucleic acid sequence.

"Specifically hybridizable" and "specifically complementary" are terms that indicate a sufficient degree of complementarity such that stable and specific binding occurs between the oligonucleotide (or its analog) and the DNA or RNA target. The oligonucleotide or oligonucleotide analog need not be 100% complementary to its target sequence to be specifically hybridizable. An oligonucleotide or analog is specifically hybridizable when binding of the oligonucleotide or analog to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide or analog to non-target sequences under conditions where specific binding is desired, for example under physiological conditions in the case of in vivo assays or systems. Such binding is referred to as specific hybridization.

Hybridization conditions resulting in particular degrees of stringency will vary depending upon the nature of the hybridization method of choice and the composition and length of the hybridizing nucleic acid sequences. Generally, the temperature of hybridization and the ionic strength (especially the Na⁺ and/or Mg⁺⁺ concentration) of the hybridization buffer will determine the stringency of hybridization, though wash times also influence stringency. Calculations regarding hybridization conditions required for attaining particular degrees of stringency are discussed by Sambrook et al. (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, chapters 9 and 11; and Ausubel et al. *Short Protocols in Molecular Biology*, 4th ed., John Wiley & Sons, Inc., 1999.

For purposes of the present disclosure, "stringent conditions" encompass conditions under which hybridization will only occur if there is less than 25% mismatch between the hybridization molecule and the target sequence. "Stringent conditions" may be broken down into particular levels of stringency for more precise definition. Thus, as used herein, "moderate stringency" conditions are those under which molecules with more than 25% sequence mismatch will not hybridize; conditions of "medium stringency" are those under which molecules with more than 15% mismatch will not hybridize, and conditions of "high stringency" are those under which sequences with more than 10% mismatch will not hybridize. Conditions of "very high stringency" are those under which sequences with more than 6% mismatch will not hybridize.

Immune Stimulatory Composition: A term used herein to mean a composition useful for stimulating or eliciting a specific immune response (or immunogenic response) in a vertebrate. In some embodiments, the immunogenic response is protective or provides protective immunity, in that it enables the vertebrate animal to better resist infection with or disease progression from the organism against which the vaccine is directed.

Without wishing to be bound by a specific theory, it is believed that an immunogenic response may arise from the generation of an antibody specific to one or more of the epitopes provided in the immune stimulatory composition. Alternatively, the response may comprise a T-helper or cytotoxic cell-based response to one or more of the epitopes provided in the immune stimulatory composition. All three of these responses may originate from naïve or memory cells. One specific example of a type of immune stimulatory composition is a vaccine.

In some embodiments, an "effective amount" or "immune-stimulatory amount" of an immune stimulatory composition is an amount which, when administered to a

subject, is sufficient to engender a detectable immune response. Such a response may comprise, for instance, generation of an antibody specific to one or more of the epitopes provided in the immune stimulatory composition. Alternatively, the response may comprise a T-helper or CTL-based response to one or more of the epitopes provided in the immune stimulatory composition. All three of these responses may originate from naïve or memory cells. In other embodiments, a "protective effective amount" of an immune stimulatory composition is an amount which, when administered to a subject, is sufficient to confer protective immunity upon the subject.

Inhibiting or Treating a Disease: Inhibiting the full development of a disease or condition, for example, in a subject who is at risk for a disease such as SARS. "Treatment" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. As used herein, the term "ameliorating," with reference to a disease, pathological condition or symptom, refers to any observable beneficial effect of the treatment. The beneficial effect can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease, a reduction in the number of relapses of the disease, an improvement in the overall health or well-being of the subject, or by other parameters well known in the art that are specific to the particular disease.

Isolated: An "isolated" microorganism (such as a virus, bacterium, fungus, or protozoan) has been substantially separated or purified away from microorganisms of different types, strains, or species. Microorganisms can be isolated by a variety of techniques, including serial dilution and culturing.

An "isolated" biological component (such as a nucleic acid molecule, protein or organelle) has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, such as other chromosomal and extrachromosomal DNA and RNA, proteins, and organelles. Nucleic acids and proteins that have been "isolated" include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant expression in a host cell, as well as chemically synthesized nucleic acids or proteins, or fragments thereof.

Label: A detectable compound or composition that is conjugated directly or indirectly to another molecule to facilitate detection of that molecule. Specific, non-limiting examples of labels include fluorescent tags, enzymatic linkages, and radioactive isotopes.

Nucleic Acid Molecule: A polymeric form of nucleotides, which may include both sense and anti-sense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. A nucleotide refers to a ribonucleotide, deoxynucleotide or a modified form of either type of nucleotide. A "nucleic acid molecule" as used herein is synonymous with "nucleic acid" and "polynucleotide." A nucleic acid molecule is usually at least 10 bases in length, unless otherwise specified. The term includes single- and double-stranded forms of DNA. A polynucleotide may include either or both naturally occurring and modified nucleotides linked together by naturally occurring and/or non-naturally occurring nucleotide linkages.

Oligonucleotide: A nucleic acid molecule generally comprising a length of 300 bases or fewer. The term often refers to single-stranded deoxyribonucleotides, but it can refer as well to single- or double-stranded ribonucleotides, RNA: DNA hybrids and double-stranded DNAs, among others. The term "oligonucleotide" also includes oligonucleosides

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(that is, an oligonucleotide minus the phosphate) and any other organic base polymer. In some examples, oligonucleotides are about 10 to about 90 bases in length, for example, 12, 13, 14, 15, 16, 17, 18, 19 or 20 bases in length. Other oligonucleotides are about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60 bases, about 65 bases, about 70 bases, about 75 bases or about 80 bases in length. Oligonucleotides may be single-stranded, for example, for use as probes or primers, or may be double-stranded, for example, for use in the construction of a mutant gene. Oligonucleotides can be either sense or anti-sense oligonucleotides. An oligonucleotide can be modified as discussed above in reference to nucleic acid molecules. Oligonucleotides can be obtained from existing nucleic acid sources (for example, genomic or cDNA), but can also be synthetic (for example, produced by laboratory or in vitro oligonucleotide synthesis).

Open Reading Frame (ORF): A series of nucleotide triplets (codons) coding for amino acids without any internal termination codons. These sequences are usually translatable into a peptide/polypeptide/protein/polypeptide.

Operably Linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame. If introns are present, the operably linked DNA sequences may not be contiguous.

Pharmaceutically Acceptable Carriers: The pharmaceutically acceptable carriers useful in this disclosure are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, Pa., 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of one or more therapeutic compounds or molecules, such as one or more SARS-CoV nucleic acid molecules, proteins or antibodies that bind these proteins, and additional pharmaceutical agents.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Polypeptide: A polymer in which the monomers are amino acid residues which are joined together through amide bonds. When the amino acids are alpha-amino acids, either the L-optical isomer or the D-optical isomer can be used, the L-isomers being preferred. The terms "polypeptide" or "protein" as used herein are intended to encompass any amino acid sequence and include modified sequences such as glycoproteins. The term "polypeptide" is specifically intended to cover naturally occurring proteins, as well as those which are recombinantly or synthetically produced.

Conservative amino acid substitutions are those substitutions that, when made, least interfere with the properties of the original protein, that is, the structure and especially the

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function of the protein is conserved and not significantly changed by such substitutions. Examples of conservative substitutions are shown below.

Original Residue	Conservative Substitutions
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

The substitutions which in general are expected to produce the greatest changes in protein properties will be non-conservative, for instance changes in which (a) a hydrophilic residue, for example, seryl or threonyl, is substituted for (or by) a hydrophobic residue, for example, leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, for example, lysyl, arginyl, or histadyl, is substituted for (or by) an electronegative residue, for example, glutamyl or aspartyl; or (d) a residue having a bulky side chain, for example, phenylalanine, is substituted for (or by) one not having a side chain, for example, glycine.

Probes and Primers: A probe comprises an isolated nucleic acid attached to a detectable label or other reporter molecule. Typical labels include radioactive isotopes, enzyme substrates, co-factors, ligands, chemiluminescent or fluorescent agents, haptens, and enzymes. Methods for labeling and guidance in the choice of labels appropriate for various purposes are discussed, for example, in Sambrook et al. (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989 and Ausubel et al. *Short Protocols in Molecular Biology*, 4th ed., John Wiley & Sons, Inc., 1999.

Primers are short nucleic acid molecules, for instance DNA oligonucleotides 10 nucleotides or more in length, for example that hybridize to contiguous complementary nucleotides or a sequence to be amplified. Longer DNA oligonucleotides may be about 15, 20, 25, 30 or 50 nucleotides or more in length. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then the primer extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, for example, by the PCR or other nucleic-acid amplification methods known in the art, as described above.

Methods for preparing and using nucleic acid probes and primers are described, for example, in Sambrook et al. (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor,

N.Y., 1989; Ausubel et al. *Short Protocols in Molecular Biology*, 4th ed., John Wiley & Sons, Inc., 1999; and Innis et al. *PCR Protocols, A Guide to Methods and Applications*, Academic Press, Inc., San Diego, Calif., 1990. Amplification primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, © 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.). One of ordinary skill in the art will appreciate that the specificity of a particular probe or primer increases with its length. Thus, in order to obtain greater specificity, probes and primers can be selected that comprise at least 20, 25, 30, 35, 40, 45, 50 or more consecutive nucleotides of a target nucleotide sequences.

Protein: A biological molecule, particularly a polypeptide, expressed by a gene and comprised of amino acids. A "polypeptide" is a protein that, after synthesis, is cleaved to produce several functionally distinct polypeptides.

Purified: The term "purified" does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified protein preparation is one in which the subject protein is more pure than in its natural environment within a cell. Generally, a protein preparation is purified such that the protein represents at least 50% of the total protein content of the preparation.

Recombinant Nucleic Acid: A sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic engineering techniques such as those described in Sambrook et al. (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid.

Sample: A portion, piece, or segment that is representative of a whole. This term encompasses any material, including for instance samples obtained from an animal, a plant, or the environment.

An "environmental sample" includes a sample obtained from inanimate objects or reservoirs within an indoor or outdoor environment. Environmental samples include, but are not limited to: soil, water, dust, and air samples; bulk samples, including building materials, furniture, and landfill contents; and other reservoir samples, such as animal refuse, harvested grains, and foodstuffs.

A "biological sample" is a sample obtained from a plant or animal subject. As used herein, biological samples include all samples useful for detection of viral infection in subjects, including, but not limited to: cells, tissues, and bodily fluids, such as blood; derivatives and fractions of blood (such as serum); extracted galls; biopsied or surgically removed tissue, including tissues that are, for example, unfixed, frozen, fixed in formalin and/or embedded in paraffin; tears; milk; skin scrapes; surface washings; urine; sputum; cerebrospinal fluid; prostate fluid; pus; bone marrow aspirates; BAL; saliva; cervical swabs; vaginal swabs; and oropharyngeal wash.

Sequence Identity: The similarity between two nucleic acid sequences, or two amino acid sequences, is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment

algorithms are described in: Smith and Waterman (*Adv. Appl. Math.*, 2:482, 1981); Needleman and Wunsch (*J. Mol. Biol.*, 48:443, 1970); Pearson and Lipman (*Proc. Natl. Acad. Sci.*, 85:2444, 1988); Higgins and Sharp (*Gene*, 73:237-44, 1988); Higgins and Sharp (*CABIOS*, 5:151-53, 1989); Corpet et al. (*Nuc. Acids Res.*, 16:10881-90, 1988); Huang et al. (*Comp. Appls Biosci.*, 8:155-65, 1992); and Pearson et al. (*Meth. Mol. Biol.*, 24:307-31, 1994). Altschul et al. (*Nature Genet.*, 6:119-29, 1994) presents a detailed consideration of sequence alignment methods and homology calculations.

The alignment tools ALIGN (Myers and Miller, *CABIOS* 4:11-17, 1989) or LFASTA (Pearson and Lipman, 1988) may be used to perform sequence comparisons (Internet Program© 1996, W. R. Pearson and the University of Virginia, "fasta20u63" version 2.0u63, release date December 1996). ALIGN compares entire sequences against one another, while LFASTA compares regions of local similarity. These alignment tools and their respective tutorials are available on the Internet at the NCSA website. Alternatively, for comparisons of amino acid sequences of greater than about 30 amino acids, the "Blast 2 sequences" function can be employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the "Blast 2 sequences" function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). The BLAST sequence comparison system is available, for instance, from the NCBI web site; see also Altschul et al., *J. Mol. Biol.*, 215:403-10, 1990; Gish and States, *Nature Genet.*, 3:266-72, 1993; Madden et al., *Meth. Enzymol.*, 266:131-41, 1996; Altschul et al., *Nucleic Acids Res.*, 25:3389-402, 1997; and Zhang and Madden, *Genome Res.*, 7:649-56, 1997.

Orthologs (equivalent to proteins of other species) of proteins are in some instances characterized by possession of greater than 75% sequence identity counted over the full-length alignment with the amino acid sequence of specific protein using ALIGN set to default parameters. Proteins with even greater similarity to a reference sequence will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, or at least 98% sequence identity. In addition, sequence identity can be compared over the full length of one or both binding domains of the disclosed fusion proteins.

When significantly less than the entire sequence is being compared for sequence identity, homologous sequences will typically possess at least 80% sequence identity over short windows of 10-20, and may possess sequence identities of at least 85%, at least 90%, at least 95%, or at least 99% depending on their similarity to the reference sequence. Sequence identity over such short windows can be determined using LFASTA; methods are described at the NCSA website. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided. Similar homology concepts apply for nucleic acids as are described for protein.

An alternative indication that two nucleic acid molecules are closely related is that the two molecules hybridize to each other under stringent conditions. Representative hybridization conditions are discussed above.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences, due to the degeneracy of the genetic code. It is understood that changes in nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that each encode substantially the same protein.

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Specific Binding Agent: An agent that binds substantially only to a defined target. Thus a protein-specific binding agent binds substantially only the defined protein, or to a specific region within the protein. As used herein, a protein-specific binding agent includes antibodies and other agents that bind substantially to a specified polypeptide. The antibodies may be monoclonal or polyclonal antibodies that are specific for the polypeptide, as well as immunologically effective portions ("fragments") thereof.

The determination that a particular agent binds substantially only to a specific polypeptide may readily be made by using or adapting routine procedures. One suitable in vitro assay makes use of the Western blotting procedure (described in many standard texts, including Harlow and Lane, *Using Antibodies: A Laboratory Manual*, CSHL, New York, 1999).

Transformed: A "transformed" cell is a cell into which has been introduced a nucleic acid molecule by molecular biology techniques. The term encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration.

Vector: A nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art.

Virus: Microscopic infectious organism that reproduces inside living cells. A virus typically consists essentially of a core of a single nucleic acid surrounded by a protein coat, and has the ability to replicate only inside a living cell. "Viral replication" is the production of additional virus by the occurrence of at least one viral life cycle. A virus may subvert the host cells' normal functions, causing the cell to behave in a manner determined by the virus. For example, a viral infection may result in a cell producing a cytokine, or responding to a cytokine, when the uninfected cell does not normally do so.

"Coronaviruses" are large, enveloped, RNA viruses that cause respiratory and enteric diseases in humans and other animals. Coronavirus genomes are non-segmented, single-stranded, positive-sense RNA, approximately 27–31 kb in length. Genomes have a 5' methylated cap and 3' poly-A tail, and function directly as mRNA. Host cell entry occurs via endocytosis and membrane fusion, and replication occurs in the cytoplasm. Initially, the 5' 20 kb of the positive-sense genome is translated to produce a viral polymerase, which then produces a full-length negative-sense strand used as a template to produce subgenomic mRNA as a "nested set" of transcripts. Assembly occurs by budding into the golgi apparatus, and particles are transported to the surface of the cell and released.

III. Overview of Several Embodiments

A newly isolated human coronavirus (SARS-CoV) is disclosed herein. The entire genomic nucleic acid sequence of this virus is also provided herein. Also disclosed are the nucleic acid sequences of the SARS-CoV ORFs, and the polypeptide sequences encoded by these ORFs. Pharmaceutical and immune stimulatory compositions are also disclosed that include one or more SARS-CoV viral nucleic acids, polypeptides encoded by these viral nucleic acids and antibodies that bind to a SARS-CoV polypeptide or SARS-CoV polypeptide fragment.

In one embodiment, a method is provided for detecting the presence of SARS-CoV in a sample. This method includes contacting the sample with a pair of nucleic acid

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primers that hybridize to a SARS-CoV nucleic acid, wherein at least one primer is 5'-end labeled with a reporter dye, amplifying the SARS-CoV nucleic acid or a fragment thereof from the sample utilizing the pair of nucleic acid primers, electrophoresing the amplified products, and detecting the 5'-end labeled reporter dye, thereby detecting a SARS-CoV. In a specific, non-limiting example, the amplification utilizes RT-PCR. In a further specific example of the provided method, at least one of the nucleic acid primers that hybridize to a SARS-CoV nucleic acid includes a sequence as set forth in any one of SEQ ID NOs: 13–15.

In another example of the provided method, detecting the presence of SARS-CoV in a sample includes contacting the sample with a pair of nucleic acid primers that hybridize to a SARS-CoV nucleic acid, amplifying the SARS-CoV nucleic acid or a fragment thereof from the sample utilizing the pair of nucleic acid primers, adding to the amplified SARS-CoV nucleic acid or the fragment thereof a TaqMan SARS-CoV probe that hybridizes to the SARS-CoV nucleic acid, wherein the TaqMan SARS-CoV probe is labeled with a 5'-reporter dye and a 3'-quencher dye, performing one or more additional rounds of amplification, and detecting fluorescence of the 5'-reporter dye, thereby detecting a SARS-CoV. In a specific, non-limiting example, the amplification utilizes RT-PCR. In a further specific example of the provided method, at least one of the nucleic acid primers that hybridize to a SARS-CoV nucleic acid and/or the TaqMan SARS-CoV probe that hybridizes to the SARS-CoV nucleic acid includes a sequence as set forth in any one of SEQ ID NOs: 16–33.

In another embodiment, a method is provided for detecting a SARS-CoV in a biological sample that contains antibodies. This method includes contacting the biological sample with a SARS-CoV-specific antigen, wherein the antigen includes a SARS-CoV organism and determining whether a binding reaction occurs between the SARS-CoV-specific antigen and an antibody in the biological sample if such is present, thereby detecting SARS-CoV.

In a further embodiment, a method is provided for detecting a SARS-CoV in a biological sample that contains polypeptides and/or fragments thereof. This method includes contacting the biological sample with a SARS-CoV-specific antibody and determining whether a binding reaction occurs between the SARS-CoV-specific antibody and a SARS-CoV polypeptide or fragment thereof in the biological sample if such is present, thereby detecting SARS-CoV. In a specific, non-limiting example, determining whether a binding reaction occurs between the SARS-CoV-specific antibody and a SARS-CoV polypeptide or fragment thereof is carried out in situ or in a tissue sample. In a further specific example, determining whether a binding reaction occurs between the SARS-CoV-specific antibody and a SARS-CoV polypeptide or fragment thereof includes an immunohistochemical assay.

An additional embodiment includes a kit for detecting a SARS-CoV in a sample, including a pair of nucleic acid primers that hybridize under stringent conditions to a SARS-CoV nucleic acid, wherein one primer is 5'-end labeled with a reporter dye. In a specific, non-limiting example, at least one of the nucleic acid primers that hybridize to a SARS-CoV nucleic acid includes a sequence as set forth in any one of SEQ ID NOs: 13–15.

Another example of the provided kit includes a pair of nucleic acid primers that hybridize under high stringency conditions to a SARS-CoV nucleic acid and a TaqMan SARS-CoV probe that hybridizes to the SARS-CoV nucleic acid, wherein the TaqMan SARS-CoV probe is labeled with a 5'-reporter dye and a 3'-quencher dye. In a specific, non-limiting example, at least one of the nucleic acid primers that hybridize to a SARS-CoV nucleic acid and/or

the TaqMan SARS-CoV probe that hybridizes to the SARS-CoV nucleic acid includes a sequence as set forth in any one of SEQ ID NOs: 16–33.

Also disclosed herein is a composition including an isolated SARS-CoV organism. In one embodiment, the isolated SARS-CoV organism is an inactive isolated SARS-CoV organism. In another embodiment, the composition includes at least one component selected from the group consisting of pharmaceutically acceptable carriers, adjuvants and combinations of two or more thereof. In yet another embodiment, the composition is introduced into a subject, thereby eliciting an immune response against a SARS-CoV antigenic epitope in a subject.

IV. SARS-CoV Nucleotide and Amino Acid Sequences

The current disclosure provides an isolated SARS-CoV genome, isolated SARS-CoV polypeptides, and isolated nucleic acid molecules encoding the same. In one embodiment, the isolated SARS-CoV genome has a sequence as shown in SEQ ID NO: 1 or an equivalent thereof. Polynucleotides encoding a SARS-CoV polypeptide (encoded by an ORF from within the genome) are also provided, and are termed SARS-CoV nucleic acid molecules. These nucleic acid molecules include DNA, cDNA and RNA sequences which encode a SARS-CoV polypeptide. Specific, non-limiting examples of a SARS-CoV nucleic acid molecule encoding an ORF are nucleic acid 265 to nucleic acid 13,398 of SEQ ID NO: 1 (encoding SARS-CoV 1a, SEQ ID NO: 2), nucleic acid 13,398 to 21,482 of SEQ ID NO: 1 (encoding SARS-CoV 1b, SEQ ID NO: 3), nucleic acid 21,492 to 25,256 of SEQ ID NO: 1 (encoding SARS-CoV S, SEQ ID NO: 4), nucleic acid 25,268 to 26,089 of SEQ ID NO: 1 (encoding SARS-CoV X1, SEQ ID NO: 5), nucleic acid 25,689 to 26,150 of SEQ ID NO: 1 (encoding SARS-CoV X2, SEQ ID NO: 6), nucleic acid 26,117 to 26,344 of SEQ ID NO: 1 (encoding SARS-CoV E, SEQ ID NO: 7), nucleic acid 26,398 to 27,060 of SEQ ID NO: 1 (encoding SARS-CoV M, SEQ ID NO: 8), nucleic acid 27,074 to 27,262 of SEQ ID NO: 1 (encoding SARS-CoV X3, SEQ ID NO: 9), nucleic acid 27,273 to 27,638 of SEQ ID NO: 1 (encoding SARS-CoV X4, SEQ ID NO: 10), nucleic acid 27,864 to 28,115 of SEQ ID NO: 1 (encoding SARS-CoV X5, SEQ ID NO: 11), and nucleic acid 28,120 to 29,385 of SEQ ID NO: 1 (encoding SARS-CoV N, SEQ ID NO: 12).

