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Rota et al.

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(54) **CORONAVIRUS ISOLATED FROM HUMANS**

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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.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2005/0181357 A1* 8/2005 Peiris et al. 435/5

FOREIGN PATENT DOCUMENTS

WO WO 2004/085633 * 10/2004
WO WO 2004/092360 * 10/2004

OTHER PUBLICATIONS

GenBank Accession No. AY274119, "SARS Coronavirus Tor2, complete genome," version AY274119.1, Apr. 14, 2003.*
GenBank Accession No. AY278487, "SARS coronavirus BJ02, partial genome," version AY278487.1, Apr. 21, 2003.*
Genbank Accession No. AY278554, "SARS coronavirus CUHK-W1, complete genome.," version AY278554.1, Apr. 18, 2003.*
GenBank Accession No. AY278491, "SARS Coronavirus HKU-39849, complete genome," version AY278491.1, Apr. 18, 2003.*
SARS-associated Coronavirus. Genomic Sequence Availability. [online] [retreived on Aug. 8, 2005]. Retreived from the Internet <URL: http://www.bcgsc.ca/bioinfo/SARS>.*
GenBank Accession No. AY274119, Apr. 14, 2003.
GenBank Accession No. AY278741, Apr. 21, 2003.
"Update: Outbreak of Severe Acute Respiratory Syndrome—Worldwide, 2003," *MMWR Weekly 52*:241-248 (2003).
Emery et al., "Real-Time Reverse Transcription-Polymerase Chain Reaction Assay for SARS-Associated Coronavirus," *Emerg. Infect. Diseases 10*:311-316 (2004).
Goldsmith et al., "Ultrastructural Characterization of SARS Coronavirus," *Emerg. Infect. Diseases 10*:320-326 (2004).
Ksiazek et al., "A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome," *N. Eng. J. Med. 348*:1953-1966 (2003).
Luo and Luo, "Initial SARS Coronavirus Genome Sequence Analysis Using a Bioinformatics Platform," 2nd *Asia-Pacific Bioinformatics Conference* (*APBC2004*), Dunedin, New Zealand (2004).
Marra et al., "The Genome Sequence of the SARS-Associated Coronavirus," *Science 300*:1393-1404 (2003).
Supplementary Online Material for Marra et al. <<www.sciencemag.org/cgi/content/full/1085953/DC1>>.
Rota et al., "Characterization of a Novel Coronavirus Associated with Severe Acute Respiratory Syndrome," *Science 300*:1394-1399 (2003).
Supplementary Online Material for Rota et al. <<www.sciencemag.org/cgi/content/full/1085952/DC1>>.

* cited by examiner

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(74) *Attorney, Agent, or Firm*—Klarquist Sparkman LLP

(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.