Oligonucleotide primers and probes derived from the SARS-CoV genome (SEQ ID NO: 1) are also encompassed within the scope of the present disclosure. Such oligonucleotide primers and probes may comprise a sequence of at least about 15 consecutive nucleotides of the SARS-CoV nucleic acid sequence, such as at least about 20, 25, 30, 35, 40, 45, or 50 or more consecutive nucleotides. These primers and probes may be obtained from any region of the disclosed SARS-CoV genome (SEQ ID NO: 1), including particularly from any of the ORFs disclosed herein. Specific, non-limiting examples of oligonucleotide primers derived from the SARS-CoV genome (SEQ ID NO: 1) include: Cor-p-F2 (SEQ ID NO: 13), Cor-p-F3 (SEQ ID NO: 14), Cor-p-R1 (SEQ ID NO: 15), SARS1-F (SEQ ID NO: 16), SARS1-R (SEQ ID NO: 17), SARS2-F (SEQ ID NO: 19), SARS2-R (SEQ ID NO: 20), SARS3-F (SEQ ID NO: 22), SARS3-R (SEQ ID NO: 23), N3-F (SEQ ID NO: 25), N3-R (SEQ ID NO: 26), 3'NTR-F (SEQ ID NO: 28), 3'NTR-R (SEQ ID NO: 29), M-F (SEQ ID NO: 31), and M-R (SEQ ID NO: 32). Specific, non-limiting examples of oligonucleotide probes derived from the SARS-CoV genome (SEQ ID NO: 1) include: SARS1-P (SEQ ID NO: 18), SARS2-P (SEQ ID NO: 21), SARS3-P (SEQ ID NO: 24), N3-P (SEQ ID NO: 27), 3'NTR-P (SEQ ID NO: 30), and M-P (SEQ ID NO: 33).

Nucleic acid molecules encoding a SARS-CoV polypeptide can be operatively linked to regulatory sequences or

elements. Regulatory sequences or elements include, but are not limited to promoters, enhancers, transcription terminators, a start codon (for example, ATG), stop codons, and the like.

Additionally, nucleic acid molecules encoding a SARS-CoV polypeptide can be inserted into an expression vector. Specific, non-limiting examples of vectors include, plasmids, bacteriophages, cosmids, animal viruses and yeast artificial chromosomes (YACs) (Burke et al., *Science* 236: 806–12, 1987). Such vectors may then be introduced into a variety of hosts including somatic cells, and simple or complex organisms, such as bacteria, fungi (Timberlake and Marshall, *Science* 244:1313–17, 1989), invertebrates, plants (Gasser et al., *Plant Cell* 1:15–24, 1989), and animals (Pursel et al., *Science* 244:1281–88, 1989).

Transformation of a host cell with an expression vector carrying a nucleic acid molecule encoding a SARS-CoV polypeptide may be carried out by conventional techniques, as are well known to those skilled in the art. By way of example, where the host is prokaryotic, such as *E. coli*, competent cells that are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl_2 method using procedures well known in the art. Alternatively, MgCl_2 or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired, or by electroporation.

When the host is a eukaryote, methods of transfection of DNA, such as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors, may be used. Eukaryotic cells can also be cotransformed with SARS-CoV nucleic acid molecules, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein (see, for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman et al., 1982).

The SARS-CoV polypeptides of this disclosure include proteins encoded by any of the ORFs disclosed herein, and equivalents thereof. Specific, non-limiting examples of SARS-CoV proteins are provided in SEQ ID NOs: 2–12. Fusion proteins are also contemplated that include a heterologous amino acid sequence chemically linked to a SARS-CoV polypeptide. Exemplary heterologous sequences include short amino acid sequence tags (such as six histidine residues), as well a fusion of other proteins (such as c-myc or green fluorescent protein fusions). Epitopes of the SARS-CoV proteins, that are recognized by an antibody or that bind the major histocompatibility complex, and can be used to induce a SARS-CoV-specific immune response, are also encompassed by this disclosure.

Methods for expressing large amounts of protein from a cloned gene introduced into *E. coli* may be utilized for the purification and functional analysis of proteins. For example, fusion proteins consisting of amino terminal peptides encoded by a portion of the *E. coli* lacZ or trpE gene linked to SARS-CoV proteins may be used to prepare polyclonal and monoclonal antibodies against these proteins.

Intact native protein may also be produced in *E. coli* in large amounts for functional studies. Methods and plasmid vectors for producing fusion proteins and intact native proteins in bacteria are described by Sambrook et al. (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1–3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989. Such fusion proteins may be made in large amounts, are easy to purify, and can be used to elicit

antibody response. Native proteins can be produced in bacteria by placing a strong, regulated promoter and an efficient ribosome-binding site upstream of the cloned gene. If low levels of protein are produced, additional steps may be taken to increase protein production; if high levels of protein are produced, purification is relatively easy. Suitable methods are presented by Sambrook et al. (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1–3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, and are well known in the art. Often, proteins expressed at high levels are found in insoluble inclusion bodies. Methods for extracting proteins from these aggregates are described by Sambrook et al. (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1–3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

Isolation and purification of recombinantly expressed proteins may be carried out by conventional means including preparative chromatography and immunological separations. Additionally, the proteins can be chemically synthesized by any of a number of manual or automated methods of synthesis known in the art.

V. Specific Binding Agents

The disclosure provides specific binding agents that bind to SARS-CoV polypeptides disclosed herein. The binding agent may be useful for purifying and detecting the polypeptides, as well as for detection and diagnosis of SARS-CoV. Examples of the binding agents are a polyclonal or monoclonal antibody, and fragments thereof, that bind to any of the SARS-CoV polypeptides disclosed herein.

Monoclonal or polyclonal antibodies may be raised to recognize a SARS-CoV polypeptide described herein, or a fragment or variant thereof. Optimally, antibodies raised against these polypeptides would specifically detect the polypeptide with which the antibodies are generated. That is, antibodies raised against a specific SARS-CoV polypeptide will recognize and bind that polypeptide, and will not substantially recognize or bind to other polypeptides or antigens. The determination that an antibody specifically binds to a target polypeptide is made by any one of a number of standard immunoassay methods; for instance, the Western blotting technique (Sambrook et al. (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1–3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989).

Substantially pure SARS-CoV recombinant polypeptide antigens suitable for use as immunogen may be isolated from the transformed cells described above, using methods well known in the art. Monoclonal or polyclonal antibodies to the antigens may then be prepared.

Monoclonal antibodies to the polypeptides can be prepared from murine hybridomas according to the classic method of Kohler & Milstein (*Nature* 256:495–97, 1975), or a derivative method thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein immunogen (for example, a polypeptide comprising at least one SARS-CoV-specific epitope, a portion of a polypeptide comprising at least one SARS-CoV-specific epitope, or a synthetic peptide comprising at least one SARS-CoV-specific epitope) over a period of a few weeks. The mouse is then sacrificed, and the antibody-producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as

originally described by Engvall (*Meth. Enzymol.*, 70:419–39, 1980), or a derivative method thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Harlow and Lane, *Using Antibodies: A Laboratory Manual*, CSHL, New York, 1999.

Polyclonal antiserum containing antibodies can be prepared by immunizing suitable animals with a polypeptide comprising at least one SARS-CoV-specific epitope, a portion of a polypeptide comprising at least one SARS-CoV-specific epitope, or a synthetic peptide comprising at least one SARS-CoV-specific epitope, which can be unmodified or modified, to enhance immunogenicity.

Effective antibody production (whether monoclonal or polyclonal) is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than others and may require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with either inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appear to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis et al. (*J. Clin. Endocrinol. Metab.*, 33:988–91, 1971).

Booster injections can be given at regular intervals, and antiserum harvested when the antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony et al., *Handbook of Experimental Immunology*, Wier, D. (ed.), Chapter 19, Blackwell, 1973. A plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12 μ M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher (*Manual of Clinical Immunology*, Ch. 42, 1980).

Antibody fragments may be used in place of whole antibodies and may be readily expressed in prokaryotic host cells. Methods of making and using immunologically effective portions of monoclonal antibodies, also referred to as “antibody fragments,” are well known and include those described in Better & Horowitz, *Methods Enzymol.* 178: 476–96, 1989; Glockshuber et al., *Biochemistry* 29:1362–67, 1990; and U.S. Pat. No. 5,648,237 (Expression of Functional Antibody Fragments); U.S. Pat. No. 4,946,778 (Single Polypeptide Chain Binding Molecules); and U.S. Pat. No. 5,455,030 (Immunotherapy Using Single Chain Polypeptide Binding Molecules), and references cited therein. Conditions whereby a polypeptide/binding agent complex can form, as well as assays for the detection of the formation of a polypeptide/binding agent complex and quantitation of binding affinities of the binding agent and polypeptide, are standard in the art. Such assays can include, but are not limited to, Western blotting, immunoprecipitation, immunofluorescence, immunocytochemistry, immunohistochemistry, fluorescence activated cell sorting (FACS), fluorescence in situ hybridization (FISH), immunomagnetic assays, ELISA, ELISPOT (Coligan et al., *Current Protocols in Immunology*, Wiley, NY, 1995), agglutination assays, flocculation assays, cell panning, and the like, as are well known to one of skill in the art.

Binding agents of this disclosure can be bound to a substrate (for example, beads, tubes, slides, plates, nitrocellulose sheets, and the like) or conjugated with a detectable moiety, or both bound and conjugated. The detectable moieties contemplated for the present disclosure can include, but are not limited to, an immunofluorescent moiety (for example, fluorescein, rhodamine), a radioactive moiety (for

example, ^{32}P , ^{125}I , ^{35}S), an enzyme moiety (for example, horseradish peroxidase, alkaline phosphatase), a colloidal gold moiety, and a biotin moiety. Such conjugation techniques are standard in the art (for example, see Harlow and Lane, *Using Antibodies: A Laboratory Manual*, CSHL, New York, 1999; Yang et al., *Nature*, 382:319–24, 1996).

VI. Detection and Diagnosis of SARS-CoV

A. Nucleic Acid Based Methods of Detection and Diagnosis

A major application of the SARS-CoV sequence information presented herein is in the area of detection and diagnostic testing for SARS-CoV infection. Methods for screening a subject to determine if the subject has been or is currently infected with SARS-CoV are disclosed herein.

One such method includes providing a sample, which sample includes a nucleic acid such as DNA or RNA, and providing an assay for detecting in the sample the presence of a SARS-CoV nucleic acid molecule. Suitable samples include all biological samples useful for detection of viral infection in subjects, including, but not limited to, cells, tissues (for example, lung and kidney), bodily fluids (for example, blood, serum, urine, saliva, sputum, and cerebrospinal fluid), bone marrow aspirates, BAL, and oropharyngeal wash. Additional suitable samples include all environmental samples useful for detection of viral presence in the environment, including, but not limited to, a sample obtained from inanimate objects or reservoirs within an indoor or outdoor environment. The detection in the sample of a SARS-CoV nucleic acid molecule may be performed by a number of methodologies, non-limiting examples of which are outlined below.

In one embodiment, detecting in the sample the presence of a SARS-CoV nucleic acid molecule includes the amplification of a SARS-CoV nucleic acid sequence (or a fragment thereof). Any nucleic acid amplification method can be used. In one specific, non-limiting example, PCR is used to amplify the SARS-CoV nucleic acid sequence(s). In another non-limiting example, RT-PCR can be used to amplify the SARS-CoV nucleic acid sequences. In an additional non-limiting example, transcription-mediated amplification can be used to amplify the SARS-CoV nucleic acid sequences.

In some embodiments, a pair of SARS-CoV-specific primers are utilized in the amplification reaction. One or both of the primers can be end-labeled (for example, radio-labeled, fluoresceinated, or biotinylated). In one specific, non-limiting example, at least one of the primers is 5'-end labeled with the reporter dye 6-carboxyfluorescein (6-FAM). The pair of primers includes an upstream primer (which binds 5' to the downstream primer) and a downstream primer (which binds 3' to the upstream primer). In one embodiment, either the upstream primer or the downstream primer is labeled. Specific, non-limiting examples of SARS-CoV-specific primers include, but are not limited to: Cor-p-F2 (SEQ ID NO: 13), Cor-p-F3 (SEQ ID NO: 14), Cor-p-R1 (SEQ ID NO: 15), SARS1-F (SEQ ID NO: 16), SARS1-R (SEQ ID NO: 17), SARS2-F (SEQ ID NO: 19), SARS2-R (SEQ ID NO: 20), SARS3-F (SEQ ID NO: 22), SARS3-R (SEQ ID NO: 23), N3-F (SEQ ID NO: 25), N3-R (SEQ ID NO: 26), 3'NTR-F (SEQ ID NO: 28), 3'NTR-R (SEQ ID NO: 29), M-F (SEQ ID NO: 31), and M-R (SEQ ID NO: 32). Additional primer pairs can be generated, for instance, to amplify any of the specific ORFs described herein, using well known primer design principles and methods.

In one specific, non-limiting example, electrophoresis is used to detect amplified SARS-CoV-specific sequences. Electrophoresis can be automated using many methods well known in the art. In one embodiment, a genetic analyzer is used, such as an ABI 3100 Prism Genetic Analyzer (PE

Applied Biosystems, Foster City, Calif.), wherein the bands are analyzed using GeneScan software (PE Applied Biosystems, Foster City, Calif.).

In another specific, non-limiting example, hybridization assays are used to detect amplified SARS-CoV-specific sequences using distinguishing oligonucleotide probes. Such probes include "TaqMan" probes. TaqMan probes consist of an oligonucleotide with a reporter at the 5'-end and a quencher at the 3'-end. In one specific, non-limiting example, the reporter is 6-FAM and the quencher is Black-hole Quencher (Biosearch Tech., Inc., Novato, Calif.). When the probe is intact, the proximity of the reporter to the quencher results in suppression of reporter fluorescence, primarily by fluorescence resonance energy transfer. If the target of interest is present, the TaqMan probe specifically hybridizes between the forward and reverse primer sites during the PCR annealing step. In the process of PCR elongation, the 5'-3' nucleolytic activity of the Taq DNA polymerase cleaves the hybridized probe between the reporter and the quencher. The probe fragments are then displaced from the target, and polymerization of the strand continues. Taq DNA polymerase does not cleave non-hybridized probe, and cleaves the hybridized probe only during polymerization. The 3'-end of the probe is blocked to prevent extension of the probe during PCR. The 5'-3' nuclease cleavage of the hybridized probe occurs in every cycle and does not interfere with the exponential accumulation of PCR product. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the released reporter. The increase in fluorescence signal is detected only if the target sequence is complementary to the probe and is amplified during PCR. Therefore, non-specific amplification is not detected. SARS-CoV-specific TaqMan probes of the present disclosure include, but are not limited to: SARS1-P (SEQ ID NO: 18), SARS2-P (SEQ ID NO: 21), SARS3-P (SEQ ID NO: 24), N3-P (SEQ ID NO: 27), 3'NTR-P (SEQ ID NO: 30), and M-P (SEQ ID NO: 33), and hybridization assays include, but are not limited to, a real-time RT-PCR assay.

B. Protein Based Methods of Detection and Diagnosis

The present disclosure further provides methods of detecting a SARS-CoV antigen in a sample, and/or diagnosing SARS-CoV infection in a subject by detecting a SARS-CoV antigen. Examples of such methods comprise contacting the sample with a SARS-CoV-specific binding agent under conditions whereby an antigen/binding agent complex can form; and detecting formation of the complex, thereby detecting SARS-CoV antigen in a sample and/or diagnosing SARS-CoV infection in a subject. It is contemplated that at least certain antigens will be on an intact SARS-CoV virion, will be a SARS-CoV-encoded protein displayed on the surface of a SARS-CoV-infected cell expressing the antigen, or will be a fragment of the antigen. Contemplated samples subject to analysis by these methods can comprise any sample, such as a clinical sample, useful for detection of viral infection in a subject.

Methods for detecting antigens in a sample are discussed, for example, in Ausubel et al. *Short Protocols in Molecular Biology*, 4th ed., John Wiley & Sons, Inc., 1999. Enzyme immunoassays such as IFA, ELISA and immunoblotting can be readily adapted to accomplish the detection of SARS-CoV antigens according to the methods of this disclosure. An ELISA method effective for the detection of soluble SARS-CoV antigens is the direct competitive ELISA. This method is most useful when a specific SARS-CoV antibody and purified SARS-CoV antigen are available. Briefly: 1) coat a substrate (for example, a microtiter plate) with a sample suspected of containing a SARS-CoV antigen; 2) contact the bound SARS-CoV antigen with a SARS-CoV-specific antibody bound to a detectable moiety (for example,

horseradish peroxidase enzyme or alkaline phosphatase enzyme); 3) add purified inhibitor SARS-CoV antigen; 4) contact the above with the substrate for the enzyme; and 5) observe/measure inhibition of color change or fluorescence and quantitate antigen concentration (for example, using a microtiter plate reader).

An additional ELISA method effective for the detection of soluble SARS-CoV antigens is the antibody-sandwich ELISA. This method is frequently more sensitive in detecting antigen than the direct competitive ELISA method. Briefly: 1) coat a substrate (for example, a microtiter plate) with a SARS-CoV-specific antibody; 2) contact the bound SARS-CoV antibody with a sample suspected of containing a SARS-CoV antigen; 3) contact the above with SARS-CoV-specific antibody bound to a detectable moiety (for example, horseradish peroxidase enzyme or alkaline phosphatase enzyme); 4) contact the above with the substrate for the enzyme; and 5) observe/measure color change or fluorescence and quantitate antigen concentration (for example, using a microtiter plate reader).

An ELISA method effective for the detection of cell-surface SARS-CoV antigens is the direct cellular ELISA. Briefly, cells suspected of exhibiting a cell-surface SARS-CoV antigen are fixed (for example, using glutaraldehyde) and incubated with a SARS-CoV-specific antibody bound to a detectable moiety (for example, horseradish peroxidase enzyme or alkaline phosphatase enzyme). Following a wash to remove unbound antibody, substrate for the enzyme is added and color change or fluorescence is observed/measured.

The present disclosure further provides methods of detecting a SARS-CoV-reactive antibody in a sample, and/or diagnosing SARS-CoV infection in a subject by detecting a SARS-CoV-reactive antibody. Examples of such methods comprise contacting the sample with a SARS-CoV polypeptide of this disclosure under conditions whereby a polypeptide/antibody complex can form; and detecting formation of the complex, thereby detecting SARS-CoV antibody in a sample and/or diagnosing SARS-CoV infection in a subject. Contemplated samples subject to analysis by these methods can comprise any sample, such as a clinical sample, as described herein as being useful for detection of viral infection in a subject.

Methods for detecting antibodies in a sample are discussed, for example, in Ausubel et al. *Short Protocols in Molecular Biology*, 4th ed., John Wiley & Sons, Inc., 1999. Enzyme immunoassays such as IFA, ELISA and immunoblotting can be readily adapted to accomplish the detection of SARS-CoV antibodies according to the methods of this disclosure. An ELISA method effective for the detection of specific SARS-CoV antibodies is the indirect ELISA method. Briefly: 1) bind a SARS-CoV polypeptide to a substrate (for example, a microtiter plate); 2) contact the bound polypeptide with a sample suspected of containing SARS-CoV antibody; 3) contact the above with a secondary antibody bound to a detectable moiety which is reactive with the bound antibody (for example, horseradish peroxidase enzyme or alkaline phosphatase enzyme); 4) contact the above with the substrate for the enzyme; and 5) observe/measure color change or fluorescence.

Another immunologic technique that can be useful in the detection of SARS-CoV antibodies uses monoclonal antibodies for detection of antibodies specifically reactive with SARS-CoV polypeptides in a competitive inhibition assay. Briefly, a sample suspected of containing SARS-CoV antibodies is contacted with a SARS-CoV polypeptide of this disclosure which is bound to a substrate (for example, a microtiter plate). Excess sample is thoroughly washed away. A labeled (for example, enzyme-linked, fluorescent, radioactive, and the like) monoclonal antibody specific for the

SARS-CoV polypeptide is then contacted with any previously formed polypeptide-antibody complexes and the amount of monoclonal antibody binding is measured. The amount of inhibition of monoclonal antibody binding is measured relative to a control (no monoclonal antibody), allowing for detection and measurement of antibody in the sample. The degree of monoclonal antibody inhibition can be a very specific assay for detecting SARS-CoV. Monoclonal antibodies can also be used for direct detection of SARS-CoV in cells or tissue samples by, for example, IFA analysis according to standard methods.

As a further example, a micro-agglutination test can be used to detect the presence of SARS-CoV antibodies in a sample. Briefly, latex beads, red blood cells or other agglutinable particles are coated with a SARS-CoV polypeptide of this disclosure and mixed with a sample, such that antibodies in the sample that are specifically reactive with the antigen crosslink with the antigen, causing agglutination. The agglutinated polypeptide-antibody complexes form a precipitate, visible with the naked eye or measurable by spectrophotometer. In a modification of the above test, SARS-CoV-specific antibodies of this disclosure can be bound to the agglutinable particles and SARS-CoV antigen in the sample thereby detected.

VII. Pharmaceutical and Immune Stimulatory Compositions and Uses Thereof

Pharmaceutical compositions including SARS-CoV nucleic acid sequences, SARS-CoV polypeptides, or antibodies that bind these polypeptides, are also encompassed by the present disclosure. These pharmaceutical compositions include a therapeutically effective amount of one or more SARS-CoV polypeptides, one or more nucleic acid molecules encoding a SARS-CoV polypeptide, or an antibody that binds a SARS-CoV polypeptide, in conjunction with a pharmaceutically acceptable carrier.

Disclosed herein are substances suitable for use as immune stimulatory compositions for the inhibition or treatment of SARS. Particular immune stimulatory compositions are directed against SARS-CoV, and include antigens obtained from SARS-CoV. In one embodiment, an immune stimulatory composition contains attenuated SARS-CoV. Methods of viral attenuation are well known in the art, and include, but are not limited to, high serial passage (for example, in susceptible host cells under specific environmental conditions to select for attenuated virions), exposure to a mutagenic agent (for example, a chemical mutagen or radiation), genetic engineering using recombinant DNA technology (for example, using gene replacement or gene knockout to disable one or more viral genes), or some combination thereof.

In another embodiment, the immune stimulatory composition contains inactivated SARS-CoV. Methods of viral inactivation are well known in the art, and include, but are not limited to, heat and chemicals (for example, formalin, β -propiolactone, and ethylenimines).

In yet another embodiment, the immune stimulatory composition contains a nucleic acid vector that includes SARS-CoV nucleic acid molecules described herein, or that includes a nucleic acid sequence encoding an immunogenic polypeptide or polypeptide fragment of SARS-CoV or derived from SARS-CoV, such as a polypeptide that encodes a surface protein of SARS-CoV.

In a further embodiment, the immune stimulatory composition contains a SARS-CoV subunit, such as glycoprotein, major capsid protein, or other gene products found to elicit humoral and/or cell mediated immune responses.

The provided immune stimulatory SARS-CoV polypeptides, constructs or vectors encoding such polypeptides, are combined with a pharmaceutically acceptable carrier or

vehicle for administration as an immune stimulatory composition to human or animal subjects. In some embodiments, more than one immune stimulatory SARS-CoV polypeptide may be combined to form a single preparation.

The immunogenic formulations may be conveniently presented in unit dosage form and prepared using conventional pharmaceutical techniques. Such techniques include the step of bringing into association the active ingredient and the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of a sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets commonly used by one of ordinary skill in the art.

In certain embodiments, unit dosage formulations are those containing a dose or unit, or an appropriate fraction thereof, of the administered ingredient. It should be understood that in addition to the ingredients particularly mentioned above, formulations encompassed herein may include other agents commonly used by one of ordinary skill in the art.

The compositions provided herein, including those for use as immune stimulatory compositions, may be administered through different routes, such as oral, including buccal and sublingual, rectal, parenteral, aerosol, nasal, intramuscular, subcutaneous, intradermal, and topical. They may be administered in different forms, including but not limited to solutions, emulsions and suspensions, microspheres, particles, microparticles, nanoparticles, and liposomes.

The volume of administration will vary depending on the route of administration. By way of example, intramuscular injections may range from about 0.1 ml to about 1.0 ml. Those of ordinary skill in the art will know appropriate volumes for different routes of administration.

A relatively recent development in the field of immune stimulatory compounds (for example, vaccines) is the direct injection of nucleic acid molecules encoding peptide antigens (broadly described in Janeway & Travers, *Immunobiology: The Immune System In Health and Disease*, page 13.25, Garland Publishing, Inc., New York, 1997; and McDonnell & Askari, *N. Engl. J. Med.* 334:42-45, 1996). Vectors that include nucleic acid molecules described herein, or that include a nucleic acid sequence encoding an immunogenic SARS-CoV polypeptide may be utilized in such DNA vaccination methods.

Thus, the term "immune stimulatory composition" as used herein also includes nucleic acid vaccines in which a nucleic acid molecule encoding a SARS-CoV polypeptide is administered to a subject in a pharmaceutical composition. For genetic immunization, suitable delivery methods known to those skilled in the art include direct injection of plasmid DNA into muscles (Wolff et al., *Hum. Mol. Genet.* 1:363, 1992), delivery of DNA complexed with specific protein carriers (Wu et al., *J. Biol. Chem.* 264:16985, 1989), coprecipitation of DNA with calcium phosphate (Benvenisty and Reshef, *Proc. Natl. Acad. Sci.* 83:9551, 1986), encapsulation of DNA in liposomes (Kaneda et al., *Science* 243:375, 1989), particle bombardment (Tang et al., *Nature*

356:152, 1992; Eisenbraun et al., *DNA Cell Biol.* 12:791, 1993), and in vivo infection using cloned retroviral vectors (Seeger et al., *Proc. Natl. Acad. Sci.* 81:5849, 1984). Similarly, nucleic acid vaccine preparations can be administered via viral carrier.

The amount of immunostimulatory compound in each dose of an immune stimulatory composition is selected as an amount that induces an immunostimulatory or immunoprotective response without significant, adverse side effects. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Initial injections may range from about 1 µg to about 1 mg, with some embodiments having a range of about 10 µg to about 800 µg, and still other embodiments a range of from about 25 µg to about 500 µg. Following an initial administration of the immune stimulatory composition, subjects may receive one or several booster administrations, adequately spaced. Booster administrations may range from about 1 µg to about 1 mg, with other embodiments having a range of about 10 µg to about 750 µg, and still others a range of about 50 µg to about 500 µg. Periodic boosters at intervals of 1-5 years, for instance three years, may be desirable to maintain the desired levels of protective immunity.

It is also contemplated that the provided immunostimulatory molecules and compositions can be administered to a subject indirectly, by first stimulating a cell in vitro, which stimulated cell is thereafter administered to the subject to elicit an immune response. Additionally, the pharmaceutical or immune stimulatory compositions or methods of treatment may be administered in combination with other therapeutic treatments.

VIII. Kits

Also provided herein are kits useful in the detection and/or diagnosis of SARS-CoV. This includes kits for use with nucleic acid and protein detection methods, such as those disclosed herein.

The SARS-CoV-specific oligonucleotide primers and probes described herein can be supplied in the form of a kit for use in detection of SARS-CoV. In such a kit, an appropriate amount of one or more of the oligonucleotides is provided in one or more containers, or held on a substrate. An oligonucleotide primer or probe can be provided in an aqueous solution or as a freeze-dried or lyophilized powder, for instance. The container(s) in which the oligonucleotide(s) are supplied can be any conventional container that is capable of holding the supplied form, for instance, microfuge tubes, ampoules, or bottles. In some applications, pairs of primers are provided in pre-measured single use amounts in individual (typically disposable) tubes or equivalent containers. With such an arrangement, the sample to be tested for the presence of a SARS-CoV nucleic acid can be added to the individual tubes and amplification carried out directly.

The amount of each oligonucleotide supplied in the kit can be any appropriate amount, and can depend on the market to which the product is directed. For instance, if the kit is adapted for research or clinical use, the amount of each oligonucleotide primer provided would likely be an amount sufficient to prime several PCR amplification reactions. General guidelines for determining appropriate amounts can be found, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; Ausubel et al. (eds.), *Short Protocols in Molecular Biology*, John Wiley and Sons, New York, N.Y., 1999; and Innis et al., *PCR Applications, Protocols for Functional Genomics*, Academic Press, Inc., San Diego, Calif., 1999. A kit can include more than two primers, in order to facilitate the amplification of a larger number of SARS-CoV nucleotide sequences.

In some embodiments, kits also include one or more reagents necessary to carry out in vitro amplification reactions, including DNA sample preparation reagents, appropriate buffers (for example, polymerase buffer), salts (for example, magnesium chloride), and deoxyribonucleotides (dNTPs).

Kits can include either labeled or unlabeled oligonucleotide primers and/or probes for use in detection of SARS-CoV nucleotide sequences. The appropriate sequences for such a probe will be any sequence that falls between the annealing sites of the two provided oligonucleotide primers, such that the sequence that the probe is complementary to is amplified during the amplification reaction.

One or more control sequences for use in the amplification reactions also can be supplied in the kit. In other particular embodiments, the kit includes equipment, reagents, and instructions for extracting and/or purifying nucleotides from a sample.

Kits for the detection of SARS-CoV antigen include for instance at least one SARS-CoV antigen-specific binding agent (for example, a polyclonal or monoclonal antibody or antibody fragment). The kits may also include means for detecting antigen:specific binding agent complexes, for instance the specific binding agent may be detectably labeled. If the specific binding agent is not labeled, it may be detected by second antibodies or protein A, for example, which may also be provided in some kits in one or more separate containers. Such techniques are well known.

Another example of an assay kit provided herein is a recombinant SARS-CoV-specific polypeptide (or fragment thereof) as an antigen and an enzyme-conjugated anti-human antibody as a second antibody. Examples of such kits also can include one or more enzymatic substrates. Such kits can be used to test if a sample from a subject contains antibodies against a SARS-CoV-specific protein.

The subject matter of the present disclosure is further illustrated by the following non-limiting Examples.

EXAMPLES

Example 1

Isolation and Characterization of SARS-CoV

Virus Isolation and Ultrastructural Characterization

This example describes the original isolation and characterization of a new human coronavirus from patients with SARS.

A variety of clinical specimens (blood, serum, material from oropharyngeal swabs or washings, material from nasopharyngeal swabs, and tissues of major organs collected at autopsy) from patients meeting the case definition of SARS were sent to the Centers for Disease Control and Prevention (CDC) as part of the etiologic investigation of SARS. These samples were inoculated onto a number of continuous cell lines, including Vero E6, NCI-H292, MDCK, LLC-MK2, and B95-8 cells, and into suckling ICR mice by the intracranial and intraperitoneal routes. All cultures were observed daily for CPE. Maintenance medium was replenished at day seven, and cultures were terminated fourteen days after inoculation. Any cultures exhibiting identifiable CPE were subjected to several procedures to identify the cause of the effect. Suckling mice were observed daily for fourteen days, and any sick or dead mice were further tested by preparing a brain suspension that was filtered and subcultured. Mice that remained well after fourteen days were killed, and their test results were recorded as negative.

Two cell lines, Vero E6 cells and NCI-H292 cells, inoculated with oropharyngeal specimens from Patient 16 (a 46 year old male physician with an epidemiologic link to a hospital with multiple SARS patients) initially showed CPE (Table 1)

TABLE 1

Specimens from patients with SARS that were positive for SARS-CoV by one or more methods*.								
Patient No.	Exposure and Setting	Age/Sex	Findings on Chest Radiograph	Hospitalization	Serologic Results	Specimen	Isolation	RT-PCR
1	Singapore, hospital	53 yr/F	Pneumonia	Yes	+	Nasal, oropharyngeal swabs	—	Not done
2†	Hong Kong, hotel	36 yr/F	Pneumonia	Yes	+	Nasal, swab	—	Not done
3	Hong Kong, hotel	22 yr/M	Pneumonia	Yes	+	Swab	—	—
4†	Hong Kong, hotel	39 yr/M	Pneumonia	Yes	+	Nasal, pharyngeal swab	—	—
5	Hong Kong, hotel	49 Yr/M	Pneumonia	Yes	Not done	Sputum	+	+
6‡	Hong Kong, hotel	46 yr/M	Pneumonia	Yes	+	Kidney, lung, bronchoalveolar lavage	+§	+
7	Vietnam, hospital	Adult/unknown	Pneumonia	Yes	—	Oropharyngeal wash	+	+
8	Vietnam, hospital	Adult/unknown	Pneumonia	Yes	—	Oropharyngeal wash	—	+
9	Vietnam, hospital	Adult/unknown	Pneumonia	Yes	—	Oropharyngeal wash	—	+
10	Vietnam, hospital	Adult/unknown	Pneumonia	Yes	—	Oropharyngeal wash	—	+
11	Vietnam, hospital	Adult/unknown	Pneumonia	Yes	—	Oropharyngeal wash	—	+

TABLE 1-continued

Specimens from patients with SARS that were positive for SARS-CoV by one or more methods*.								
Patient No.	Exposure and Setting	Age/Sex	Findings on Chest Radiograph	Hospitalization	Serologic Results	Specimen	Isolation	RT-PCR
12	Vietnam, hospital	Adult/unknown	Pneumonia	Yes	–	Oropharyngeal wash	–	+
13	Vietnam, hospital	Adult/unknown	Pneumonia	Yes	–	Oropharyngeal wash	+	+
14	Vietnam, hospital	Adult/unknown	Pneumonia	Yes	–	Oropharyngeal wash	–	+
15	Vietnam, hospital	Adult/unknown	Pneumonia	Yes	–	Oropharyngeal wash	–	+
16	Vietnam, hospital	46 yr/M	Pneumonia	Yes	+	Nasal, oropharyngeal swabs	+¶	+
17	Canada, family	43 yr/M	Pneumonia	Yes	Not done	Lung, bone marrow	–	
18	Taiwan, family	51 yr/F	Pneumonia	Yes	–	Sputum	–	+
19	Hong Kong, hotel	Adult/F	Pneumonia	Yes	+	Oropharyngeal wash	–	+

*Plus signs denote positive results, and minus signs negative results. The serologic and RT-PCR assays were not necessarily performed on samples obtained at the same time.

†This was a late specimen, antibody positive at first sample.

‡Travel included China, Hong Kong (hotel), and Hanoi (the patient was the index patient in the French Hospital).

§Isolation was from the kidney only.

¶Isolation was from the oropharyngeal only.

The CPE in the Vero E6 cells was first noted on the fifth day post-inoculation; it was focal, with cell rounding and a refractive appearance in the affected cells that was soon followed by cell detachment (FIG. 1A). The CPE spread quickly to involve the entire cell monolayer within 24 to 48 hours. Subculture of material after preparation of a master seed stock (used for subsequent antigen production) resulted in the rapid appearance of CPE, as noted above, and in complete destruction of the monolayer in the inoculated flasks within 48 hours. Similar CPE was also noted in four additional cultures: three cultures of respiratory specimens (two oropharyngeal washes and one sputum specimen) and one culture of a suspension of kidney tissue obtained at autopsy. In these specimens, the initial CPE was observed between day two and day four and, as noted above, the CPE rapidly progressed to involve the entire cell monolayer.

Tissue culture samples showing CPE were prepared for electron-microscopical examination. Negative-stain electron-microscopical specimens were prepared by drying culture supernatant, mixed 1:1 with 2.5% paraformaldehyde, onto Formvarcarbon-coated grids and staining with 2% methylamine tungstate. Thin-section electron-microscopical specimens were prepared by fixing a washed cell pellet with 2.5% glutaraldehyde and embedding the cell pellet in epoxy resin. In addition, a master seed stock was prepared from the remaining culture supernatant and cells by freeze-thawing the culture flask, clarifying the thawed contents by centrifugation at 1000×g, and dispensing the supernatant into aliquots stored in gas phase over liquid nitrogen. The master seed stock was subcultured into 850-cm² roller bottles of Vero E6 cells for the preparation of formalin-fixed positive control cells for immunohistochemical analysis, mixed with normal Vero E6 cells, and gamma-irradiated for preparation of spot slides for IFA tests or extracted with detergent and gamma-irradiated for use as an ELISA antigen for antibody tests.

Examination of CPE-positive Vero E6 cells by thin-section electron microscopy revealed characteristic coronavirus particles within the cisternae of the rough endoplasmic reticulum and in vesicles (FIG. 2A) (Becker et al., *J.*

Virol. 1:1019–27, 1967; Oshiro et al. *J. Gen. Virol.* 12:161–8, 1971). Extracellular particles were found in large clusters and adhering to the surface of the plasma membrane. Negative-stain electron microscopy identified coronavirus particles, 80 to 140 nm in diameter, with 20- to 40-nm complex surface projections surrounding the periphery (FIG. 2B). Hemagglutinin esterase-type glycoprotein projections were not seen.

The isolation and growth of a human-derived coronavirus in Vero E6 cells were unexpected. The previously known human coronaviruses are notably fastidious, preferring select cell lines, organ culture, or suckling mice for propagation. The only human or animal coronavirus which has been shown to grow in Vero E6 cells is PEDV, and it requires the addition of trypsin to culture medium for growth in the cells. Moreover, PEDV adapted to growth in Vero E6 cells results in a strikingly different CPE, with cytoplasmic vacuoles and the formation of large syncytia. Syncytial cells were only observed occasionally in monolayers of Vero E6 cells infected with the SARS-CoV; they clearly do not represent the dominant CPE.

Reverse Transcription-Polymerase Chain Reaction and Sequencing

For RT-PCR assays, cell-culture supernatants were placed in lysis buffer. RNA extracts were prepared from 100 µl of each specimen (or culture supernatant) with the automated NucliSens extraction system (bioMérieux, Durham, N.C.). Initially, degenerate, inosine-containing primers IN-2 (+) 5'GGGTTGGGACTA TCCTAAGTGTGA3' (SEQ ID NO: 34) and IN-4 (–) 5'TAACACACAACICCATCA TCA3' (SEQ ID NO: 35) were designed to anneal to sites encoding conserved amino acid motifs that were identified on the basis of alignments of available coronavirus ORF1a, ORF1b, S, HE, M, and N gene sequences. Additional, SARS-specific, primers Cor-p-F2 (+) 5'CTAACATGCT-TAGGATAATGG3' (SEQ ID NO: 13), Cor-p-F3 (+) 5'GCCTCTCTTGTCTTGCTCGC3' (SEQ ID NO: 14), and Cor-p-R1 (–) 5'CAGGTAAGCGTAAACTCATC3 (SEQ ID NO: 15) were designed as sequences were gener-

ated from RT-PCR products amplified with the degenerate primers. These SARS-specific primers were used to test patient specimens for SARS (see below). Primers used for specific amplification of human metapneumovirus have been described by Falsey et al. (*J. Infect. Dis.* 87:785–90, 2003).

For RT-PCR products of less than 3 kb, cDNA was synthesized in a 20 μ l reaction mixture containing 500 ng of RNA, 200 U of SuperscriptTM II reverse transcriptase (Invitrogen Life Technologies, Carlsbad, Calif.), 40 U of RNasin (Promega Corp., Madison, Wis.), 100 mM each dNTP (Roche Molecular Biochemicals, Indianapolis, Ind.), 4 μ l of 5 \times reaction buffer (Invitrogen Life Technologies, Carlsbad, Calif.), and 200 pmol of the reverse primer. The reaction mixture, except for the reverse transcriptase, was heated to 70° C. for 2 minutes, cooled to 4° C. for 5 minutes and then heated to 42° C. in a thermocycler. The mixture was held at 42° C. for 4 minutes, and then the reverse transcriptase was added, and the reactions were incubated at 42° C. for 45 minutes. Two microliters of the cDNA reaction was used in a 50 μ l PCR reaction containing 67 mM Tris-HCl (pH 8.8), 1 mM each primer, 17 mM ammonium sulfate, 6 mM EDTA, 2 mM MgCl₂, 200 mM each dNTP, and 2.5 U of Taq DNA polymerase (Roche Molecular Biochemicals, Indianapolis, Ind.). The thermocycler program for the PCR consisted of 40 cycles of denaturation at 95° C. for 30 seconds, annealing at 42° C. for 30 seconds, and extension at 65° C. for 30 seconds. For SARS-CoV-specific primers, the annealing temperature was increased to 55° C.

For amplification of fragments longer than 3 kb, regions of the genome between sections of known sequence were amplified by means of a long RT-PCR protocol and SARS-CoV-specific primers. First-strand cDNA synthesis was performed at 42° C. or 50° C. using SuperscriptTM II RNase H reverse transcriptase (Invitrogen Life Technologies, Carlsbad, Calif.) according to the manufacturer's instructions with minor modifications. Coronavirus-specific primers (500 ng) and SARS-CoV RNA (350 ng) were combined with the PCR Nucleotide Mix (Roche Molecular Biochemicals, Indianapolis, Ind.), heated for 1 minute at 94° C., and cooled to 4° C. in a thermocycler. The 5 \times first-strand buffer, dithiothreitol (Invitrogen Life Technologies, Carlsbad, Calif.), and Protector RNase Inhibitor (Roche Molecular Biochemicals, Indianapolis, Ind.) were added, and the samples were incubated at 42° C. or 50° C. for 2 minutes. After reverse transcriptase (200 U) was added, the samples were incubated at 42° C. or 50° C. for 1.5 to 2 hours. Samples were inactivated at 70° C. for 15 minutes and subsequently treated with 2 U of RNase H (Roche Molecular Biochemicals, Indianapolis, Ind.) at 37° C. for 30 minutes. Long RT-PCR amplification of 5- to 8-kb fragments was performed using Taq Plus Precision (Stratagene, La Jolla, Calif.) and AmpliWax PCR Gem 100 beads (Applied Biosystems; Foster City, Calif.) for "hot start" PCR with the following thermocycling parameters: denaturation at 94° C. for 1 minute followed by 35 cycles of 94° C. for 30 seconds, 55° C. for 30 seconds, an increase of 0.4 degrees per second up to 72° C., and 72° C. for 7 to 10 minutes, with a final extension at 72° C. for 10 minutes. RT-PCR products were separated by electrophoresis on 0.9% agarose TAE gels and purified by use of a QIAquick Gel Extraction Kit (Qiagen, Inc., Santa Clarita, Calif.).

In all cases, the RT-PCR products were gel-isolated and purified for sequencing by means of a QIAquick Gel Extraction kit (Qiagen, Inc., Santa Clarita, Calif.). Both strands were sequenced by automated methods, using fluorescent dideoxy-chain terminators (Applied Biosystems; Foster City, Calif.).

The sequence of the leader was obtained from the sub-genomic mRNA coding for the N gene and from the 5'

terminus of genomic RNA. The 5' rapid amplification of cDNA ends (RACE) technique (Harcourt et al., *Virology* 271:334–49, 2000) was used with reverse primers specific for the N gene or for the 5' untranslated region. RACE products were either sequenced directly or were cloned into a plasmid vector before sequencing. A primer that was specific for the leader of SARS-CoV was used to amplify the region between the 5'-terminus of the genome and known sequences in the rep gene. The 3'-terminus of the genome was amplified for sequencing by use of an oligo-(dT) primer and primers specific for the N gene.

Once the complete SARS-CoV genomic sequence had been determined, it was confirmed by sequencing a series of independently amplified RT-PCR products spanning the entire genome. Positive- and negative-sense sequencing primers, at intervals of approximately 300 nt, were used to generate a confirmatory sequence with an average redundancy of 9.1. The confirmatory sequence was identical to the original sequence. The genomic sequence (SEQ ID NO: 1) was published in the GenBank sequence database (Accession No. AY278741) on Apr. 21, 2003.

Sequence Analysis

Predicted amino acid sequences were compared with those from reference viruses representing each species for which complete genomic sequence information was available: group 1 representatives included human coronavirus 229E (GenBank Accession No. AF304460), porcine epidemic diarrhea virus (GenBank Accession No. AF353511), and transmissible gastroenteritis virus (GenBank Accession No. AF271965); group 2 representatives included bovine coronavirus (GenBank Accession No. AF220295) and mouse hepatitis virus (GenBank Accession No. AF201929); group 3 was represented by infectious bronchitis virus (GenBank Accession No. M95169). Sequences for representative strains of other coronavirus species for which partial sequence information was available were included for some of the structural protein comparisons: group 1 representative strains included canine coronavirus (GenBank Accession No. D13096), feline coronavirus (GenBank Accession No. AY204704), and porcine respiratory coronavirus (GenBank Accession No. Z24675); and group 2 representatives included three strains of human coronavirus OC43 (GenBank Accession Nos. M76373, L14643 and M93390), porcine hemagglutinating encephalomyelitis virus (GenBank Accession No. AY078417), and rat coronavirus (GenBank Accession No. AF207551).

Partial nucleotide sequences of the polymerase gene were aligned with published coronavirus sequences, using CLUSTAL W for Unix (version 1.7; Thompson et al., *Nucleic Acids Res.* 22:4673–80, 1994). Phylogenetic trees were computed by maximum parsimony, distance, and maximum likelihood-based criteria analysis with PAUP (version 4.0.d10; Swofford ed., *Phylogenetic Analysis using Parsimony and other Methods*, Sinauer Associates, Sunderland, Mass.). When compared with other human and animal coronaviruses, the nucleotide and deduced amino acid sequence from this region had similarity scores ranging from 0.56 to 0.63 and from 0.57 to 0.74, respectively. The highest sequence similarity was obtained with group II coronaviruses. The maximum-parsimony tree obtained from the nucleotide-sequence alignment is shown in FIG. 3. Bootstrap analyses of the internal nodes at the internal branches of the tree provided strong evidence that the SARS-CoV is genetically distinct from other known coronaviruses.

Microarray analyses (using a long oligonucleotide DNA microarray with array elements derived from highly conserved regions within viral families) of samples from infected and uninfected cell cultures gave a positive signal for a group of eight oligonucleotides derived from two virus

families: Coronaviridae and Astroviridae (Wang et al., *PNAS* 99:15687–92, 2002). All of the astroviruses and two of the coronavirus oligonucleotides share a consensus sequence motif that maps to the extreme 3'-end of astroviruses and two members of the coronavirus family: avian infectious bron-

chitis and turkey coronavirus (Jonassen et al., *J. Gen. Virol.* 79:715–8, 1998). Results were consistent with the identity of the isolate as a coronavirus.

Additional sequence alignments and neighbor-joining trees were generated by using ClustalX (Thompson et al., *Nucleic Acids Res.* 25:4876–82, 1997), version 1.83, with the Gonnet protein comparison matrix. The resulting trees were adjusted for final output by using treetool version 2.0.1. Uncorrected pairwise distances were calculated from the aligned sequences by using the Distances program from the Wisconsin Sequence Analysis Package, version 10.2 (Accelrys, Burlington, Mass.). Distances were converted to percent identity by subtracting from 100. The amino acid sequences for three well-defined enzymatic proteins encoded by the rep gene and the four major structural proteins of SARS-CoV were compared with those from representative viruses for each of the species of coronavirus for which complete genomic sequence information was available (FIG. 4, Table 2). The topologies of the resulting phylograms are remarkably similar (FIG. 4). For each protein analyzed, the species formed monophyletic clusters consistent with the established taxonomic groups. In all cases, SARS-CoV sequences segregated into a fourth, well-resolved branch. These clusters were supported by bootstrap values above 90% (1000 replicates). Consistent with pairwise comparisons between the previously characterized coronavirus species (Table 2), there was greater sequence conservation in the enzymatic proteins (3CL^{PRO}, polymerase (POL), and helicase (HEL)) than among the structural proteins (S, E, M, and N). These results indicate that SARS-CoV is not closely related to any of the previously characterized coronaviruses and forms a distinct group within the genus Coronavirus.