1 Claim, 7 Drawing Sheets

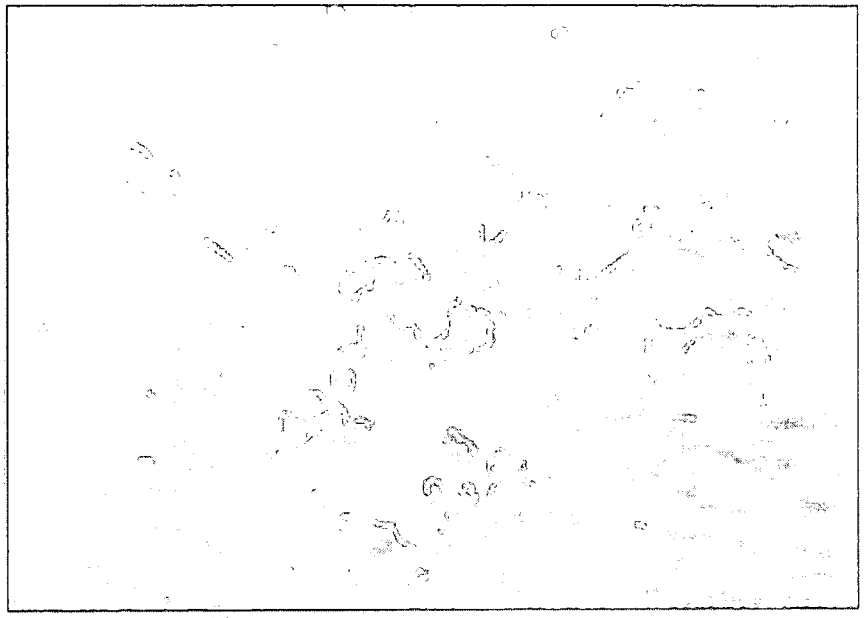


FIG. 1A

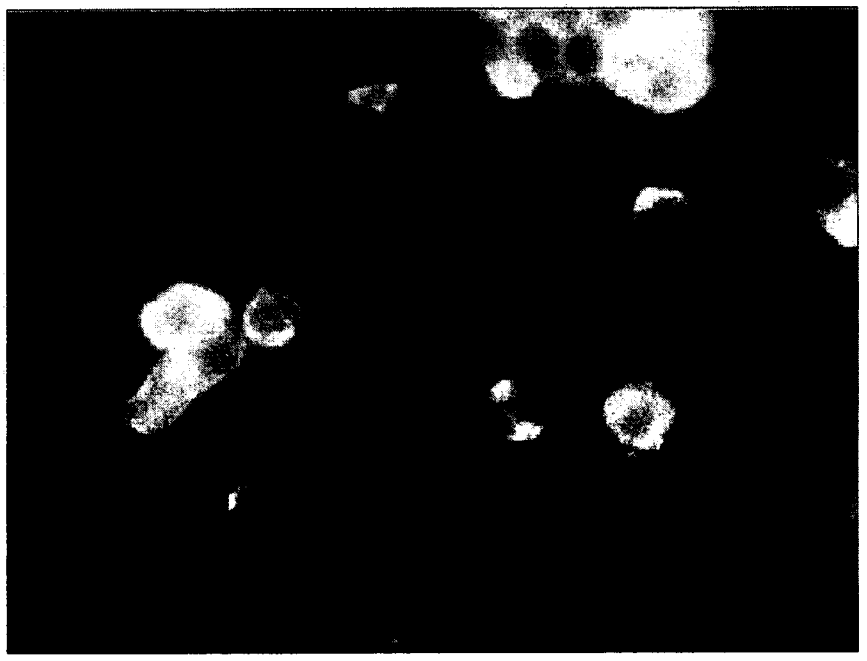


FIG. 1B

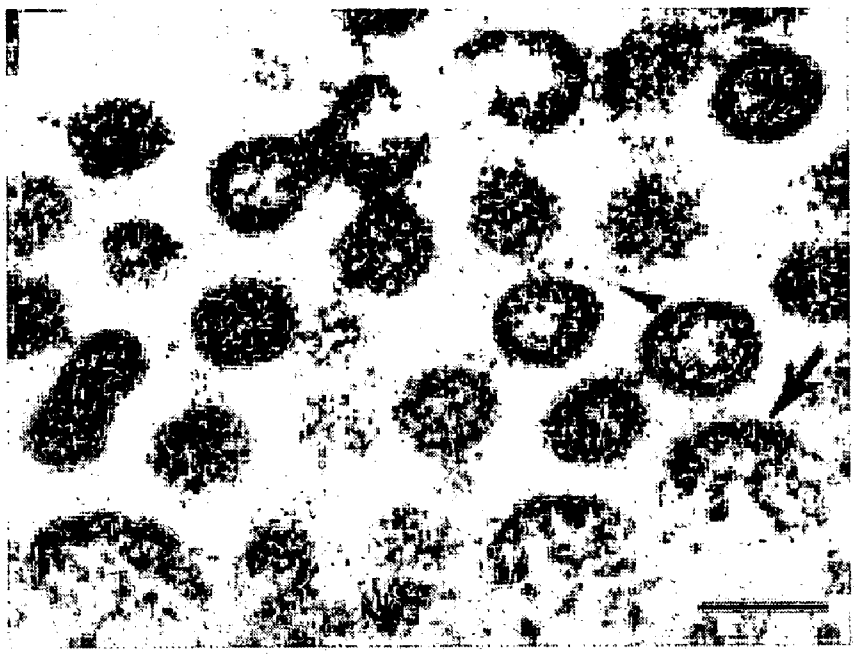


FIG. 2A