TABLE 2

Pairwise amino acid identities of coronavirus proteins.								
Group	Virus	3CLPRO	POL	HEL	S	E	M	N
Pairwise Amino Acid Identity (Percent)								
G1	HCoV-229E	40.1	58.8	59.7	23.9	22.7	28.8	23.0
	PEDV	44.4	59.5	61.7	21.7	17.6	31.8	22.6
	TGEV	44.0	59.4	61.2	20.6	22.4	30.0	25.6
G2	BCoV	48.8	66.3	68.3	27.1	20.0	39.7	31.9
	MHV	49.2	66.5	67.3	26.5	21.1	39.0	33.0
G3	IBV	41.3	62.5	58.6	21.8	18.4	27.2	24.0
Predicted Protein Length (aa)								
	SARS-CoV	306	932	601	1255	76	221	422
	CoV Range	302–307	923–940	506–600	1173–1452	76–108	225–262	377–454

Example 2

Detection of SARS-CoV in a Subject

This example demonstrates the detection of SARS-CoV in patient specimens using SARS-CoV-specific primers.

The SARS-specific primers Cor-p-F2 (SEQ ID NO: 13), Cor-p-F3 (SEQ ID NO: 14) and Cor-p-R1 (SEQ ID NO: 15) were used to test patient specimens for SARS. One primer for each set was 5'-end-labeled with 6-FAM to facilitate GeneScan analysis. One-step amplification reactions were performed with the Access RT-PCR System (Promega, Madison, Wis.) as described by Falsey et al., *J. Infect. Dis.*

87:785–90, 2003. Positive and negative RT-PCR controls, containing standardized viral RNA extracts, and nuclease-free water were included in each run. Amplified 6-FAM-labeled products were analyzed by capillary electrophoresis on an ABI 3100 Prism Genetic Analyzer with GeneScan software (version 3.1.2; Applied Biosystems; Foster City, Calif.). Specimens were considered positive for SARS-CoV if the amplification products were within one nucleotide of the expected product size (368 nucleotides for Cor-p-F2 or Cor-p-R1 and 348 nucleotides for Cor-p-F3 or Cor-p-R1) for both specific primer sets, as confirmed by a second PCR reaction from another aliquot of RNA extract in a separate laboratory. Where DNA yield was sufficient, the amplified products were also sequenced. Additionally, as described above, microarray-based detection of SARS-CoV in patient specimens was carried out (Wang et al., *PNAS* 99:15687–92, 2002 and Bohlander et al., *Genomics* 13:1322–24, 1992).

Example 3

Immunohistochemical and Histopathological Analysis, and Electron-Microscopical Analysis of Bronchoalveolar Lavage Fluid

This example illustrates immunohistochemical, histopathological and electron-microscopical analysis of Vero E6 cells infected with the SARS-CoV and tissue samples from SARS patients.

Formalin-fixed, paraffin-embedded Vero E6 cells infected with the SARS-CoV and tissues obtained from patients with SARS were stained with hematoxylin and eosin and various immunohistochemical stains. Immunohistochemical assays were based on a method described previously for hantavirus (Zaki et al., *Amer. J. Pathol.* 146:552–79, 1995). Briefly, 4-μm sections were deparaffinized, rehydrated, and digested in Proteinase K for 15 minutes. Slides were then incubated for 60 minutes at room temperature with monoclonal anti-

bodies, polyclonal antiserum or ascitic fluids derived from animal species with reactivities to various known coronaviruses, and with a convalescent-phase serum specimen from a patient with SARS.

Optimal dilutions of the primary antibodies were determined by titration experiments with coronavirus-infected cells from patients with SARS and with noninfected cells or, when available, with concentrations recommended by the manufacturers. After sequential application of the appropriate biotinylated link antibody, avidin-alkaline phosphatase complex, and naphthol-fast red substrate, sections were counterstained in Mayer's hematoxylin and mounted with aqueous mounting medium. The following antibody and tissue controls were used: serum specimens from nonin-

fectured animals, various coronavirus-infected cell cultures and animal tissues, noninfected cell cultures, and normal human and animal tissues. Tissues from patients were also tested by immunohistochemical assays for various other viral and bacterial pulmonary pathogens. In addition, a BAL specimen was available from one patient for thin-section electron-microscopical evaluation.

Lung tissues were obtained from the autopsy of three patients and by open lung biopsy of one patient, 14–19 days following onset of SARS symptoms. Confirmatory laboratory evidence of infection with coronavirus was available for two patients (patients 6 and 17) and included PCR amplification of coronavirus nucleic acids from tissues, viral isolation from BAL fluid or detection of serum antibodies reactive with coronavirus (Table 1). For two patients, no samples were available for molecular, cell culture, or serological analysis; however, both patients met the CDC definition for probable SARS cases and had strong epidemiologic links with laboratory-confirmed SARS cases. Histopathologic evaluation of lung tissues of the four patients showed diffuse alveolar damage at various levels of progression and severity. Changes included hyaline membrane formation, interstitial mononuclear inflammatory infiltrates, and desquamation of pneumocytes in alveolar spaces (FIG. 5A). Other findings identified in some patients included focal intraalveolar hemorrhage, necrotic inflammatory debris in small airways, and organizing pneumonia. Multinucleated syncytial cells were identified in the intraalveolar spaces of two patients who died 14 and 17 days, respectively, after onset of illness. These cells contained abundant vacuolated cytoplasm with cleaved and convoluted nuclei. No obvious intranuclear or intracytoplasmic viral inclusions were identified (FIG. 5B), and electron-microscopical examination of a limited number of these syncytial cells revealed no coronavirus particles. No definitive immunostaining was identified in tissues from SARS patients with the use of a battery of immunohistochemical stains reactive with coronaviruses from antigenic groups I, II, and III. In addition, no staining of patient tissues was identified with the use of immunohistochemical stains for influenzaviruses A and B, adenoviruses, Hendra and Nipah viruses, human metapneumovirus, respiratory syncytial virus, measles virus, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*.

Evaluation of Vero E6 cells infected with coronavirus isolated from a patient with SARS revealed viral CPE that included occasional multinucleated syncytial cells but no obvious viral inclusions (FIG. 5C). Immunohistochemical assays with various antibodies reactive with coronaviruses from antigenic group I, including HCoV-229E, FIPV and TGEV, and with an immune serum specimen from a patient with SARS, demonstrated strong cytoplasmic and membranous staining of infected cells (FIG. 5C and Table 3); however, cross-reactivity with the same immune human serum sample and FIPV antigen was not observed. No staining was identified with any of several monoclonal or polyclonal antibodies reactive with coronaviruses in antigenic group II (HCoV-OC43, BCoV and MHV) or group III (TCoV and IBV-Avian). Electron microscopical examination of a BAL fluid from one patient revealed many coronavirus-infected cells (FIGS. 6A–B).

TABLE 3

Immunohistochemical reactivities of various polyclonal group I anti-coronavirus reference antiserum samples with a coronavirus isolated from a patient with SARS and with selected antigenic group I coronaviruses.

Antiserum	Immunohistochemical reactivity of antiserum with coronavirus-infected culture cells		
	SARS-CoV (Vero E6)	HCoV-229E (mouse 3T3-hAPN)	FIPV-1 (BHK-fAPN)
Convalescent-phase SARS (patient 3)	+	+	–
Guinea pig anti-HCoV-229E	+	+	–
Rabbit anti-HCoV-229E	+	+	+
Feline anti-FIPV-1	+	+	+
Porcine anti-TGEV	+	–	+

Example 4

SARS-CoV Serologic Analysis

This example illustrates representative methods of performing serological analysis of SARS-CoV.

Spot slides were prepared by applying 15 µl of the suspension of gamma-irradiated mixed infected and noninfected cells onto 12-well Teflon-coated slides. Slides were allowed to air dry before being fixed in acetone. Slides were then stored at –70° C. until used for IFA tests (Wulff and Lange, *Bull. WHO* 52:429–36, 1975). An ELISA antigen was prepared by detergent extraction and subsequent gamma irradiation of infected Vero E6 cells (Ksiazek et al., *J. Infect. Dis.* 179 (suppl. 1):S191–8, 1999). The optimal dilution (1:1000) for the use of this antigen was determined by checkerboard titration against SARS patient serum from the convalescent phase; a control antigen, similarly prepared from uninfected Vero E6 cells, was used to control for specific reactivity of tested sera. The conjugates used were goat antihuman IgG, IgA, and IgM conjugated to fluorescein isothiocyanate and horseradish peroxidase (Kirkegaard and Perry, Gaithersburg, Md.), for the IFA test and ELISA, respectively. Specificity and cross-reactivity of a variety of serum samples to the newly identified virus were evaluated by using the tests described herein. For this evaluation, serum from SARS patients in Singapore, Bangkok and Hong Kong was used, along with serum from healthy blood donors from the CDC serum bank and from persons infected with known human coronavirus (human coronaviruses OC43 and 229E) (samples provided by E. Walsh and A. Falsey, University of Rochester School of Medicine and Dentistry, Rochester, N.Y.).

Spot slides with infected cells reacted with serum from patients with probable SARS in the convalescent phase (FIG. 1B). Screening of a panel of serum from patients with suspected SARS from Hong Kong, Bangkok, Singapore as well as the United States showed a high level of specific reaction with infected cells, and conversion from negative to positive reactivity or diagnostic rises in the IFA test by a factor of four. Similarly, tests of these same serum samples with the ELISA antigen showed high specific signal in the convalescent-phase samples and conversion from negative to positive antibody reactivity or diagnostic increases in titer (Table 4).

TABLE 4

Results of serological testing with both IFA assay and ELISA in SARS patients tested against the newly isolated human coronavirus.

Source	Serum No.	Days After Onset	ELISA Titer*	IFA Titer*
Hong Kong	1.1	4	<100	<25
Hong Kong	1.2	13	≥6400	1600
Hong Kong	2.1	11	400	100
Hong Kong	2.2	16	1600	200
Hong Kong	3.1	7	<100	<25
Hong Kong	3.2	17	≥6400	800
Hong Kong	4.1	8	<100	<25
Hong Kong	4.2	13	1600	50
Hong Kong	5.1	10	100	<25
Hong Kong	5.2	17	≥6400	1600
Hong Kong	6.1	12	1600	200
Hong Kong	6.2	20	≥6400	6400
Hong Kong	7.1	17	400	50
Hong Kong	7.2	24	≥6400	3200
Hong Kong	8.1	3	<100	<25
Hong Kong	8.2	15	≥6400	200
Hong Kong	9.1	5	<100	<25
(Hanoi)				
Hong Kong	9.2	11	≥6400	1600
Bangkok	1.1	2	<100	<25
Bangkok	1.2	4	<100	<25
Bangkok	1.3	7	<100	<25
Bangkok	1.4	15	1600	200
United States	1.1	2	<100	<25
United States	1.2	6	400	50
United States	1.3	13	≥6400	800
Singapore	1.1	2	100	<25
Singapore	1.2	11	≥6400	800
Singapore	2.1	6	100	<25
Singapore	2.2	25	≥6400	400
Singapore	3.1	6	100	<25
Singapore	3.2	14	≥6400	400
Singapore	4.1	5	100	<25
Singapore	4.2	16	1600	400

*Reciprocal of dilution

Information from the limited numbers of samples tested suggests that antibody is first detectable by IFA assay and ELISA between one and two weeks after the onset of symptoms in the patient. IFA testing and ELISA of a panel of 384 randomly selected serum samples (from U.S. blood donors) were negative for antibodies to the new coronavirus, with the exception of one specimen that had minimal reactivity on ELISA. A panel of paired human serum samples with diagnostic increases (by a factor of four or more) in antibody (with very high titers to the homologous viral antigen in the convalescent-phase serum) to the two known human coronaviruses, OC43 (13 pairs) and 229E (14 pairs), showed no reactivity in either acute- or convalescent-phase serum with the newly isolated coronavirus by either the IFA test or the ELISA.

Example 5

Poly(A)⁺ RNA Isolation and Northern Hybridization

This example illustrates a representative method of Northern hybridization to detect SARS-CoV messages in Vero E6 cells.

Total RNA from infected or uninfected Vero E6 cells was isolated with Trizol reagent (Invitrogen Life Technologies, Carlsbad, Calif.) used according to the manufacturer's recommendations. Poly(A)⁺ RNA was isolated from total RNA by use of the Oligotex Direct mRNA Kit (Qiagen, Inc., Santa Clarita, Calif.), following the instructions for the batch protocol, followed by ethanol precipitation. RNA isolated from 1 cm² of cells was separated by electrophoresis on a

0.9% agarose gel containing 3.7% formaldehyde, followed by partial alkaline hydrolysis (Ausubel et al. eds. *Current Protocols in Molecular Biology*, vol. 1, John Wiley & Sons, Inc., NY, N.Y., Ch. 4.9, 1996). RNA was transferred to a nylon membrane (Roche Molecular Biochemicals, Indianapolis, Ind.) by vacuum blotting (Bio-Rad, Hercules, Calif.) and fixed by UV cross-linking. The DNA template for probe synthesis was generated by RT-PCR amplification of SARS-CoV nt 29,083 to 29,608 (SEQ ID NO: 1), by using a reverse primer containing a T7 RNA polymerase promoter to facilitate generation of a negative-sense riboprobe. In vitro transcription of the digoxigenin-labeled riboprobe, hybridization, and detection of the bands were carried out with the digoxigenin system by using manufacturer's recommended procedures (Roche Molecular Biochemicals, Indianapolis, Ind.). Signals were visualized by chemiluminescence and detected with x-ray film.

Example 6

SARS-CoV Genome Organization

This example illustrates the genomic organization of the SARS-CoV genome, including the location of SARS-CoV ORFs.

The genome of SARS-CoV is a 29,727-nucleotide, polyadenylated RNA, and 41% of the residues are G or C (range for published coronavirus complete genome sequences, 37% to 42%). The genomic organization is typical of coronaviruses, having the characteristic gene order [5'-replicase (rep), spike (S), envelope (E), membrane (M), nucleocapsid (N)-3'] and short untranslated regions at both termini (FIG. 7A, Table 5). The SARS-CoV rep gene, which comprises approximately two-thirds of the genome, encodes two polyproteins (encoded by ORF1a and ORF1b) that undergo co-translational proteolytic processing. There are four ORFs downstream of rep that encode the structural proteins, S, E, M, and N, which are common to all known coronaviruses. The hemagglutinin-esterase gene, which is present between ORF1b and S in group 2 and some group 3 coronaviruses (Lai and Holmes, in *Fields Virology*, eds. Knipe and Howley, Lippincott Williams and Wilkins, New York, 4th edition, 2001, Ch. 35), was not found in SARS-CoV.

Coronaviruses also encode a number of non-structural proteins that are located between S and E, between M and N, or downstream of N. These non-structural proteins, which vary widely among the different coronavirus species, are of unknown function and are dispensable for virus replication (Lai and Holmes, in *Fields Virology*, eds. Knipe and Howley, Lippincott Williams and Wilkins, New York, 4th edition, 2001, Ch. 35). The genome of SARS-CoV contains ORFs for five non-structural proteins of greater than 50 amino acids (FIG. 7B, Table 5). Two overlapping ORFs encoding proteins of 274 and 154 amino acids (termed X1 (SEQ ID NO: 5) and X2 (SEQ ID NO: 6), respectively) are located between S (SEQ ID NO: 4) and E (SEQ ID NO: 7). Three additional non-structural genes, X3 (SEQ ID NO: 9), X4 (SEQ ID NO: 10), and X5 (SEQ ID NO: 11) (encoding proteins of 63, 122, and 84 amino acids, respectively), are located between M (SEQ ID NO: 8) and N (SEQ ID NO: 12). In addition to the five ORFs encoding the non-structural proteins described above, there are also two smaller ORFs between M and N, encoding proteins of less than 50 amino acids. Searches of the GenBank database (BLAST and FastA) indicated that there is no significant sequence similarity between these non-structural proteins of SARS-CoV and any other proteins.

The coronavirus rep gene products are translated from genomic RNA, but the remaining viral proteins are translated from subgenomic mRNAs that form a 3'-coterminally nested set, each with a 5'-end derived from the genomic 5'-leader sequence. The coronavirus subgenomic mRNAs are synthesized through a discontinuous transcription process, the mechanism of which has not been unequivocally established (Lai and Holmes, in *Fields Virology*, eds. Knipe and Howley, Lippincott Williams and Wilkins, New York, 4th edition, 2001, Ch. 35; Sawicki and Sawicki, *Adv. Exp. Med. Biol.* 440:215–19, 1998). The SARS-CoV leader sequence was mapped by comparing the sequence of 5'-RACE products synthesized from the N gene mRNA with those synthesized from genomic RNA. A sequence, AAAC-GAAC (nucleotides 65–72 of SEQ ID NO: 1), was identified immediately upstream of the site where the N gene mRNA and genomic sequences diverged. This sequence was also present upstream of ORF1a and immediately upstream of five other ORFs (Table 5), suggesting that it functions as the conserved core of the transcriptional regulatory sequence (TRS).

In addition to the site at the 5'-terminus of the genome, the TRS conserved core sequence appears six times in the remainder of the genome. The positions of the TRS in the genome of SARS-CoV predict that subgenomic mRNAs of 8.3, 4.5, 3.4, 2.5, 2.0, and 1.7 kb, not including the poly(A) tail, should be produced (FIGS. 7A–B, Table 5). At least five subgenomic mRNAs were detected by Northern hybridization of RNA from SARS-CoV-infected cells, using a probe derived from the 3'-untranslated region (FIG. 7C). The calculated sizes of the five predominant bands correspond to the sizes of five of the predicted subgenomic mRNAs of SARS-CoV; the possibility that other, low-abundance mRNAs are present cannot be excluded. By analogy with other coronaviruses (Lai and Holmes, in *Fields Virology*, eds. Knipe and Howley, Lippincott Williams and Wilkins, New York, 4th edition, 2001, Ch. 35), the 8.3-kb and 1.7-kb subgenomic mRNAs are monocistronic, directing translation of S and N, respectively, whereas multiple proteins are translated from the 4.5-kb (X1, X2, and E), 3.4-kb (M and X3), and 2.5-kb (X4 and X5) mRNAs. A consensus TRS is not found directly upstream of the ORF encoding the predicted E protein, and a monocistronic mRNA that would be predicted to code for E could not be clearly identified by Northern blot analysis. It is possible that the 3.6-kb band contains more than one mRNA species or that the monocistronic mRNA for E is a low-abundance message.

TABLE 5

Locations of SARS-CoV ORFs and sizes of proteins and mRNAs					
ORF	Genome Location			Predicted Size	
	TRS ^a	ORF Start	ORF End	Protein (aa)	mRNA (nt) ^b
1a	72	265	13,398	4,378	29,727
1b		13,398	21,482	2,695	
S	21,491	21,492	25,256	1,255	8,308 ^c
X1	25,265	25,268	26,089	274	4,534 ^c
X2		25,689	26,150	154	
E		26,117	26,344	76	
M	26,353	26,398	27,060	221	3,446 ^c
X3		27,074	27,262	63	
X4	27,272	27,273	27,638	122	2,527 ^c

TABLE 5-continued

Locations of SARS-CoV ORFs and sizes of proteins and mRNAs					
ORF	Genome Location			Predicted Size	
	TRS ^a	ORF Start	ORF End	Protein (aa)	mRNA (nt) ^b
X5	27,778	27,864	28,115	84	2,021 ^d
N	28,111	28,120	29,385	422	1,688 ^e

^aThe location is the 3'-most nucleotide in the consensus TRS, AAAC-GAAC.

^bNot including poly(A). Predicted size is based on the position of the conserved TRS.

^cCorresponding mRNA detected by Northern blot analysis (FIG. 7C)

^dNo mRNA corresponding to utilization of this consensus TRS was detected by Northern blot analysis (FIG. 7C)

Example 7

Real-Time RT-PCR Assay for SARS-CoV Detection

This example demonstrates the use of SARS-CoV-specific primers and probes in a real-time RT-PCR assay to detect SARS-CoV in patient specimens.

A variant of the real-time format, based on TaqMan probe hydrolysis technology (Applied Biosystems, Foster City, Calif.), was used to analyze a total of 340 clinical specimens collected from 246 persons with confirmed or suspected SARS-CoV infection. Specimens included oro- and nasopharyngeal swabs (dry and in viral transport media), sputa, nasal aspirates and washes, BAL, and lung tissue specimens collected at autopsy.

Nucleic Acid Extraction

SARS-CoV nucleic acids were recovered from clinical specimens using the automated NucliSens extraction system (bioMérieux, Durham, N.C.). Following manufacturer's instructions, specimens received in NucliSens lysis buffer were incubated at 37° C. for 30 min with intermittent mixing, and 50 µL of silica suspension, provided in the extraction kit, was added and mixed. The contents of the tube were then transferred to a nucleic acid extraction cartridge and processed on an extractor workstation. Approximately 40–50 µL of total nucleic acid eluate was recovered into nuclease-free vials and either tested immediately or stored at –70° C.

Primers and Probes

Multiple primer and probe sets were designed from the SARS-CoV polymerase 1b (nucleic acid 13,398 to 21,482 of SEQ ID NO: 1) and nucleocapsid gene (nucleic acid 28,120 to 29,385 of SEQ ID NO: 1) sequences by using Primer Express software version 1.5 or 2.0.0 (Applied Biosystems, Foster City, Calif.) with the following default settings: primer melting temperature (T_M) set at 60° C.; probe T_M set at 10° C. greater than the primers at approximately 70° C.; and no guanine residues permitted at the 5' probe termini. All primers and probes were synthesized by standard phosphoramidite chemistry techniques. TaqMan probes were labeled at the 5'-end with the reporter 6-FAM and at the 3'-end with the quencher Blackhole Quencher 1 (Biosearch Technologies, Inc., Novato, Calif.). Optimal primer and probe concentrations were determined by cross-titration of serial twofold dilutions of each primer against a constant amount of purified SARS-CoV RNA. Primer and probe concentrations that gave the highest amplification efficiencies were selected for further study (Table 6).

TABLE 6

Primers and probes used for real-time RT-PCR assays ^a			
Assay ID	Primer/probe	Sequence	Genomic Region
Primary diagnostic assay			
SARS1	F	CATGTGTGGCGGCTCACTATAT (SEQ ID NO: 16)	RNA Pol
	R	GACACTATTAGCATAAGCAGTTGTAGCA (SEQ ID NO: 17)	
	P	TTAAACCAAGGTGGAACATCATCCGGTG (SEQ ID NO: 18)	
SARS2	F	GGAGCCTTGAATACACCCAAAG (SEQ ID NO: 19)	Nucleocapsid
	R	GCACGGTGGCAGCATTG (SEQ ID NO: 20)	
	P	CCACATTGGCACCCGCAATCC (SEQ ID NO: 21)	
SARS3	F	CAAACATTGGCCGCAAAIT (SEQ ID NO: 22)	Nucleocapsid
	R	CAATGCGTGACATTCCAAAGA (SEQ ID NO: 23)	
	P	CACAATTTGCTCCAAGTGCCTCTGCA (SEQ ID NO: 24)	
To confirm positive results			
N3	F	GAAGTACCATCTGGGGCTGAG (SEQ ID NO: 25)	Nucleocapsid
	R	CCGAAGAGCTACCCGACG (SEQ ID NO: 26)	
	P	CTCTTTTCATTTTGCCGTCACCACCAC (SEQ ID NO: 27)	
3'-NTR	F	AGCTCTCCCTAGCATTATTCCTAGT (SEQ ID NO: 28)	3'-NTR
	R	CACCACATTTTCATCGAGGC (SEQ ID NO: 29)	
	P	TACCCTCGATCGTACTCCGCGT (SEQ ID NO: 30)	
M	F	TGTAGGCACTGATTCAGGTTTGT (SEQ ID NO: 31)	M protein
	R	CGGCGTGGTCTGTATTAAATTA (SEQ ID NO: 32)	
	P	CTGCATACAACCGCTACCGTATTGGAA (SEQ ID NO: 33)	

^aRT-PCR, reverse transcription-polymerase chain reaction; F, forward primer; R, reverse primer; P, probe; NTR, nontranslated region.

Real-Time RT-PCR Assay

The real-time RT-PCR assay was performed by using the Real-Time One-Step RT-PCR Master Mix (Applied Biosystems, Foster City, Calif.). Each 25-μL reaction mixture contained 12.5 μL of 2× Master Mix, 0.625 μL of the 40× MultiScribe and RNase Inhibitor mix, 0.25 μL of 10 μM probe, 0.25 μL each of 50 μM forward and reverse primers, 6.125 μL of nuclease-free water, and 5 μL of nucleic acid extract. Amplification was carried out in 96-well plates on an iCycler iQ Real-Time Detection System (Bio-Rad, Hercules, Calif.). Thermocycling conditions consisted of 30 minutes at 48° C. for reverse transcription, 10 minutes at 95° C. for activation of the AmpliTaq Gold DNA polymerase, and 45 cycles of 15 seconds at 95° C. and 1 minute at 60° C. Each run included one SARS-CoV genomic template control and at least two no-template controls for the extraction (to check for contamination during sample processing) and one no-template control for the PCR-amplification step. As a control for PCR inhibitors, and to monitor nucleic acid extraction efficiency, each sample was tested by real-time RT-PCR for the presence of the human ribonuclease (RNase) P gene (GenBank Accession No. NM_006413) by using the fol-

lowing primers and probe: forward primer 5'-AGATTTG-GACCTGCGAGCG-3' (SEQ ID NO: 36); reverse primer 5'-GAGCGGCTGTCTCCACAAGT-3' (SEQ ID NO: 37); probe 5'-TTCTGACC TGAAGGCTCTGCGCG-3' (SEQ ID NO: 38). The assay reaction was performed identically to that described above except that primer concentrations used were 30 μM each. Fluorescence measurements were taken and the threshold cycle (C_T) value for each sample was calculated by determining the point at which fluorescence exceeded a threshold limit set at the mean plus 10 standard deviations above the baseline. A test result was considered positive if two or more of the SARS genomic targets showed positive results ($C_T \leq 45$ cycles) and all positive and negative control reactions gave expected values.

While this disclosure has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations and equivalents of the preferred embodiments may be used and it is intended that the disclosure may be practiced otherwise than as specifically described herein. Accordingly, this disclosure includes all modifications encompassed within the spirit and scope of the disclosure as defined by the claims below.

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His Gly His Lys Val Val Glu Leu Val Ala Glu Met Asp Gly Ile Gln
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Trp Asn Thr Lys His Gly Ser Gly Ala Leu Arg Glu Leu Thr Arg Glu
165          170          175

Leu Asn Gly Gly Ala Val Thr Arg Tyr Val Asp Asn Asn Phe Cys Gly
180          185          190

Pro Asp Gly Tyr Pro Leu Asp Cys Ile Lys Asp Phe Leu Ala Arg Ala
195          200          205

Gly Lys Ser Met Cys Thr Leu Ser Glu Gln Leu Asp Tyr Ile Glu Ser
210          215          220

Lys Arg Gly Val Tyr Cys Cys Arg Asp His Glu His Glu Ile Ala Trp
225          230          235          240

Phe Thr Glu Arg Ser Asp Lys Ser Tyr Glu His Gln Thr Pro Phe Glu
245          250          255

Ile Lys Ser Ala Lys Lys Phe Asp Thr Phe Lys Gly Glu Cys Pro Lys
260          265          270

Phe Val Phe Pro Leu Asn Ser Lys Val Lys Val Ile Gln Pro Arg Val
275          280          285

Glu Lys Lys Lys Thr Glu Gly Phe Met Gly Arg Ile Arg Ser Val Tyr
290          295          300

Pro Val Ala Ser Pro Gln Glu Cys Asn Asn Met His Leu Ser Thr Leu
305          310          315          320

Met Lys Cys Asn His Cys Asp Glu Val Ser Trp Gln Thr Cys Asp Phe
325          330          335

Leu Lys Ala Thr Cys Glu His Cys Gly Thr Glu Asn Leu Val Ile Glu
340          345          350

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Gly	Pro	Thr	Thr	Cys	Gly	Tyr	Leu	Pro	Thr	Asn	Ala	Val	Val	Lys	Met
	355						360					365			
Pro	Cys	Pro	Ala	Cys	Gln	Asp	Pro	Glu	Ile	Gly	Pro	Glu	His	Ser	Val
	370					375					380				
Ala	Asp	Tyr	His	Asn	His	Ser	Asn	Ile	Glu	Thr	Arg	Leu	Arg	Lys	Gly
385					390					395					400
Gly	Arg	Thr	Arg	Cys	Phe	Gly	Gly	Cys	Val	Phe	Ala	Tyr	Val	Gly	Cys
				405					410					415	
Tyr	Asn	Lys	Arg	Ala	Tyr	Trp	Val	Pro	Arg	Ala	Ser	Ala	Asp	Ile	Gly
			420					425					430		
Ser	Gly	His	Thr	Gly	Ile	Thr	Gly	Asp	Asn	Val	Glu	Thr	Leu	Asn	Glu
		435					440					445			
Asp	Leu	Leu	Glu	Ile	Leu	Ser	Arg	Glu	Arg	Val	Asn	Ile	Asn	Ile	Val
	450					455					460				
Gly	Asp	Phe	His	Leu	Asn	Glu	Glu	Val	Ala	Ile	Ile	Leu	Ala	Ser	Phe
465					470					475					480
Ser	Ala	Ser	Thr	Ser	Ala	Phe	Ile	Asp	Thr	Ile	Lys	Ser	Leu	Asp	Tyr
				485					490					495	
Lys	Ser	Phe	Lys	Thr	Ile	Val	Glu	Ser	Cys	Gly	Asn	Tyr	Lys	Val	Thr
			500					505					510		
Lys	Gly	Lys	Pro	Val	Lys	Gly	Ala	Trp	Asn	Ile	Gly	Gln	Gln	Arg	Ser
		515					520					525			
Val	Leu	Thr	Pro	Leu	Cys	Gly	Phe	Pro	Ser	Gln	Ala	Ala	Gly	Val	Ile
	530					535					540				
Arg	Ser	Ile	Phe	Ala	Arg	Thr	Leu	Asp	Ala	Ala	Asn	His	Ser	Ile	Pro
545					550				555						560
Asp	Leu	Gln	Arg	Ala	Ala	Val	Thr	Ile	Leu	Asp	Gly	Ile	Ser	Glu	Gln
				565					570					575	
Ser	Leu	Arg	Leu	Val	Asp	Ala	Met	Val	Tyr	Thr	Ser	Asp	Leu	Leu	Thr
		580						585					590		
Asn	Ser	Val	Ile	Ile	Met	Ala	Tyr	Val	Thr	Gly	Gly	Leu	Val	Gln	Gln
		595				600						605			
Thr	Ser	Gln	Trp	Leu	Ser	Asn	Leu	Leu	Gly	Thr	Thr	Val	Glu	Lys	Leu
	610					615						620			
Arg	Pro	Ile	Phe	Glu	Trp	Ile	Glu	Ala	Lys	Leu	Ser	Ala	Gly	Val	Glu
625					630				635						640
Phe	Leu	Lys	Asp	Ala	Trp	Glu	Ile	Leu	Lys	Phe	Leu	Ile	Thr	Gly	Val
			645						650					655	
Phe	Asp	Ile	Val	Lys	Gly	Gln	Ile	Gln	Val	Ala	Ser	Asp	Asn	Ile	Lys
		660						665					670		
Asp	Cys	Val	Lys	Cys	Phe	Ile	Asp	Val	Val	Asn	Lys	Ala	Leu	Glu	Met
		675					680					685			
Cys	Ile	Asp	Gln	Val	Thr	Ile	Ala	Gly	Ala	Lys	Leu	Arg	Ser	Leu	Asn
	690					695					700				
Leu	Gly	Glu	Val	Phe	Ile	Ala	Gln	Ser	Lys	Gly	Leu	Tyr	Arg	Gln	Cys
705					710					715					720
Ile	Arg	Gly	Lys	Glu	Gln	Leu	Gln	Leu	Leu	Met	Pro	Leu	Lys	Ala	Pro
			725						730					735	
Lys	Glu	Val	Thr	Phe	Leu	Glu	Gly	Asp	Ser	His	Asp	Thr	Val	Leu	Thr
			740					745					750		
Ser	Glu	Glu	Val	Val	Leu	Lys	Asn	Gly	Glu	Leu	Glu	Ala	Leu	Glu	Thr
	755						760					765			
Pro	Val	Asp	Ser	Phe	Thr	Asn	Gly	Ala	Ile	Val	Gly	Thr	Pro	Val	Cys

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770	775	780
Val Asn Gly Leu Met 785	Leu Leu Glu Ile Lys Asp 790	Lys Glu Gln Tyr Cys 795 800
Ala Leu Ser Pro Gly 805	Leu Leu Ala Thr Asn Asn Val Phe Arg Leu Lys 810 815	
Gly Gly Ala Pro Ile Lys Gly Val Thr Phe Gly Glu Asp Thr Val Trp 820 825 830		
Glu Val Gln Gly Tyr Lys Asn Val Arg Ile Thr Phe Glu Leu Asp Glu 835 840 845		
Arg Val Asp Lys Val Leu Asn Glu Lys Cys Ser Val Tyr Thr Val Glu 850 855 860		
Ser Gly Thr Glu Val Thr Glu Phe Ala Cys Val Val Ala Glu Ala Val 865 870 875 880		
Val Lys Thr Leu Gln Pro Val Ser Asp Leu Leu Thr Asn Met Gly Ile 885 890 895		
Asp Leu Asp Glu Trp Ser Val Ala Thr Phe Tyr Leu Phe Asp Asp Ala 900 905 910		
Gly Glu Glu Asn Phe Ser Ser Arg Met Tyr Cys Ser Phe Tyr Pro Pro 915 920 925		
Asp Glu Glu Glu Glu Asp Asp Ala Glu Cys Glu Glu Glu Glu Ile Asp 930 935 940		
Glu Thr Cys Glu His Glu Tyr Gly Thr Glu Asp Asp Tyr Gln Gly Leu 945 950 955 960		
Pro Leu Glu Phe Gly Ala Ser Ala Glu Thr Val Arg Val Glu Glu Glu 965 970 975		
Glu Glu Glu Asp Trp Leu Asp Asp Thr Thr Glu Gln Ser Glu Ile Glu 980 985 990		
Pro Glu Pro Glu Pro Thr Pro Glu Glu Pro Val Asn Gln Phe Thr Gly 995 1000 1005		
Tyr Leu Lys Leu Thr Asp Asn Val Ala Ile Lys Cys Val Asp Ile 1010 1015 1020		
Val Lys Glu Ala Gln Ser Ala Asn Pro Met Val Ile Val Asn Ala 1025 1030 1035		
Ala Asn Ile His Leu Lys His Gly Gly Gly Val Ala Gly Ala Leu 1040 1045 1050		
Asn Lys Ala Thr Asn Gly Ala Met Gln Lys Glu Ser Asp Asp Tyr 1055 1060 1065		
Ile Lys Leu Asn Gly Pro Leu Thr Val Gly Gly Ser Cys Leu Leu 1070 1075 1080		
Ser Gly His Asn Leu Ala Lys Lys Cys Leu His Val Val Gly Pro 1085 1090 1095		
Asn Leu Asn Ala Gly Glu Asp Ile Gln Leu Leu Lys Ala Ala Tyr 1100 1105 1110		
Glu Asn Phe Asn Ser Gln Asp Ile Leu Leu Ala Pro Leu Leu Ser 1115 1120 1125		
Ala Gly Ile Phe Gly Ala Lys Pro Leu Gln Ser Leu Gln Val Cys 1130 1135 1140		
Val Gln Thr Val Arg Thr Gln Val Tyr Ile Ala Val Asn Asp Lys 1145 1150 1155		
Ala Leu Tyr Glu Gln Val Val Met Asp Tyr Leu Asp Asn Leu Lys 1160 1165 1170		
Pro Arg Val Glu Ala Pro Lys Gln Glu Glu Pro Pro Asn Thr Glu 1175 1180 1185		

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Asp	Ser	Lys	Thr	Glu	Glu	Lys	Ser	Val	Val	Gln	Lys	Pro	Val	Asp
1190						1195					1200			
Val	Lys	Pro	Lys	Ile	Lys	Ala	Cys	Ile	Asp	Glu	Val	Thr	Thr	Thr
1205						1210					1215			
Leu	Glu	Glu	Thr	Lys	Phe	Leu	Thr	Asn	Lys	Leu	Leu	Leu	Phe	Ala
1220						1225					1230			
Asp	Ile	Asn	Gly	Lys	Leu	Tyr	His	Asp	Ser	Gln	Asn	Met	Leu	Arg
1235						1240					1245			
Gly	Glu	Asp	Met	Ser	Phe	Leu	Glu	Lys	Asp	Ala	Pro	Tyr	Met	Val
1250						1255					1260			
Gly	Asp	Val	Ile	Thr	Ser	Gly	Asp	Ile	Thr	Cys	Val	Val	Ile	Pro
1265						1270					1275			
Ser	Lys	Lys	Ala	Gly	Gly	Thr	Thr	Glu	Met	Leu	Ser	Arg	Ala	Leu
1280						1285					1290			
Lys	Lys	Val	Pro	Val	Asp	Glu	Tyr	Ile	Thr	Thr	Tyr	Pro	Gly	Gln
1295						1300					1305			
Gly	Cys	Ala	Gly	Tyr	Thr	Leu	Glu	Glu	Ala	Lys	Thr	Ala	Leu	Lys
1310						1315					1320			
Lys	Cys	Lys	Ser	Ala	Phe	Tyr	Val	Leu	Pro	Ser	Glu	Ala	Pro	Asn
1325						1330					1335			
Ala	Lys	Glu	Glu	Ile	Leu	Gly	Thr	Val	Ser	Trp	Asn	Leu	Arg	Glu
1340						1345					1350			
Met	Leu	Ala	His	Ala	Glu	Glu	Thr	Arg	Lys	Leu	Met	Pro	Ile	Cys
1355						1360					1365			
Met	Asp	Val	Arg	Ala	Ile	Met	Ala	Thr	Ile	Gln	Arg	Lys	Tyr	Lys
1370						1375					1380			
Gly	Ile	Lys	Ile	Gln	Glu	Gly	Ile	Val	Asp	Tyr	Gly	Val	Arg	Phe
1385						1390					1395			
Phe	Phe	Tyr	Thr	Ser	Lys	Glu	Pro	Val	Ala	Ser	Ile	Ile	Thr	Lys
1400						1405					1410			
Leu	Asn	Ser	Leu	Asn	Glu	Pro	Leu	Val	Thr	Met	Pro	Ile	Gly	Tyr
1415						1420					1425			
Val	Thr	His	Gly	Phe	Asn	Leu	Glu	Glu	Ala	Ala	Arg	Cys	Met	Arg
1430						1435					1440			
Ser	Leu	Lys	Ala	Pro	Ala	Val	Val	Ser	Val	Ser	Ser	Pro	Asp	Ala
1445						1450					1455			
Val	Thr	Thr	Tyr	Asn	Gly	Tyr	Leu	Thr	Ser	Ser	Ser	Lys	Thr	Ser
1460						1465					1470			
Glu	Glu	His	Phe	Val	Glu	Thr	Val	Ser	Leu	Ala	Gly	Ser	Tyr	Arg
1475						1480					1485			
Asp	Trp	Ser	Tyr	Ser	Gly	Gln	Arg	Thr	Glu	Leu	Gly	Val	Glu	Phe
1490						1495					1500			
Leu	Lys	Arg	Gly	Asp	Lys	Ile	Val	Tyr	His	Thr	Leu	Glu	Ser	Pro
1505						1510					1515			
Val	Glu	Phe	His	Leu	Asp	Gly	Glu	Val	Leu	Ser	Leu	Asp	Lys	Leu
1520						1525					1530			
Lys	Ser	Leu	Leu	Ser	Leu	Arg	Glu	Val	Lys	Thr	Ile	Lys	Val	Phe
1535						1540					1545			
Thr	Thr	Val	Asp	Asn	Thr	Asn	Leu	His	Thr	Gln	Leu	Val	Asp	Met
1550						1555					1560			
Ser	Met	Thr	Tyr	Gly	Gln	Gln	Phe	Gly	Pro	Thr	Tyr	Leu	Asp	Gly
1565						1570					1575			

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Ala Asp	Val Thr Lys Ile	Lys Pro His Val Asn His	Glu Gly Lys
1580		1585	1590
Thr Phe	Phe Val Leu Pro Ser	Asp Asp Thr Leu Arg	Ser Glu Ala
1595		1600	1605
Phe Glu	Tyr Tyr His Thr Leu	Asp Glu Ser Phe Leu	Gly Arg Tyr
1610		1615	1620
Met Ser	Ala Leu Asn His Thr	Lys Lys Trp Lys Phe	Pro Gln Val
1625		1630	1635
Gly Gly	Leu Thr Ser Ile Lys	Trp Ala Asp Asn Asn	Cys Tyr Leu
1640		1645	1650
Ser Ser	Val Leu Leu Ala Leu	Gln Gln Leu Glu Val	Lys Phe Asn
1655		1660	1665
Ala Pro	Ala Leu Gln Glu Ala	Tyr Tyr Arg Ala Arg	Ala Gly Asp
1670		1675	1680
Ala Ala	Asn Phe Cys Ala Leu	Ile Leu Ala Tyr Ser	Asn Lys Thr
1685		1690	1695
Val Gly	Glu Leu Gly Asp Val	Arg Glu Thr Met Thr	His Leu Leu
1700		1705	1710
Gln His	Ala Asn Leu Glu Ser	Ala Lys Arg Val Leu	Asn Val Val
1715		1720	1725
Cys Lys	His Cys Gly Gln Lys	Thr Thr Thr Leu Thr	Gly Val Glu
1730		1735	1740
Ala Val	Met Tyr Met Gly Thr	Leu Ser Tyr Asp Asn	Leu Lys Thr
1745		1750	1755
Gly Val	Ser Ile Pro Cys Val	Cys Gly Arg Asp Ala	Thr Gln Tyr
1760		1765	1770
Leu Val	Gln Gln Glu Ser Ser	Phe Val Met Met Ser	Ala Pro Pro
1775		1780	1785
Ala Glu	Tyr Lys Leu Gln Gln	Gly Thr Phe Leu Cys	Ala Asn Glu
1790		1795	1800
Tyr Thr	Gly Asn Tyr Gln Cys	Gly His Tyr Thr His	Ile Thr Ala
1805		1810	1815
Lys Glu	Thr Leu Tyr Arg Ile	Asp Gly Ala His Leu	Thr Lys Met
1820		1825	1830
Ser Glu	Tyr Lys Gly Pro Val	Thr Asp Val Phe Tyr	Lys Glu Thr
1835		1840	1845
Ser Tyr	Thr Thr Thr Ile Lys	Pro Val Ser Tyr Lys	Leu Asp Gly
1850		1855	1860
Val Thr	Tyr Thr Glu Ile Glu	Pro Lys Leu Asp Gly	Tyr Tyr Lys
1865		1870	1875
Lys Asp	Asn Ala Tyr Tyr Thr	Glu Gln Pro Ile Asp	Leu Val Pro
1880		1885	1890
Thr Gln	Pro Leu Pro Asn Ala	Ser Phe Asp Asn Phe	Lys Leu Thr
1895		1900	1905
Cys Ser	Asn Thr Lys Phe Ala	Asp Asp Leu Asn Gln	Met Thr Gly
1910		1915	1920
Phe Thr	Lys Pro Ala Ser Arg	Glu Leu Ser Val Thr	Phe Phe Pro
1925		1930	1935
Asp Leu	Asn Gly Asp Val Val	Ala Ile Asp Tyr Arg	His Tyr Ser
1940		1945	1950
Ala Ser	Phe Lys Lys Gly Ala	Lys Leu Leu His Lys	Pro Ile Val
1955		1960	1965
Trp His	Ile Asn Gln Ala Thr	Thr Lys Thr Thr Phe	Lys Pro Asn

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1970	1975	1980
Thr Trp Cys Leu Arg Cys 1985	Leu Trp Ser Thr Lys 1990	Pro Val Asp Thr 1995
Ser Asn Ser Phe Glu Val 2000	Leu Ala Val Glu Asp 2005	Thr Gln Gly Met 2010
Asp Asn Leu Ala Cys Glu 2015	Ser Gln Gln Pro Thr 2020	Ser Glu Glu Val 2025
Val Glu Asn Pro Thr Ile 2030	Gln Lys Glu Val Ile 2035	Glu Cys Asp Val 2040
Lys Thr Thr Glu Val Val 2045	Gly Asn Val Ile Leu 2050	Lys Pro Ser Asp 2055
Glu Gly Val Lys Val Thr 2060	Gln Glu Leu Gly His 2065	Glu Asp Leu Met 2070
Ala Ala Tyr Val Glu Asn 2075	Thr Ser Ile Thr Ile 2080	Lys Lys Pro Asn 2085
Glu Leu Ser Leu Ala Leu 2090	Gly Leu Lys Thr Ile 2095	Ala Thr His Gly 2100
Ile Ala Ala Ile Asn Ser 2105	Val Pro Trp Ser Lys 2110	Ile Leu Ala Tyr 2115
Val Lys Pro Phe Leu Gly 2120	Gln Ala Ala Ile Thr 2125	Thr Ser Asn Cys 2130
Ala Lys Arg Leu Ala Gln 2135	Arg Val Phe Asn Asn 2140	Tyr Met Pro Tyr 2145
Val Phe Thr Leu Leu Phe 2150	Gln Leu Cys Thr Phe 2155	Thr Lys Ser Thr 2160
Asn Ser Arg Ile Arg Ala 2165	Ser Leu Pro Thr Thr 2170	Ile Ala Lys Asn 2175
Ser Val Lys Ser Val Ala 2180	Lys Leu Cys Leu Asp 2185	Ala Gly Ile Asn 2190
Tyr Val Lys Ser Pro Lys 2195	Phe Ser Lys Leu Phe 2200	Thr Ile Ala Met 2205
Trp Leu Leu Leu Leu Ser 2210	Ile Cys Leu Gly Ser 2215	Leu Ile Cys Val 2220
Thr Ala Ala Phe Gly Val 2225	Leu Leu Ser Asn Phe 2230	Gly Ala Pro Ser 2235
Tyr Cys Asn Gly Val Arg 2240	Glu Leu Tyr Leu Asn 2245	Ser Ser Asn Val 2250
Thr Thr Met Asp Phe Cys 2255	Glu Gly Ser Phe Pro 2260	Cys Ser Ile Cys 2265
Leu Ser Gly Leu Asp Ser 2270	Leu Asp Ser Tyr Pro 2275	Ala Leu Glu Thr 2280
Ile Gln Val Thr Ile Ser 2285	Ser Tyr Lys Leu Asp 2290	Leu Thr Ile Leu 2295
Gly Leu Ala Ala Glu Trp 2300	Val Leu Ala Tyr Met 2305	Leu Phe Thr Lys 2310
Phe Phe Tyr Leu Leu Gly 2315	Leu Ser Ala Ile Met 2320	Gln Val Phe Phe 2325
Gly Tyr Phe Ala Ser His 2330	Phe Ile Ser Asn Ser 2335	Trp Leu Met Trp 2340
Phe Ile Ile Ser Ile Val 2345	Gln Met Ala Pro Val 2350	Ser Ala Met Val 2355
Arg Met Tyr Ile Phe Phe 2360	Ala Ser Phe Tyr Tyr 2365	Ile Trp Lys Ser 2370

Tyr	Val	His	Ile	Met	Asp	Gly	Cys	Thr	Ser	Ser	Thr	Cys	Met	Met
	2375					2380					2385			
Cys	Tyr	Lys	Arg	Asn	Arg	Ala	Thr	Arg	Val	Glu	Cys	Thr	Thr	Ile
	2390					2395					2400			
Val	Asn	Gly	Met	Lys	Arg	Ser	Phe	Tyr	Val	Tyr	Ala	Asn	Gly	Gly
	2405					2410					2415			
Arg	Gly	Phe	Cys	Lys	Thr	His	Asn	Trp	Asn	Cys	Leu	Asn	Cys	Asp
	2420					2425					2430			
Thr	Phe	Cys	Thr	Gly	Ser	Thr	Phe	Ile	Ser	Asp	Glu	Val	Ala	Arg
	2435					2440					2445			
Asp	Leu	Ser	Leu	Gln	Phe	Lys	Arg	Pro	Ile	Asn	Pro	Thr	Asp	Gln
	2450					2455					2460			
Ser	Ser	Tyr	Ile	Val	Asp	Ser	Val	Ala	Val	Lys	Asn	Gly	Ala	Leu
	2465					2470					2475			
His	Leu	Tyr	Phe	Asp	Lys	Ala	Gly	Gln	Lys	Thr	Tyr	Glu	Arg	His
	2480					2485					2490			
Pro	Leu	Ser	His	Phe	Val	Asn	Leu	Asp	Asn	Leu	Arg	Ala	Asn	Asn
	2495					2500					2505			
Thr	Lys	Gly	Ser	Leu	Pro	Ile	Asn	Val	Ile	Val	Phe	Asp	Gly	Lys
	2510					2515					2520			
Ser	Lys	Cys	Asp	Glu	Ser	Ala	Ser	Lys	Ser	Ala	Ser	Val	Tyr	Tyr
	2525					2530					2535			
Ser	Gln	Leu	Met	Cys	Gln	Pro	Ile	Leu	Leu	Leu	Asp	Gln	Val	Leu
	2540					2545					2550			
Val	Ser	Asp	Val	Gly	Asp	Ser	Thr	Glu	Val	Ser	Val	Lys	Met	Phe
	2555					2560					2565			
Asp	Ala	Tyr	Val	Asp	Thr	Phe	Ser	Ala	Thr	Phe	Ser	Val	Pro	Met
	2570					2575					2580			
Glu	Lys	Leu	Lys	Ala	Leu	Val	Ala	Thr	Ala	His	Ser	Glu	Leu	Ala
	2585					2590					2595			
Lys	Gly	Val	Ala	Leu	Asp	Gly	Val	Leu	Ser	Thr	Phe	Val	Ser	Ala
	2600					2605					2610			
Ala	Arg	Gln	Gly	Val	Val	Asp	Thr	Asp	Val	Asp	Thr	Lys	Asp	Val
	2615					2620					2625			
Ile	Glu	Cys	Leu	Lys	Leu	Ser	His	His	Ser	Asp	Leu	Glu	Val	Thr
	2630					2635					2640			
Gly	Asp	Ser	Cys	Asn	Asn	Phe	Met	Leu	Thr	Tyr	Asn	Lys	Val	Glu
	2645					2650					2655			
Asn	Met	Thr	Pro	Arg	Asp	Leu	Gly	Ala	Cys	Ile	Asp	Cys	Asn	Ala
	2660					2665					2670			
Arg	His	Ile	Asn	Ala	Gln	Val	Ala	Lys	Ser	His	Asn	Val	Ser	Leu
	2675					2680					2685			
Ile	Trp	Asn	Val	Lys	Asp	Tyr	Met	Ser	Leu	Ser	Glu	Gln	Leu	Arg
	2690					2695					2700			
Lys	Gln	Ile	Arg	Ser	Ala	Ala	Lys	Lys	Asn	Asn	Ile	Pro	Phe	Arg
	2705					2710					2715			
Leu	Thr													

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Cys Tyr	Ile Val Met Pro Val	His Thr Leu Ser Ile	His Asp Gly
2765	2770	2775	
Tyr Thr	Asn Glu Ile Ile Gly	Tyr Lys Ala Ile Gln	Asp Gly Val
2780	2785	2790	
Thr Arg	Asp Ile Ile Ser Thr	Asp Asp Cys Phe Ala	Asn Lys His
2795	2800	2805	
Ala Gly	Phe Asp Ala Trp Phe	Ser Gln Arg Gly Gly	Ser Tyr Lys
2810	2815	2820	
Asn Asp	Lys Ser Cys Pro Val	Val Ala Ala Ile Ile	Thr Arg Glu
2825	2830	2835	
Ile Gly	Phe Ile Val Pro Gly	Leu Pro Gly Thr Val	Leu Arg Ala
2840	2845	2850	
Ile Asn	Gly Asp Phe Leu His	Phe Leu Pro Arg Val	Phe Ser Ala
2855	2860	2865	
Val Gly	Asn Ile Cys Tyr Thr	Pro Ser Lys Leu Ile	Glu Tyr Ser
2870	2875	2880	
Asp Phe	Ala Thr Ser Ala Cys	Val Leu Ala Ala Glu	Cys Thr Ile
2885	2890	2895	
Phe Lys	Asp Ala Met Gly Lys	Pro Val Pro Tyr Cys	Tyr Asp Thr
2900	2905	2910	
Asn Leu	Leu Glu Gly Ser Ile	Ser Tyr Ser Glu Leu	Arg Pro Asp
2915	2920	2925	
Thr Arg	Tyr Val Leu Met Asp	Gly Ser Ile Ile Gln	Phe Pro Asn
2930	2935	2940	
Thr Tyr	Leu Glu Gly Ser Val	Arg Val Val Thr Thr	Phe Asp Ala
2945	2950	2955	
Glu Tyr	Cys Arg His Gly Thr	Cys Glu Arg Ser Glu	Val Gly Ile
2960	2965	2970	
Cys Leu	Ser Thr Ser Gly Arg	Trp Val Leu Asn Asn	Glu His Tyr
2975	2980	2985	
Arg Ala	Leu Ser Gly Val Phe	Cys Gly Val Asp Ala	Met Asn Leu
2990	2995	3000	
Ile Ala	Asn Ile Phe Thr Pro	Leu Val Gln Pro Val	Gly Ala Leu
3005	3010	3015	
Asp Val	Ser Ala Ser Val Val	Ala Gly Gly Ile Ile	Ala Ile Leu
3020	3025	3030	
Val Thr	Cys Ala Ala Tyr Tyr	Phe Met Lys Phe Arg	Arg Val Phe
3035	3040	3045	
Gly Glu	Tyr Asn His Val Val	Ala Ala Asn Ala Leu	Leu Phe Leu
3050	3055	3060	
Met Ser	Phe Thr Ile Leu Cys	Leu Val Pro Ala Tyr	Ser Phe Leu
3065	3070	3075	
Pro Gly	Val Tyr Ser Val Phe	Tyr Leu Tyr Leu Thr	Phe Tyr Phe
3080	3085	3090	
Thr Asn	Asp Val Ser Phe Leu	Ala His Leu Gln Trp	Phe Ala Met
3095	3100	3105	
Phe Ser	Pro Ile Val Pro Phe	Trp Ile Thr Ala Ile	Tyr Val Phe
3110	3115	3120	
Cys Ile	Ser Leu Lys His Cys	His Trp Phe Phe Asn	Asn Tyr Leu
3125	3130	3135	
Arg Lys	Arg Val Met Phe Asn	Gly Val Thr Phe Ser	Thr Phe Glu
3140	3145	3150	
Glu Ala	Ala Leu Cys Thr Phe	Leu Leu Asn Lys Glu	Met Tyr Leu

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3155	3160	3165
Lys Leu Arg Ser Glu Thr 3170	Leu Leu Pro Leu Thr 3175	Gln Tyr Asn Arg 3180
Tyr Leu Ala Leu Tyr Asn 3185	Lys Tyr Lys Tyr Phe 3190	Ser Gly Ala Leu 3195
Asp Thr Thr Ser Tyr Arg 3200	Glu Ala Ala Cys Cys 3205	His Leu Ala Lys 3210
Ala Leu Asn Asp Phe Ser 3215	Asn Ser Gly Ala Asp 3220	Val Leu Tyr Gln 3225
Pro Pro Gln Thr Ser Ile 3230	Thr Ser Ala Val Leu 3235	Gln Ser Gly Phe 3240
Arg Lys Met Ala Phe Pro 3245	Ser Gly Lys Val Glu 3250	Gly Cys Met Val 3255
Gln Val Thr Cys Gly Thr 3260	Thr Thr Leu Asn Gly 3265	Leu Trp Leu Asp 3270
Asp Thr Val Tyr Cys Pro 3275	Arg His Val Ile Cys 3280	Thr Ala Glu Asp 3285
Met Leu Asn Pro Asn Tyr 3290	Glu Asp Leu Leu Ile 3295	Arg Lys Ser Asn 3300
His Ser Phe Leu Val Gln 3305	Ala Gly Asn Val Gln 3310	Leu Arg Val Ile 3315
Gly His Ser Met Gln Asn 3320	Cys Leu Leu Arg Leu 3325	Lys Val Asp Thr 3330
Ser Asn Pro Lys Thr Pro 3335	Lys Tyr Lys Phe Val 3340	Arg Ile Gln Pro 3345
Gly Gln Thr Phe Ser Val 3350	Leu Ala Cys Tyr Asn 3355	Gly Ser Pro Ser 3360
Gly Val Tyr Gln Cys Ala 3365	Met Arg Pro Asn His 3370	Thr Ile Lys Gly 3375
Ser Phe Leu Asn Gly Ser 3380	Cys Gly Ser Val Gly 3385	Phe Asn Ile Asp 3390
Tyr Asp Cys Val Ser Phe 3395	Cys Tyr Met His His 3400	Met Glu Leu Pro 3405
Thr Gly Val His Ala Gly 3410	Thr Asp Leu Glu Gly 3415	Lys Phe Tyr Gly 3420
Pro Phe Val Asp Arg Gln 3425	Thr Ala Gln Ala Ala 3430	Gly Thr Asp Thr 3435
Thr Ile Thr Leu Asn Val 3440	Leu Ala Trp Leu Tyr 3445	Ala Ala Val Ile 3450
Asn Gly Asp Arg Trp Phe 3455	Leu Asn Arg Phe Thr 3460	Thr Thr Leu Asn 3465
Asp Phe Asn Leu Val Ala 3470	Met Lys Tyr Asn Tyr 3475	Glu Pro Leu Thr 3480
Gln Asp His Val Asp Ile 3485	Leu Gly Pro Leu Ser 3490	Ala Gln Thr Gly 3495
Ile Ala Val Leu Asp Met 3500	Cys Ala Ala Leu Lys 3505	Glu Leu Leu Gln 3510
Asn Gly Met Asn Gly Arg 3515	Thr Ile Leu Gly Ser 3520	Thr Ile Leu Glu 3525
Asp Glu Phe Thr Pro Phe 3530	Asp Val Val Arg Gln 3535	Cys Ser Gly Val 3540
Thr Phe Gln Gly Lys Phe 3545	Lys Lys Ile Val Lys 3550	Gly Thr His His 3555

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Trp Met	Leu Leu Thr Phe	Leu Thr Ser Leu Leu Ile	Leu Val Gln
3560		3565	3570
Ser Thr	Gln Trp Ser Leu Phe	Phe Phe Val Tyr Glu	Asn Ala Phe
3575		3580	3585
Leu Pro	Phe Thr Leu Gly Ile	Met Ala Ile Ala Ala	Cys Ala Met
3590		3595	3600
Leu Leu	Val Lys His Lys His	Ala Phe Leu Cys Leu	Phe Leu Leu
3605		3610	3615
Pro Ser	Leu Ala Thr Val Ala	Tyr Phe Asn Met Val	Tyr Met Pro
3620		3625	3630
Ala Ser	Trp Val Met Arg Ile	Met Thr Trp Leu Glu	Leu Ala Asp
3635		3640	3645
Thr Ser	Leu Ser Gly Tyr Arg	Leu Lys Asp Cys Val	Met Tyr Ala
3650		3655	3660
Ser Ala	Leu Val Leu Leu Ile	Leu Met Thr Ala Arg	Thr Val Tyr
3665		3670	3675
Asp Asp	Ala Ala Arg Arg Val	Trp Thr Leu Met Asn	Val Ile Thr
3680		3685	3690
Leu Val	Tyr Lys Val Tyr Tyr	Gly Asn Ala Leu Asp	Gln Ala Ile
3695		3700	3705
Ser Met	Trp Ala Leu Val Ile	Ser Val Thr Ser Asn	Tyr Ser Gly
3710		3715	3720
Val Val	Thr Thr Ile Met Phe	Leu Ala Arg Ala Ile	Val Phe Val
3725		3730	3735
Cys Val	Glu Tyr Tyr Pro Leu	Leu Phe Ile Thr Gly	Asn Thr Leu
3740		3745	3750
Gln Cys	Ile Met Leu Val Tyr	Cys Phe Leu Gly Tyr	Cys Cys Cys
3755		3760	3765
Cys Tyr	Phe Gly Leu Phe Cys	Leu Leu Asn Arg Tyr	Phe Arg Leu
3770		3775	3780
Thr Leu	Gly Val Tyr Asp Tyr	Leu Val Ser Thr Gln	Glu Phe Arg
3785		3790	3795
Tyr Met	Asn Ser Gln Gly Leu	Leu Pro Pro Lys Ser	Ser Ile Asp
3800		3805	3810
Ala Phe	Lys Leu Asn Ile Lys	Leu Leu Gly Ile Gly	Gly Lys Pro
3815		3820	3825
Cys Ile	Lys Val Ala Thr Val	Gln Ser Lys Met Ser	Asp Val Lys
3830		3835	3840
Cys Thr	Ser Val Val Leu Leu	Ser Val Leu Gln Gln	Leu Arg Val
3845		3850	3855
Glu Ser	Ser Ser Lys Leu Trp	Ala Gln Cys Val Gln	Leu His Asn
3860		3865	3870
Asp Ile	Leu Leu Ala Lys Asp	Thr Thr Glu Ala Phe	Glu Lys Met
3875		3880	3885
Val Ser	Leu Leu Ser Val Leu	Leu Ser Met Gln Gly	Ala Val Asp
3890		3895	3900
Ile Asn	Arg Leu Cys Glu Glu	Met Leu Asp Asn Arg	Ala Thr Leu
3905		3910	3915
Gln Ala	Ile Ala Ser Glu Phe	Ser Ser Leu Pro Ser	Tyr Ala Ala
3920		3925	3930
Tyr Ala	Thr Ala Gln Glu Ala	Tyr Glu Gln Ala Val	Ala Asn Gly
3935		3940	3945

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Asp Ser	Glu Val Val Leu	Lys Lys Leu Lys Lys	Ser Leu Asn Val
3950		3955	3960
Ala Lys	Ser Glu Phe Asp	Arg Asp Ala Ala Met	Gln Arg Lys Leu
3965		3970	3975
Glu Lys	Met Ala Asp Gln	Ala Met Thr Gln Met	Tyr Lys Gln Ala
3980		3985	3990
Arg Ser	Glu Asp Lys Arg	Ala Lys Val Thr Ser	Ala Met Gln Thr
3995		4000	4005
Met Leu	Phe Thr Met Leu	Arg Lys Leu Asp Asn	Asp Ala Leu Asn
4010		4015	4020
Asn Ile	Ile Asn Asn Ala	Arg Asp Gly Cys Val	Pro Leu Asn Ile
4025		4030	4035
Ile Pro	Leu Thr Thr Ala	Ala Lys Leu Met Val	Val Val Pro Asp
4040		4045	4050
Tyr Gly	Thr Tyr Lys Asn	Thr Cys Asp Gly Asn	Thr Phe Thr Tyr
4055		4060	4065
Ala Ser	Ala Leu Trp Glu	Ile Gln Gln Val Val	Asp Ala Asp Ser
4070		4075	4080
Lys Ile	Val Gln Leu Ser	Glu Ile Asn Met Asp	Asn Ser Pro Asn
4085		4090	4095
Leu Ala	Trp Pro Leu Ile	Val Thr Ala Leu Arg	Ala Asn Ser Ala
4100		4105	4110
Val Lys	Leu Gln Asn Asn	Glu Leu Ser Pro Val	Ala Leu Arg Gln
4115		4120	4125
Met Ser	Cys Ala Ala Gly	Thr Thr Gln Thr Ala	Cys Thr Asp Asp
4130		4135	4140
Asn Ala	Leu Ala Tyr Tyr	Asn Asn Ser Lys Gly	Gly Arg Phe Val
4145		4150	4155
Leu Ala	Leu Leu Ser Asp	His Gln Asp Leu Lys	Trp Ala Arg Phe
4160		4165	4170
Pro Lys	Ser Asp Gly Thr	Gly Thr Ile Tyr Thr	Glu Leu Glu Pro
4175		4180	4185
Pro Cys	Arg Phe Val Thr	Asp Thr Pro Lys Gly	Pro Lys Val Lys
4190		4195	4200
Tyr Leu	Tyr Phe Ile Lys	Gly Leu Asn Asn Leu	Asn Arg Gly Met
4205		4210	4215
Val Leu	Gly Ser Leu Ala	Ala Thr Val Arg Leu	Gln Ala Gly Asn
4220		4225	4230
Ala Thr	Glu Val Pro Ala	Asn Ser Thr Val Leu	Ser Phe Cys Ala
4235		4240	4245
Phe Ala	Val Asp Pro Ala	Lys Ala Tyr Lys Asp	Tyr Leu Ala Ser
4250		4255	4260
Gly Gly	Gln Pro Ile Thr	Asn Cys Val Lys Met	Leu Cys Thr His
4265		4270	4275
Thr Gly	Thr Gly Gln Ala	Ile Thr Val Thr Pro	Glu Ala Asn Met
4280		4285	4290
Asp Gln	Glu Ser Phe Gly	Gly Ala Ser Cys Cys	Leu Tyr Cys Arg
4295		4300	4305
Cys His	Ile Asp His Pro	Asn Pro Lys Gly Phe	Cys Asp Leu Lys
4310		4315	4320
Gly Lys	Tyr Val Gln Ile	Pro Thr Thr Cys Ala	Asn Asp Pro Val
4325		4330	4335
Gly Phe	Thr Leu Arg Asn	Thr Val Cys Thr Val	Cys Gly Met Trp

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4340	4345	4350
Lys Gly Tyr Gly Cys Ser Cys Asp Gln Leu Arg Glu Pro Leu Met		
4355	4360	4365
Gln Ser Ala Asp Ala Ser Thr Phe Leu Asn Gly Phe Ala Val		
4370	4375	4380

<210> SEQ ID NO 3
 <211> LENGTH: 2695
 <212> TYPE: PRT
 <213> ORGANISM: Coronavirus

<400> SEQUENCE: 3

Arg Val Cys Gly Val Ser Ala Ala Arg Leu Thr Pro Cys Gly Thr Gly		
1	5	10 15
Thr Ser Thr Asp Val Val Tyr Arg Ala Phe Asp Ile Tyr Asn Glu Lys		
	20	25 30
Val Ala Gly Phe Ala Lys Phe Leu Lys Thr Asn Cys Cys Arg Phe Gln		
	35	40 45
Glu Lys Asp Glu Glu Gly Asn Leu Leu Asp Ser Tyr Phe Val Val Lys		
	50	55 60
Arg His Thr Met Ser Asn Tyr Gln His Glu Glu Thr Ile Tyr Asn Leu		
65	70	75 80
Val Lys Asp Cys Pro Ala Val Ala Val His Asp Phe Phe Lys Phe Arg		
	85	90 95
Val Asp Gly Asp Met Val Pro His Ile Ser Arg Gln Arg Leu Thr Lys		
	100	105 110
Tyr Thr Met Ala Asp Leu Val Tyr Ala Leu Arg His Phe Asp Glu Gly		
	115	120 125
Asn Cys Asp Thr Leu Lys Glu Ile Leu Val Thr Tyr Asn Cys Cys Asp		
	130	135 140
Asp Asp Tyr Phe Asn Lys Lys Asp Trp Tyr Asp Phe Val Glu Asn Pro		
145	150	155 160
Asp Ile Leu Arg Val Tyr Ala Asn Leu Gly Glu Arg Val Arg Gln Ser		
	165	170 175
Leu Leu Lys Thr Val Gln Phe Cys Asp Ala Met Arg Asp Ala Gly Ile		
	180	185 190
Val Gly Val Leu Thr Leu Asp Asn Gln Asp Leu Asn Gly Asn Trp Tyr		
	195	200 205
Asp Phe Gly Asp Phe Val Gln Val Ala Pro Gly Cys Gly Val Pro Ile		
	210	215 220
Val Asp Ser Tyr Tyr Ser Leu Leu Met Pro Ile Leu Thr Leu Thr Arg		
225	230	235 240
Ala Leu Ala Ala Glu Ser His Met Asp Ala Asp Leu Ala Lys Pro Leu		
	245	250 255
Ile Lys Trp Asp Leu Leu Lys Tyr Asp Phe Thr Glu Glu Arg Leu Cys		
	260	265 270
Leu Phe Asp Arg Tyr Phe Lys Tyr Trp Asp Gln Thr Tyr His Pro Asn		
	275	280 285
Cys Ile Asn Cys Leu Asp Asp Arg Cys Ile Leu His Cys Ala Asn Phe		
	290	295 300
Asn Val Leu Phe Ser Thr Val Phe Pro Pro Thr Ser Phe Gly Pro Leu		
305	310	315 320
Val Arg Lys Ile Phe Val Asp Gly Val Pro Phe Val Val Ser Thr Gly		
	325	330 335

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Tyr	His	Phe	Arg	Glu	Leu	Gly	Val	Val	His	Asn	Gln	Asp	Val	Asn	Leu
			340					345					350		
His	Ser	Ser	Arg	Leu	Ser	Phe	Lys	Glu	Leu	Leu	Val	Tyr	Ala	Ala	Asp
		355					360					365			
Pro	Ala	Met	His	Ala	Ala	Ser	Gly	Asn	Leu	Leu	Leu	Asp	Lys	Arg	Thr
	370					375					380				
Thr	Cys	Phe	Ser	Val	Ala	Ala	Leu	Thr	Asn	Asn	Val	Ala	Phe	Gln	Thr
385					390				395						400
Val	Lys	Pro	Gly	Asn	Phe	Asn	Lys	Asp	Phe	Tyr	Asp	Phe	Ala	Val	Ser
				405					410					415	
Lys	Gly	Phe	Phe	Lys	Glu	Gly	Ser	Ser	Val	Glu	Leu	Lys	His	Phe	Phe
			420					425					430		
Phe	Ala	Gln	Asp	Gly	Asn	Ala	Ala	Ile	Ser	Asp	Tyr	Asp	Tyr	Tyr	Arg
		435				440						445			
Tyr	Asn	Leu	Pro	Thr	Met	Cys	Asp	Ile	Arg	Gln	Leu	Leu	Phe	Val	Val
	450					455					460				
Glu	Val	Val	Asp	Lys	Tyr	Phe	Asp	Cys	Tyr	Asp	Gly	Gly	Cys	Ile	Asn
465					470					475					480
Ala	Asn	Gln	Val	Ile	Val	Asn	Asn	Leu	Asp	Lys	Ser	Ala	Gly	Phe	Pro
				485					490					495	
Phe	Asn	Lys	Trp	Gly	Lys	Ala	Arg	Leu	Tyr	Tyr	Asp	Ser	Met	Ser	Tyr
			500					505					510		
Glu	Asp	Gln	Asp	Ala	Leu	Phe	Ala	Tyr	Thr	Lys	Arg	Asn	Val	Ile	Pro
		515					520					525			
Thr	Ile	Thr	Gln	Met	Asn	Leu	Lys	Tyr	Ala	Ile	Ser	Ala	Lys	Asn	Arg
	530					535					540				
Ala	Arg	Thr	Val	Ala	Gly	Val	Ser	Ile	Cys	Ser	Thr	Met	Thr	Asn	Arg
545					550					555					560
Gln	Phe	His	Gln	Lys	Leu	Leu	Lys	Ser	Ile	Ala	Ala	Thr	Arg	Gly	Ala
				565					570					575	
Thr	Val	Val	Ile	Gly	Thr	Ser	Lys	Phe	Tyr	Gly	Gly	Trp	His	Asn	Met
			580					585					590		
Leu	Lys	Thr	Val	Tyr	Ser	Asp	Val	Glu	Thr	Pro	His	Leu	Met	Gly	Trp
		595					600					605			
Asp	Tyr	Pro	Lys	Cys	Asp	Arg	Ala	Met	Pro	Asn	Met	Leu	Arg	Ile	Met
	610					615						620			
Ala	Ser	Leu	Val	Leu	Ala	Arg	Lys	His	Asn	Thr	Cys	Cys	Asn	Leu	Ser
625					630					635					640
His	Arg	Phe	Tyr	Arg	Leu	Ala	Asn	Glu	Cys	Ala	Gln	Val	Leu	Ser	Glu
				645					650					655	
Met	Val	Met	Cys	Gly	Gly	Ser	Leu	Tyr	Val	Lys	Pro	Gly	Gly	Thr	Ser
		660						665					670		
Ser	Gly	Asp	Ala	Thr	Thr	Ala	Tyr	Ala	Asn	Ser	Val	Phe	Asn	Ile	Cys
		675					680					685			
Gln	Ala	Val	Thr	Ala	Asn	Val	Asn	Ala	Leu	Leu	Ser	Thr	Asp	Gly	Asn
	690					695					700				
Lys	Ile	Ala	Asp	Lys	Tyr	Val	Arg	Asn	Leu	Gln	His	Arg	Leu	Tyr	Glu
705					710					715					720
Cys	Leu	Tyr	Arg	Asn	Arg	Asp	Val	Asp	His	Glu	Phe	Val	Asp	Glu	Phe
				725					730					735	
Tyr	Ala	Tyr	Leu	Arg	Lys	His	Phe	Ser	Met	Met	Ile	Leu	Ser	Asp	Asp
			740					745					750		
Ala	Val	Val	Cys	Tyr	Asn	Ser	Asn	Tyr	Ala	Ala	Gln	Gly	Leu	Val	Ala

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755	760	765
Ser Ile Lys Asn Phe Lys Ala Val Leu Tyr Tyr Gln Asn Asn Val Phe 770 775 780		
Met Ser Glu Ala Lys Cys Trp Thr Glu Thr Asp Leu Thr Lys Gly Pro 785 790 795 800		
His Glu Phe Cys Ser Gln His Thr Met Leu Val Lys Gln Gly Asp Asp 805 810 815		
Tyr Val Tyr Leu Pro Tyr Pro Asp Pro Ser Arg Ile Leu Gly Ala Gly 820 825 830		
Cys Phe Val Asp Asp Ile Val Lys Thr Asp Gly Thr Leu Met Ile Glu 835 840 845		
Arg Phe Val Ser Leu Ala Ile Asp Ala Tyr Pro Leu Thr Lys His Pro 850 855 860		
Asn Gln Glu Tyr Ala Asp Val Phe His Leu Tyr Leu Gln Tyr Ile Arg 865 870 875 880		
Lys Leu His Asp Glu Leu Thr Gly His Met Leu Asp Met Tyr Ser Val 885 890 895		
Met Leu Thr Asn Asp Asn Thr Ser Arg Tyr Trp Glu Pro Glu Phe Tyr 900 905 910		
Glu Ala Met Tyr Thr Pro His Thr Val Leu Gln Ala Val Gly Ala Cys 915 920 925		
Val Leu Cys Asn Ser Gln Thr Ser Leu Arg Cys Gly Ala Cys Ile Arg 930 935 940		
Arg Pro Phe Leu Cys Cys Lys Cys Cys Tyr Asp His Val Ile Ser Thr 945 950 955 960		
Ser His Lys Leu Val Leu Ser Val Asn Pro Tyr Val Cys Asn Ala Pro 965 970 975		
Gly Cys Asp Val Thr Asp Val Thr Gln Leu Tyr Leu Gly Gly Met Ser 980 985 990		
Tyr Tyr Cys Lys Ser His Lys Pro Pro Ile Ser Phe Pro Leu Cys Ala 995 1000 1005		
Asn Gly Gln Val Phe Gly Leu Tyr Lys Asn Thr Cys Val Gly Ser 1010 1015 1020		
Asp Asn Val Thr Asp Phe Asn Ala Ile Ala Thr Cys Asp Trp Thr 1025 1030 1035		
Asn Ala Gly Asp Tyr Ile Leu Ala Asn Thr Cys Thr Glu Arg Leu 1040 1045 1050		
Lys Leu Phe Ala Ala Glu Thr Leu Lys Ala Thr Glu Glu Thr Phe 1055 1060 1065		
Lys Leu Ser Tyr Gly Ile Ala Thr Val Arg Glu Val Leu Ser Asp 1070 1075 1080		
Arg Glu Leu His Leu Ser Trp Glu Val Gly Lys Pro Arg Pro Pro 1085 1090 1095		
Leu Asn Arg Asn Tyr Val Phe Thr Gly Tyr Arg Val Thr Lys Asn 1100 1105 1110		
Ser Lys Val Gln Ile Gly Glu Tyr Thr Phe Glu Lys Gly Asp Tyr 1115 1120 1125		
Gly Asp Ala Val Val Tyr Arg Gly Thr Thr Thr Tyr Lys Leu Asn 1130 1135 1140		
Val Gly Asp Tyr Phe Val Leu Thr Ser His Thr Val Met Pro Leu 1145 1150 1155		
Ser Ala Pro Thr Leu Val Pro Gln Glu His Tyr Val Arg Ile Thr 1160 1165 1170		

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Gly Leu Tyr Pro Thr Leu Asn Ile Ser Asp Glu Phe Ser Ser Asn	1175	1180	1185
Val Ala Asn Tyr Gln Lys Val Gly Met Gln Lys Tyr Ser Thr Leu	1190	1195	1200
Gln Gly Pro Pro Gly Thr Gly Lys Ser His Phe Ala Ile Gly Leu	1205	1210	1215
Ala Leu Tyr Tyr Pro Ser Ala Arg Ile Val Tyr Thr Ala Cys Ser	1220	1225	1230
His Ala Ala Val Asp Ala Leu Cys Glu Lys Ala Leu Lys Tyr Leu	1235	1240	1245
Pro Ile Asp Lys Cys Ser Arg Ile Ile Pro Ala Arg Ala Arg Val	1250	1255	1260
Glu Cys Phe Asp Lys Phe Lys Val Asn Ser Thr Leu Glu Gln Tyr	1265	1270	1275
Val Phe Cys Thr Val Asn Ala Leu Pro Glu Thr Thr Ala Asp Ile	1280	1285	1290
Val Val Phe Asp Glu Ile Ser Met Ala Thr Asn Tyr Asp Leu Ser	1295	1300	1305
Val Val Asn Ala Arg Leu Arg Ala Lys His Tyr Val Tyr Ile Gly	1310	1315	1320
Asp Pro Ala Gln Leu Pro Ala Pro Arg Thr Leu Leu Thr Lys Gly	1325	1330	1335
Thr Leu Glu Pro Glu Tyr Phe Asn Ser Val Cys Arg Leu Met Lys	1340	1345	1350
Thr Ile Gly Pro Asp Met Phe Leu Gly Thr Cys Arg Arg Cys Pro	1355	1360	1365
Ala Glu Ile Val Asp Thr Val Ser Ala Leu Val Tyr Asp Asn Lys	1370	1375	1380
Leu Lys Ala His Lys Asp Lys Ser Ala Gln Cys Phe Lys Met Phe	1385	1390	1395
Tyr Lys Gly Val Ile Thr His Asp Val Ser Ser Ala Ile Asn Arg	1400	1405	1410
Pro Gln Ile Gly Val Val Arg Glu Phe Leu Thr Arg Asn Pro Ala	1415	1420	1425
Trp Arg Lys Ala Val Phe Ile Ser Pro Tyr Asn Ser Gln Asn Ala	1430	1435	1440
Val Ala Ser Lys Ile Leu Gly Leu Pro Thr Gln Thr Val Asp Ser	1445	1450	1455
Ser Gln Gly Ser Glu Tyr Asp Tyr Val Ile Phe Thr Gln Thr Thr	1460	1465	1470
Glu Thr Ala His Ser Cys Asn Val Asn Arg Phe Asn Val Ala Ile	1475	1480	1485
Thr Arg Ala Lys Ile Gly Ile Leu Cys Ile Met Ser Asp Arg Asp	1490	1495	1500
Leu Tyr Asp Lys Leu Gln Phe Thr Ser Leu Glu Ile Pro Arg Arg	1505	1510	1515
Asn Val Ala Thr Leu Gln Ala Glu Asn Val Thr Gly Leu Phe Lys	1520	1525	1530
Asp Cys Ser Lys Ile Ile Thr Gly Leu His Pro Thr Gln Ala Pro	1535	1540	1545
Thr His Leu Ser Val Asp Ile Lys Phe Lys Thr Glu Gly Leu Cys	1550	1555	1560

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Val	Asp	Ile	Pro	Gly	Ile	Pro	Lys	Asp	Met	Thr	Tyr	Arg	Arg	Leu
1565						1570					1575			
Ile	Ser	Met	Met	Gly	Phe	Lys	Met	Asn	Tyr	Gln	Val	Asn	Gly	Tyr
1580						1585					1590			
Pro	Asn	Met	Phe	Ile	Thr	Arg	Glu	Glu	Ala	Ile	Arg	His	Val	Arg
1595						1600					1605			
Ala	Trp	Ile	Gly	Phe	Asp	Val	Glu	Gly	Cys	His	Ala	Thr	Arg	Asp
1610						1615					1620			
Ala	Val	Gly	Thr	Asn	Leu	Pro	Leu	Gln	Leu	Gly	Phe	Ser	Thr	Gly
1625						1630					1635			
Val	Asn	Leu	Val	Ala	Val	Pro	Thr	Gly	Tyr	Val	Asp	Thr	Glu	Asn
1640						1645					1650			
Asn	Thr	Glu	Phe	Thr	Arg	Val	Asn	Ala	Lys	Pro	Pro	Pro	Gly	Asp
1655						1660					1665			
Gln	Phe	Lys	His	Leu	Ile	Pro	Leu	Met	Tyr	Lys	Gly	Leu	Pro	Trp
1670						1675					1680			
Asn	Val	Val	Arg	Ile	Lys	Ile	Val	Gln	Met	Leu	Ser	Asp	Thr	Leu
1685						1690					1695			
Lys	Gly	Leu	Ser	Asp	Arg	Val	Val	Phe	Val	Leu	Trp	Ala	His	Gly
1700						1705					1710			
Phe	Glu	Leu	Thr	Ser	Met	Lys	Tyr	Phe	Val	Lys	Ile	Gly	Pro	Glu
1715						1720					1725			
Arg	Thr	Cys	Cys	Leu	Cys	Asp	Lys	Arg	Ala	Thr	Cys	Phe	Ser	Thr
1730						1735					1740			
Ser	Ser	Asp	Thr	Tyr	Ala	Cys	Trp	Asn	His	Ser	Val	Gly	Phe	Asp
1745						1750					1755			
Tyr	Val	Tyr	Asn	Pro	Phe	Met	Ile	Asp	Val	Gln	Gln	Trp	Gly	Phe
1760						1765					1770			
Thr	Gly	Asn	Leu	Gln	Ser	Asn	His	Asp	Gln	His	Cys	Gln	Val	His
1775						1780					1785			
Gly	Asn	Ala	His	Val	Ala	Ser	Cys	Asp	Ala	Ile	Met	Thr	Arg	Cys
1790						1795					1800			
Leu	Ala	Val	His	Glu	Cys	Phe	Val	Lys	Arg	Val	Asp	Trp	Ser	Val
1805						1810					1815			
Glu	Tyr	Pro	Ile	Ile	Gly	Asp	Glu	Leu	Arg	Val	Asn	Ser	Ala	Cys
1820						1825					1830			
Arg	Lys	Val	Gln	His	Met	Val	Val	Lys	Ser	Ala	Leu	Leu	Ala	Asp
1835						1840					1845			
Lys	Phe	Pro	Val	Leu	His	Asp	Ile	Gly	Asn	Pro	Lys	Ala	Ile	Lys
1850						1855					1860			
Cys	Val	Pro	Gln	Ala	Glu	Val	Glu	Trp	Lys	Phe	Tyr	Asp	Ala	Gln
1865						1870					1875			
Pro	Cys	Ser	Asp	Lys	Ala	Tyr	Lys	Ile	Glu	Glu	Leu	Phe	Tyr	Ser
1880						1885					1890			
Tyr	Ala	Thr	His	His	Asp	Lys	Phe	Thr	Asp	Gly	Val	Cys	Leu	Phe
1895						1900					1905			
Trp	Asn	Cys	Asn	Val	Asp	Arg	Tyr	Pro	Ala	Asn	Ala	Ile	Val	Cys
1910						1915					1920			
Arg	Phe	Asp	Thr	Arg	Val	Leu	Ser	Asn	Leu	Asn	Leu	Pro	Gly	Cys
1925						1930					1935			
Asp	Gly	Gly	Ser	Leu	Tyr	Val	Asn	Lys	His	Ala	Phe	His	Thr	Pro
1940						1945					1950			
Ala	Phe	Asp	Lys	Ser	Ala	Phe	Thr	Asn	Leu	Lys	Gln	Leu	Pro	Phe

1955					1960					1965				
Phe	Tyr	Tyr	Ser	Asp	Ser	Pro	Cys	Glu	Ser	His	Gly	Lys	Gln	Val
1970					1975					1980				
Val	Ser	Asp	Ile	Asp	Tyr	Val	Pro	Leu	Lys	Ser	Ala	Thr	Cys	Ile
1985					1990					1995				
Thr	Arg	Cys	Asn	Leu	Gly	Gly	Ala	Val	Cys	Arg	His	His	Ala	Asn
2000					2005					2010				
Glu	Tyr	Arg	Gln	Tyr	Leu	Asp	Ala	Tyr	Asn	Met	Met	Ile	Ser	Ala
2015					2020					2025				
Gly	Phe	Ser	Leu	Trp	Ile	Tyr	Lys	Gln	Phe	Asp	Thr	Tyr	Asn	Leu
2030					2035					2040				
Trp	Asn	Thr	Phe	Thr	Arg	Leu	Gln	Ser	Leu	Glu	Asn	Val	Ala	Tyr
2045					2050					2055				
Asn	Val	Val	Asn	Lys	Gly	His	Phe	Asp	Gly	His	Ala	Gly	Glu	Ala
2060					2065					2070				
Pro	Val	Ser	Ile	Ile	Asn	Asn	Ala	Val	Tyr	Thr	Lys	Val	Asp	Gly
2075					2080					2085				
Ile	Asp	Val	Glu	Ile	Phe	Glu	Asn	Lys	Thr	Thr	Leu	Pro	Val	Asn
2090					2095					2100				
Val	Ala	Phe	Glu	Leu	Trp	Ala	Lys	Arg	Asn	Ile	Lys	Pro	Val	Pro
2105					2110					2115				
Glu	Ile	Lys	Ile	Leu	Asn	Asn	Leu	Gly	Val	Asp	Ile	Ala	Ala	Asn
2120					2125					2130				
Thr	Val	Ile	Trp	Asp	Tyr	Lys	Arg	Glu	Ala	Pro	Ala	His	Val	Ser
2135					2140					2145				
Thr	Ile	Gly	Val	Cys	Thr	Met	Thr	Asp	Ile	Ala	Lys	Lys	Pro	Thr
2150					2155					2160				
Glu	Ser	Ala	Cys	Ser	Ser	Leu	Thr	Val	Leu	Phe	Asp	Gly	Arg	Val
2165					2170					2175				
Glu	Gly	Gln	Val	Asp	Leu	Phe	Arg	Asn	Ala	Arg	Asn	Gly	Val	Leu
2180					2185					2190				
Ile	Thr	Glu	Gly	Ser	Val	Lys	Gly	Leu	Thr	Pro	Ser	Lys	Gly	Pro
2195					2200					2205				
Ala	Gln	Ala	Ser	Val	Asn	Gly	Val	Thr	Leu	Ile	Gly	Glu	Ser	Val
2210					2215					2220				
Lys	Thr	Gln	Phe	Asn	Tyr	Phe	Lys	Lys	Val	Asp	Gly	Ile	Ile	Gln
2225					2230					2235				
Gln	Leu	Pro	Glu	Thr	Tyr	Phe	Thr	Gln	Ser	Arg	Asp	Leu	Glu	Asp
2240					2245					2250				
Phe	Lys	Pro	Arg	Ser	Gln	Met	Glu	Thr	Asp	Phe	Leu	Glu	Leu	Ala
2255					2260					2265				
Met	Asp	Glu	Phe	Ile	Gln	Arg	Tyr	Lys	Leu	Glu	Gly	Tyr	Ala	Phe
2270					2275					2280				
Glu	His	Ile	Val	Tyr	Gly	Asp	Phe	Ser	His	Gly	Gln	Leu	Gly	Gly
2285					2290					2295				
Leu	His	Leu	Met	Ile	Gly	Leu	Ala	Lys	Arg	Ser	Gln	Asp	Ser	Pro
2300					2305					2310				
Leu	Lys	Leu	Glu	Asp	Phe	Ile	Pro	Met	Asp	Ser	Thr	Val	Lys	Asn
2315					2320					2325				
Tyr	Phe	Ile	Thr	Asp	Ala	Gln	Thr	Gly	Ser	Ser	Lys	Cys	Val	Cys
2330					2335					2340				
Ser	Val	Ile	Asp	Leu	Leu	Leu	Asp	Asp	Phe	Val	Glu	Ile	Ile	Lys
2345					2350					2355				

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Ser Gln	Asp Leu	Ser Val	Ile	Ser Lys	Val Val	Lys	Val Thr	Ile	
2360			2365			2370			
Asp Tyr	Ala Glu	Ile Ser	Phe	Met Leu	Trp Cys	Lys	Asp Gly	His	
2375			2380			2385			
Val Glu	Thr Phe	Tyr Pro	Lys	Leu Gln	Ala Ser	Gln	Ala Trp	Gln	
2390			2395			2400			
Pro Gly	Val Ala	Met Pro	Asn	Leu Tyr	Lys Met	Gln	Arg Met	Leu	
2405			2410			2415			
Leu Glu	Lys Cys	Asp Leu	Gln	Asn Tyr	Gly Glu	Asn	Ala Val	Ile	
2420			2425			2430			
Pro Lys	Gly Ile	Met Met	Asn	Val Ala	Lys Tyr	Thr	Gln Leu	Cys	
2435			2440			2445			
Gln Tyr	Leu Asn	Thr Leu	Thr	Leu Ala	Val Pro	Tyr	Asn Met	Arg	
2450			2455			2460			
Val Ile	His Phe	Gly Ala	Gly	Ser Asp	Lys Gly	Val	Ala Pro	Gly	
2465			2470			2475			
Thr Ala	Val Leu	Arg Gln	Trp	Leu Pro	Thr Gly	Thr	Leu Leu	Val	
2480			2485			2490			
Asp Ser	Asp Leu	Asn Asp	Phe	Val Ser	Asp Ala	Asp	Ser Thr	Leu	
2495			2500			2505			
Ile Gly	Asp Cys	Ala Thr	Val	His Thr	Ala Asn	Lys	Trp Asp	Leu	
2510			2515			2520			
Ile Ile	Ser Asp	Met Tyr	Asp	Pro Arg	Thr Lys	His	Val Thr	Lys	
2525			2530			2535			
Glu Asn	Asp Ser	Lys Glu	Gly	Phe Phe	Thr Tyr	Leu	Cys Gly	Phe	
2540			2545			2550			
Ile Lys	Gln Lys	Leu Ala	Leu	Gly Gly	Ser Ile	Ala	Val Lys	Ile	
2555			2560			2565			
Thr Glu	His Ser	Trp Asn	Ala	Asp Leu	Tyr Lys	Leu	Met Gly	His	
2570			2575			2580			
Phe Ser	Trp Trp	Thr Ala	Phe	Val Thr	Asn Val	Asn	Ala Ser	Ser	
2585			2590			2595			
Ser Glu	Ala Phe	Leu Ile	Gly	Ala Asn	Tyr Leu	Gly	Lys Pro	Lys	
2600			2605			2610			
Glu Gln	Ile Asp	Gly Tyr	Thr	Met His	Ala Asn	Tyr	Ile Phe	Trp	
2615			2620			2625			
Arg Asn	Thr Asn	Pro Ile	Gln	Leu Ser	Ser Tyr	Ser	Leu Phe	Asp	
2630			2635			2640			
Met Ser	Lys Phe	Pro Leu	Lys	Leu Arg	Gly Thr	Ala	Val Met	Ser	
2645			2650			2655			
Leu Lys	Glu Asn	Gln Ile	Asn	Asp Met	Ile Tyr	Ser	Leu Leu	Glu	
2660			2665			2670			
Lys Gly	Arg Leu	Ile Ile	Arg	Glu Asn	Asn Arg	Val	Val Val	Ser	
2675			2680			2685			
Ser Asp	Ile Leu	Val Asn	Asn						
2690			2695						

<210> SEQ ID NO 4

<211> LENGTH: 1255

<212> TYPE: PRT

<213> ORGANISM: Coronavirus

<400> SEQUENCE: 4

Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu

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1	5	10	15
Asp Arg Cys Thr	Thr Phe Asp Asp	Val Gln Ala Pro	Asn Tyr Thr Gln
20		25	30
His Thr Ser	Ser Met Arg Gly	Val Tyr Tyr Pro	Asp Glu Ile Phe Arg
35		40	45
Ser Asp Thr	Leu Tyr Leu Thr	Gln Asp Leu Phe	Leu Pro Phe Tyr Ser
50		55	60
Asn Val Thr	Gly Phe His Thr	Ile Asn His Thr	Phe Gly Asn Pro Val
65		70	75
Ile Pro Phe	Lys Asp Gly Ile	Tyr Phe Ala Ala	Thr Glu Lys Ser Asn
	85	90	95
Val Val Arg	Gly Trp Val Phe	Gly Ser Thr Met	Asn Asn Lys Ser Gln
	100	105	110
Ser Val Ile	Ile Ile Asn Asn	Ser Thr Asn Val	Val Ile Arg Ala Cys
	115	120	125
Asn Phe Glu	Leu Cys Asp Asn	Pro Phe Phe Ala	Val Ser Lys Pro Met
	130	135	140
Gly Thr Gln	Thr His Thr Met	Ile Phe Asp Asn	Ala Phe Asn Cys Thr
145		150	155
Phe Glu Tyr	Ile Ser Asp Ala	Phe Ser Leu Asp	Val Ser Glu Lys Ser
	165	170	175
Gly Asn Phe	Lys His Leu Arg	Glu Phe Val Phe	Lys Asn Lys Asp Gly
	180	185	190
Phe Leu Tyr	Val Tyr Lys Gly	Tyr Gln Pro Ile	Asp Val Val Arg Asp
	195	200	205
Leu Pro Ser	Gly Phe Asn Thr	Leu Lys Pro Ile	Phe Lys Leu Pro Leu
	210	215	220
Gly Ile Asn	Ile Thr Asn Phe	Arg Ala Ile Leu	Thr Ala Phe Ser Pro
225		230	235
Ala Gln Asp	Ile Trp Gly Thr	Ser Ala Ala Ala	Tyr Phe Val Gly Tyr
	245	250	255
Leu Lys Pro	Thr Thr Phe Met	Leu Lys Tyr Asp	Glu Asn Gly Thr Ile
	260	265	270
Thr Asp Ala	Val Asp Cys Ser	Gln Asn Pro Leu	Ala Glu Leu Lys Cys
	275	280	285
Ser Val Lys	Ser Phe Glu Ile	Asp Lys Gly Ile	Tyr Gln Thr Ser Asn
	290	295	300
Phe Arg Val	Val Pro Ser Gly	Asp Val Val Arg	Phe Pro Asn Ile Thr
305		310	315
Asn Leu Cys	Pro Phe Gly Glu	Val Phe Asn Ala	Thr Lys Phe Pro Ser
	325	330	335
Val Tyr Ala	Trp Glu Arg Lys	Lys Ile Ser Asn	Cys Val Ala Asp Tyr
	340	345	350
Ser Val Leu	Tyr Asn Ser Thr	Phe Phe Ser Thr	Phe Lys Cys Tyr Gly
	355	360	365
Val Ser Ala	Thr Lys Leu Asn	Asp Leu Cys Phe	Ser Asn Val Tyr Ala
	370	375	380
Asp Ser Phe	Val Val Lys Gly	Asp Asp Val Arg	Gln Ile Ala Pro Gly
385		390	395
Gln Thr Gly	Val Ile Ala Asp	Tyr Asn Tyr Lys	Leu Pro Asp Asp Phe
	405	410	415
Met Gly Cys	Val Leu Ala Trp	Asn Thr Arg Asn	Ile Asp Ala Thr Ser
	420	425	430

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Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
 435 440 445
 Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
 450 455 460
 Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
 465 470 475 480
 Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
 485 490 495
 Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
 500 505 510
 Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
 515 520 525
 Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
 530 535 540
 Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
 545 550 555 560
 Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
 565 570 575
 Ser Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
 580 585 590
 Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
 595 600 605
 Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
 610 615 620
 Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
 625 630 635 640
 His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
 645 650 655
 Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
 660 665 670
 Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
 675 680 685
 Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
 690 695 700
 Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
 705 710 715 720
 Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
 725 730 735
 Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
 740 745 750
 Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
 755 760 765
 Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe
 770 775 780
 Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
 785 790 795 800
 Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
 805 810 815
 Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
 820 825 830
 Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
 835 840 845

Asp	Met	Ile	Ala	Ala	Tyr	Thr	Ala	Ala	Leu	Val	Ser	Gly	Thr	Ala	
850					855					860					
Thr	Ala	Gly	Trp	Thr	Phe	Gly	Ala	Gly	Ala	Leu	Gln	Ile	Pro	Phe	
865					870					875				880	
Ala	Met	Gln	Met	Ala	Tyr	Arg	Phe	Asn	Gly	Ile	Gly	Val	Thr	Gln	Asn
				885					890					895	
Val	Leu	Tyr	Glu	Asn	Gln	Lys	Gln	Ile	Ala	Asn	Gln	Phe	Asn	Lys	Ala
			900					905					910		
Ile	Ser	Gln	Ile	Gln	Glu	Ser	Leu	Thr	Thr	Thr	Ser	Thr	Ala	Leu	Gly
		915					920					925			
Lys	Leu	Gln	Asp	Val	Val	Asn	Gln	Asn	Ala	Gln	Ala	Leu	Asn	Thr	Leu
						935					940				
Val	Lys	Gln	Leu	Ser	Ser	Asn	Phe	Gly	Ala	Ile	Ser	Ser	Val	Leu	Asn
945					950					955					960
Asp	Ile	Leu	Ser	Arg	Leu	Asp	Lys	Val	Glu	Ala	Glu	Val	Gln	Ile	Asp
				965					970					975	
Arg	Leu	Ile	Thr	Gly	Arg	Leu	Gln	Ser	Leu	Gln	Thr	Tyr	Val	Thr	Gln
			980						985				990		
Gln	Leu	Ile	Arg	Ala	Ala	Glu	Ile	Arg	Ala	Ser	Ala	Asn	Leu	Ala	Ala
		995					1000					1005			
Thr	Lys	Met	Ser	Glu	Cys	Val	Leu	Gly	Gln	Ser	Lys	Arg	Val	Asp	
	1010					1015					1020				
Phe	Cys	Gly	Lys	Gly	Tyr	His	Leu	Met	Ser	Phe	Pro	Gln	Ala	Ala	
	1025					1030					1035				
Pro	His	Gly	Val	Val	Phe	Leu	His	Val	Thr	Tyr	Val	Pro	Ser	Gln	
	1040					1045					1050				
Glu	Arg	Asn	Phe	Thr	Thr	Ala	Pro	Ala	Ile	Cys	His	Glu	Gly	Lys	
	1055					1060					1065				
Ala	Tyr	Phe	Pro	Arg	Glu	Gly	Val	Phe	Val	Phe	Asn	Gly	Thr	Ser	
	1070					1075					1080				
Trp	Phe	Ile	Thr	Gln	Arg	Asn	Phe	Phe	Ser	Pro	Gln	Ile	Ile	Thr	
	1085					1090					1095				
Thr	Asp	Asn	Thr	Phe	Val	Ser	Gly	Asn	Cys	Asp	Val	Val	Ile	Gly	
	1100					1105					1110				
Ile	Ile	Asn	Asn	Thr	Val	Tyr	Asp	Pro	Leu	Gln	Pro	Glu	Leu	Asp	
	1115					1120					1125				
Ser	Phe	Lys	Glu	Glu	Leu	Asp	Lys	Tyr	Phe	Lys	Asn	His	Thr	Ser	
	1130					1135					1140				
Pro	Asp	Val	Asp	Leu	Gly	Asp	Ile	Ser	Gly	Ile	Asn	Ala	Ser	Val	
	1145					1150					1155				
Val	Asn	Ile	Gln	Lys	Glu	Ile	Asp	Arg	Leu	Asn	Glu	Val	Ala	Lys	
	1160					1165					1170				
Asn	Leu	Asn	Glu	Ser	Leu	Ile	Asp	Leu	Gln	Glu	Leu	Gly	Lys	Tyr	
	1175					1180					1185				
Glu	Gln	Tyr													

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1250 1255

<210> SEQ ID NO 5
 <211> LENGTH: 274
 <212> TYPE: PRT
 <213> ORGANISM: Coronavirus

<400> SEQUENCE: 5

Met Asp Leu Phe Met Arg Phe Phe Thr Leu Gly Ser Ile Thr Ala Gln
 1 5 10 15

Pro Val Lys Ile Asp Asn Ala Ser Pro Ala Ser Thr Val His Ala Thr
 20 25 30

Ala Thr Ile Pro Leu Gln Ala Ser Leu Pro Phe Gly Trp Leu Val Ile
 35 40 45

Gly Val Ala Phe Leu Ala Val Phe Gln Ser Ala Thr Lys Ile Ile Ala
 50 55 60

Leu Asn Lys Arg Trp Gln Leu Ala Leu Tyr Lys Gly Phe Gln Phe Ile
 65 70 75 80

Cys Asn Leu Leu Leu Leu Phe Val Thr Ile Tyr Ser His Leu Leu Leu
 85 90 95

Val Ala Ala Gly Met Glu Ala Gln Phe Leu Tyr Leu Tyr Ala Leu Ile
 100 105 110

Tyr Phe Leu Gln Cys Ile Asn Ala Cys Arg Ile Ile Met Arg Cys Trp
 115 120 125

Leu Cys Trp Lys Cys Lys Ser Lys Asn Pro Leu Leu Tyr Asp Ala Asn
 130 135 140

Tyr Phe Val Cys Trp His Thr His Asn Tyr Asp Tyr Cys Ile Pro Tyr
 145 150 155 160

Asn Ser Val Thr Asp Thr Ile Val Val Thr Glu Gly Asp Gly Ile Ser
 165 170 175

Thr Pro Lys Leu Lys Glu Asp Tyr Gln Ile Gly Gly Tyr Ser Glu Asp
 180 185 190

Arg His Ser Gly Val Lys Asp Tyr Val Val Val His Gly Tyr Phe Thr
 195 200 205

Glu Val Tyr Tyr Gln Leu Glu Ser Thr Gln Ile Thr Thr Asp Thr Gly
 210 215 220

Ile Glu Asn Ala Thr Phe Phe Ile Phe Asn Lys Leu Val Lys Asp Pro
 225 230 235 240

Pro Asn Val Gln Ile His Thr Ile Asp Gly Ser Ser Gly Val Ala Asn
 245 250 255

Pro Ala Met Asp Pro Ile Tyr Asp Glu Pro Thr Thr Thr Thr Ser Val
 260 265 270

Pro Leu

<210> SEQ ID NO 6
 <211> LENGTH: 154
 <212> TYPE: PRT
 <213> ORGANISM: Coronavirus

<400> SEQUENCE: 6

Met Met Pro Thr Thr Leu Phe Ala Gly Thr His Ile Thr Met Thr Thr
 1 5 10 15

Val Tyr His Ile Thr Val Ser Gln Ile Gln Leu Ser Leu Leu Lys Val
 20 25 30

Thr Ala Phe Gln His Gln Asn Ser Lys Lys Thr Thr Lys Leu Val Val
 35 40 45

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Ile Leu Arg Ile Gly Thr Gln Val Leu Lys Thr Met Ser Leu Tyr Met
 50                      55                      60
Ala Ile Ser Pro Lys Phe Thr Thr Ser Leu Ser Leu His Lys Leu Leu
65                      70                      75                      80
Gln Thr Leu Val Leu Lys Met Leu His Ser Ser Ser Leu Thr Ser Leu
                        85                      90                      95
Leu Lys Thr His Arg Met Cys Lys Tyr Thr Gln Ser Thr Ala Leu Gln
                        100                     105                     110
Glu Leu Leu Ile Gln Gln Trp Ile Gln Phe Met Met Ser Arg Arg Arg
                        115                     120                     125
Leu Leu Ala Cys Leu Cys Lys His Lys Lys Val Ser Thr Asn Leu Cys
                        130                     135                     140
Thr His Ser Phe Arg Lys Lys Gln Val Arg
145                      150

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<210> SEQ ID NO 7
<211> LENGTH: 76
<212> TYPE: PRT
<213> ORGANISM: Coronavirus

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<400> SEQUENCE: 7

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Met Tyr Ser Phe Val Ser Glu Glu Thr Gly Thr Leu Ile Val Asn Ser
 1                      5                      10                      15
Val Leu Leu Phe Leu Ala Phe Val Val Phe Leu Leu Val Thr Leu Ala
                        20                      25                      30
Ile Leu Thr Ala Leu Arg Leu Cys Ala Tyr Cys Cys Asn Ile Val Asn
                        35                      40                      45
Val Ser Leu Val Lys Pro Thr Val Tyr Val Tyr Ser Arg Val Lys Asn
                        50                      55                      60
Leu Asn Ser Ser Glu Gly Val Pro Asp Leu Leu Val
65                      70                      75

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<210> SEQ ID NO 8
<211> LENGTH: 221
<212> TYPE: PRT
<213> ORGANISM: Coronavirus

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<400> SEQUENCE: 8

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Met Ala Asp Asn Gly Thr Ile Thr Val Glu Glu Leu Lys Gln Leu Leu
 1                      5                      10                      15
Glu Gln Trp Asn Leu Val Ile Gly Phe Leu Phe Leu Ala Trp Ile Met
                        20                      25                      30
Leu Leu Gln Phe Ala Tyr Ser Asn Arg Asn Arg Phe Leu Tyr Ile Ile
                        35                      40                      45
Lys Leu Val Phe Leu Trp Leu Leu Trp Pro Val Thr Leu Ala Cys Phe
                        50                      55                      60
Val Leu Ala Ala Val Tyr Arg Ile Asn Trp Val Thr Gly Gly Ile Ala
65                      70                      75                      80
Ile Ala Met Ala Cys Ile Val Gly Leu Met Trp Leu Ser Tyr Phe Val
                        85                      90                      95
Ala Ser Phe Arg Leu Phe Ala Arg Thr Arg Ser Met Trp Ser Phe Asn
                        100                     105                     110
Pro Glu Thr Asn Ile Leu Leu Asn Val Pro Leu Arg Gly Thr Ile Val
                        115                     120                     125
Thr Arg Pro Leu Met Glu Ser Glu Leu Val Ile Gly Ala Val Ile Ile
                        130                     135                     140

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Arg Gly His Leu Arg Met Ala Gly His Pro Leu Gly Arg Cys Asp Ile
145           150           155           160

Lys Asp Leu Pro Lys Glu Ile Thr Val Ala Thr Ser Arg Thr Leu Ser
           165           170           175

Tyr Tyr Lys Leu Gly Ala Ser Gln Arg Val Gly Thr Asp Ser Gly Phe
           180           185           190

Ala Ala Tyr Asn Arg Tyr Arg Ile Gly Asn Tyr Lys Leu Asn Thr Asp
           195           200           205

His Ala Gly Ser Asn Asp Asn Ile Ala Leu Leu Val Gln
           210           215           220

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<210> SEQ ID NO 9
<211> LENGTH: 63
<212> TYPE: PRT
<213> ORGANISM: Coronavirus

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<400> SEQUENCE: 9

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Met Phe His Leu Val Asp Phe Gln Val Thr Ile Ala Glu Ile Leu Ile
1           5           10           15

Ile Ile Met Arg Thr Phe Arg Ile Ala Ile Trp Asn Leu Asp Val Ile
           20           25           30

Ile Ser Ser Ile Val Arg Gln Leu Phe Lys Pro Leu Thr Lys Lys Asn
           35           40           45

Tyr Ser Glu Leu Asp Asp Glu Glu Pro Met Glu Leu Asp Tyr Pro
           50           55           60

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<210> SEQ ID NO 10
<211> LENGTH: 122
<212> TYPE: PRT
<213> ORGANISM: Coronavirus

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<400> SEQUENCE: 10

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

Leu Tyr His Tyr Gln Glu Cys Val Arg Gly Thr Thr Val Leu Leu Lys
           20           25           30

Glu Pro Cys Pro Ser Gly Thr Tyr Glu Gly Asn Ser Pro Phe His Pro
           35           40           45

Leu Ala Asp Asn Lys Phe Ala Leu Thr Cys Thr Ser Thr His Phe Ala
           50           55           60

Phe Ala Cys Ala Asp Gly Thr Arg His Thr Tyr Gln Leu Arg Ala Arg
           65           70           75           80

Ser Val Ser Pro Lys Leu Phe Ile Arg Gln Glu Glu Val Gln Gln Glu
           85           90           95

Leu Tyr Ser Pro Leu Phe Leu Ile Val Ala Ala Leu Val Phe Leu Ile
           100          105          110

Leu Cys Phe Thr Ile Lys Arg Lys Thr Glu
           115          120

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<210> SEQ ID NO 11
<211> LENGTH: 84
<212> TYPE: PRT
<213> ORGANISM: Coronavirus

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<400> SEQUENCE: 11

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Met Cys Leu Lys Ile Leu Val Arg Tyr Asn Thr Arg Gly Asn Thr Tyr
1           5           10           15

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Ser Thr Ala Trp Leu Cys Ala Leu Gly Lys Val Leu Pro Phe His Arg
 20 25 30

Trp His Thr Met Val Gln Thr Cys Thr Pro Asn Val Thr Ile Asn Cys
 35 40 45

Gln Asp Pro Ala Gly Gly Ala Leu Ile Ala Arg Cys Trp Tyr Leu His
 50 55 60

Glu Gly His Gln Thr Ala Ala Phe Arg Asp Val Leu Val Val Leu Asn
 65 70 75 80

Lys Arg Thr Asn

<210> SEQ ID NO 12
 <211> LENGTH: 422
 <212> TYPE: PRT
 <213> ORGANISM: Coronavirus

<400> SEQUENCE: 12

Met Ser Asp Asn Gly Pro Gln Ser Asn Gln Arg Ser Ala Pro Arg Ile
 1 5 10 15

Thr Phe Gly Gly Pro Thr Asp Ser Thr Asp Asn Asn Gln Asn Gly Gly
 20 25 30

Arg Asn Gly Ala Arg Pro Lys Gln Arg Arg Pro Gln Gly Leu Pro Asn
 35 40 45

Asn Thr Ala Ser Trp Phe Thr Ala Leu Thr Gln His Gly Lys Glu Glu
 50 55 60

Leu Arg Phe Pro Arg Gly Gln Gly Val Pro Ile Asn Thr Asn Ser Gly
 65 70 75 80

Pro Asp Asp Gln Ile Gly Tyr Tyr Arg Arg Ala Thr Arg Arg Val Arg
 85 90 95

Gly Gly Asp Gly Lys Met Lys Glu Leu Ser Pro Arg Trp Tyr Phe Tyr
 100 105 110

Tyr Leu Gly Thr Gly Pro Glu Ala Ser Leu Pro Tyr Gly Ala Asn Lys
 115 120 125

Glu Gly Ile Val Trp Val Ala Thr Glu Gly Ala Leu Asn Thr Pro Lys
 130 135 140

Asp His Ile Gly Thr Arg Asn Pro Asn Asn Ala Ala Thr Val Leu
 145 150 155 160

Gln Leu Pro Gln Gly Thr Thr Leu Pro Lys Gly Phe Tyr Ala Glu Gly
 165 170 175

Ser Arg Gly Gly Ser Gln Ala Ser Ser Arg Ser Ser Ser Arg Ser Arg
 180 185 190

Gly Asn Ser Arg Asn Ser Thr Pro Gly Ser Ser Arg Gly Asn Ser Pro
 195 200 205

Ala Arg Met Ala Ser Gly Gly Glu Thr Ala Leu Ala Leu Leu Leu
 210 215 220

Leu Asp Arg Leu Asn Gln Leu Glu Ser Lys Val Ser Gly Lys Gly Gln
 225 230 235 240

Gln Gln Gln Gly Gln Thr Val Thr Lys Lys Ser Ala Ala Glu Ala Ser
 245 250 255

Lys Lys Pro Arg Gln Lys Arg Thr Ala Thr Lys Gln Tyr Asn Val Thr
 260 265 270

Gln Ala Phe Gly Arg Arg Gly Pro Glu Gln Thr Gln Gly Asn Phe Gly
 275 280 285

Asp Gln Asp Leu Ile Arg Gln Gly Thr Asp Tyr Lys His Trp Pro Gln
 290 295 300

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Ile Ala Gln Phe Ala Pro Ser Ala Ser Ala Phe Phe Gly Met Ser Arg
 305 310 315 320

Ile Gly Met Glu Val Thr Pro Ser Gly Thr Trp Leu Thr Tyr His Gly
 325 330 335

Ala Ile Lys Leu Asp Asp Lys Asp Pro Gln Phe Lys Asp Asn Val Ile
 340 345 350

Leu Leu Asn Lys His Ile Asp Ala Tyr Lys Thr Phe Pro Pro Thr Glu
 355 360 365

Pro Lys Lys Asp Lys Lys Lys Lys Thr Asp Glu Ala Gln Pro Leu Pro
 370 375 380

Gln Arg Gln Lys Lys Gln Pro Thr Val Thr Leu Leu Pro Ala Ala Asp
 385 390 395 400

Met Asp Asp Phe Ser Arg Gln Leu Gln Asn Ser Met Ser Gly Ala Ser
 405 410 415

Ala Asp Ser Thr Gln Ala
 420

<210> SEQ ID NO 13
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 13

ctaacatgct taggataatg g

21

<210> SEQ ID NO 14
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 14

gcctctcttg ttcttgctcg c

21

<210> SEQ ID NO 15
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 15

caggtaagcg taaaactcat c

21

<210> SEQ ID NO 16
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 16

catgtgtggc ggctcactat at

22

<210> SEQ ID NO 17
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 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic oligonucleotide.

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<400> SEQUENCE: 17

gacactatta gcataagcag ttgtagca

28

<210> SEQ ID NO 18

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 18

ttaaaccagg tggaacatca tccggtg

27

<210> SEQ ID NO 19

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 19

ggagccttga atacacccaa ag

22

<210> SEQ ID NO 20

<211> LENGTH: 17

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 20

gcacggtggc agcattg

17

<210> SEQ ID NO 21

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 21

ccacattggc acccgcaatc c

21

<210> SEQ ID NO 22

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 22

caaacattgg ccgcaaatt

19

<210> SEQ ID NO 23

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 23

caatgcgtga cattccaaag a

21

<210> SEQ ID NO 24

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<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 24

cacaatttgc tccaagtgcc tctgca 26

<210> SEQ ID NO 25
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 25

gaagtaccat ctggggctga g 21

<210> SEQ ID NO 26
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 26

ccgaagagct acccgacg 18

<210> SEQ ID NO 27
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 27

ctctttcatt ttgccgtcac caccac 26

<210> SEQ ID NO 28
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 28

agctctccct agcattattc actg 24

<210> SEQ ID NO 29
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 29

caccacattt tcatcgaggc 20

<210> SEQ ID NO 30
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 30

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taccctcgat cgtactccgc gt 22

<210> SEQ ID NO 31
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 31

tgtaggcact gattcagggtt ttg 23

<210> SEQ ID NO 32
<211> LENGTH: 23
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 32

cggcgtgggtc tgtatttaat tta 23

<210> SEQ ID NO 33
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 33

ctgcatacaa ccgctaccgt attggaa 27

<210> SEQ ID NO 34
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 34

gggttgggac tatcctaagt gtga 24

<210> SEQ ID NO 35
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: "n" equals inosine.

<400> SEQUENCE: 35

taacacacaa cnccatcatc a 21

<210> SEQ ID NO 36
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 36

agatttggac ctgcgagcg 19

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<210> SEQ ID NO 37
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 37

gagcggctgt ctccacaagt 20

<210> SEQ ID NO 38
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 38

ttctgacctg aaggctctgc gcg 23

We claim:

1. An isolated nucleic acid molecule consisting of the ²⁵
nucleotide sequence as set forth in SEQ ID NO: 1.

* * * * *