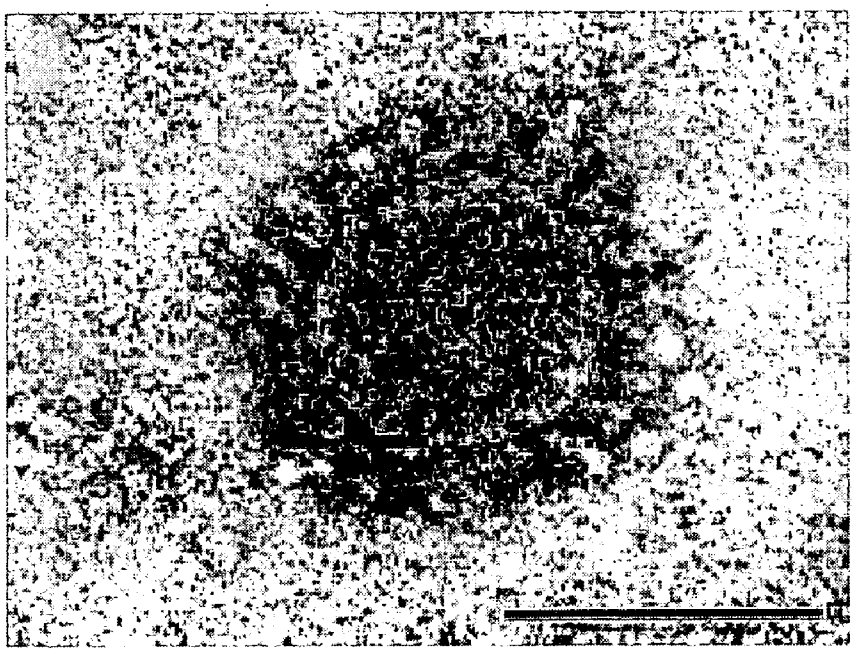


FIG. 2B

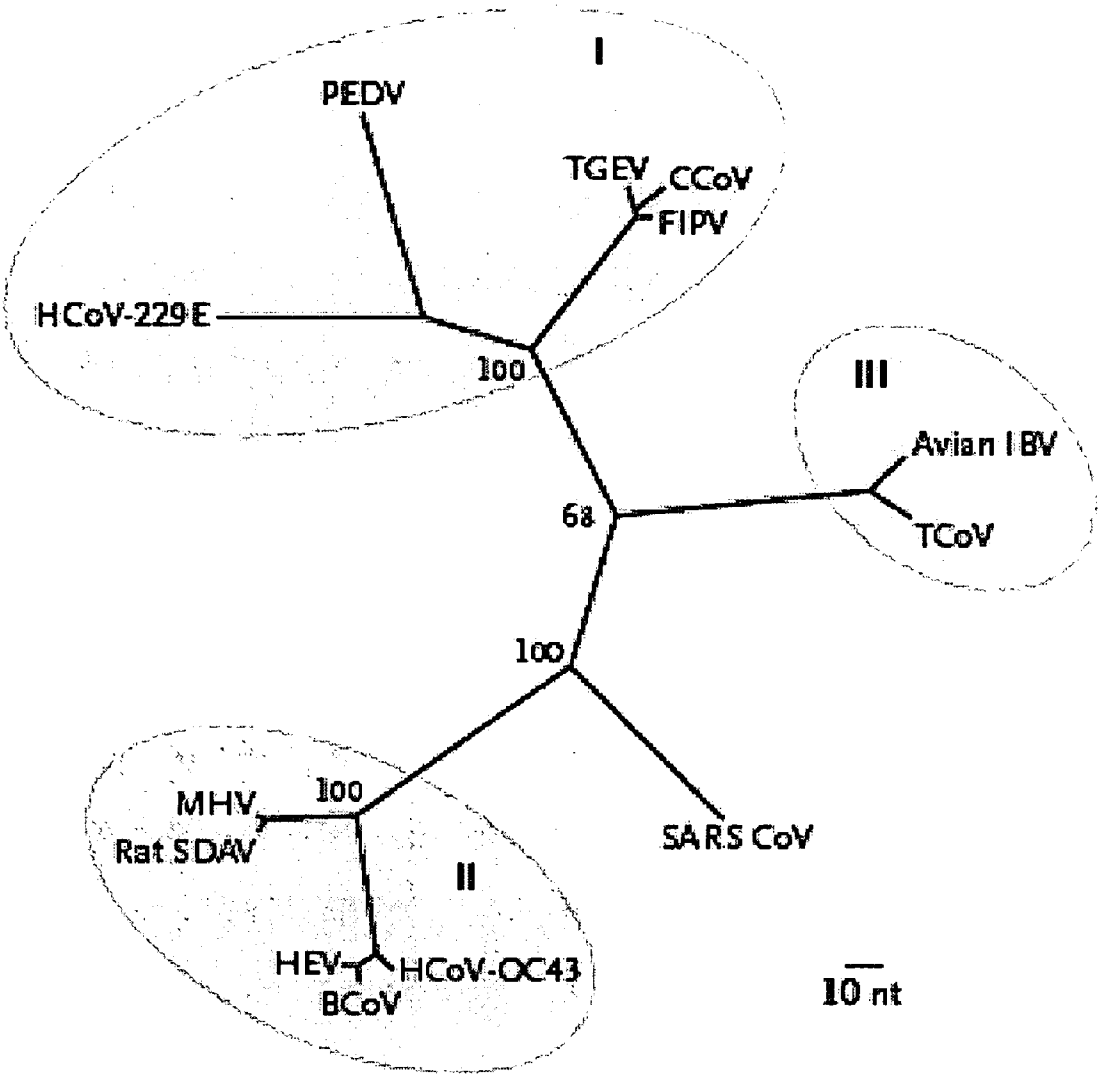

I
PEDV
TGEV
CCoV
FIPV
HCoV-229E
100
III
Avian IBV
68
TCoV
100
MHV
100
Rat SDAV
II
SARS CoV
HEV
HCoV-OC43
BCoV
10 nt

FIG. 3

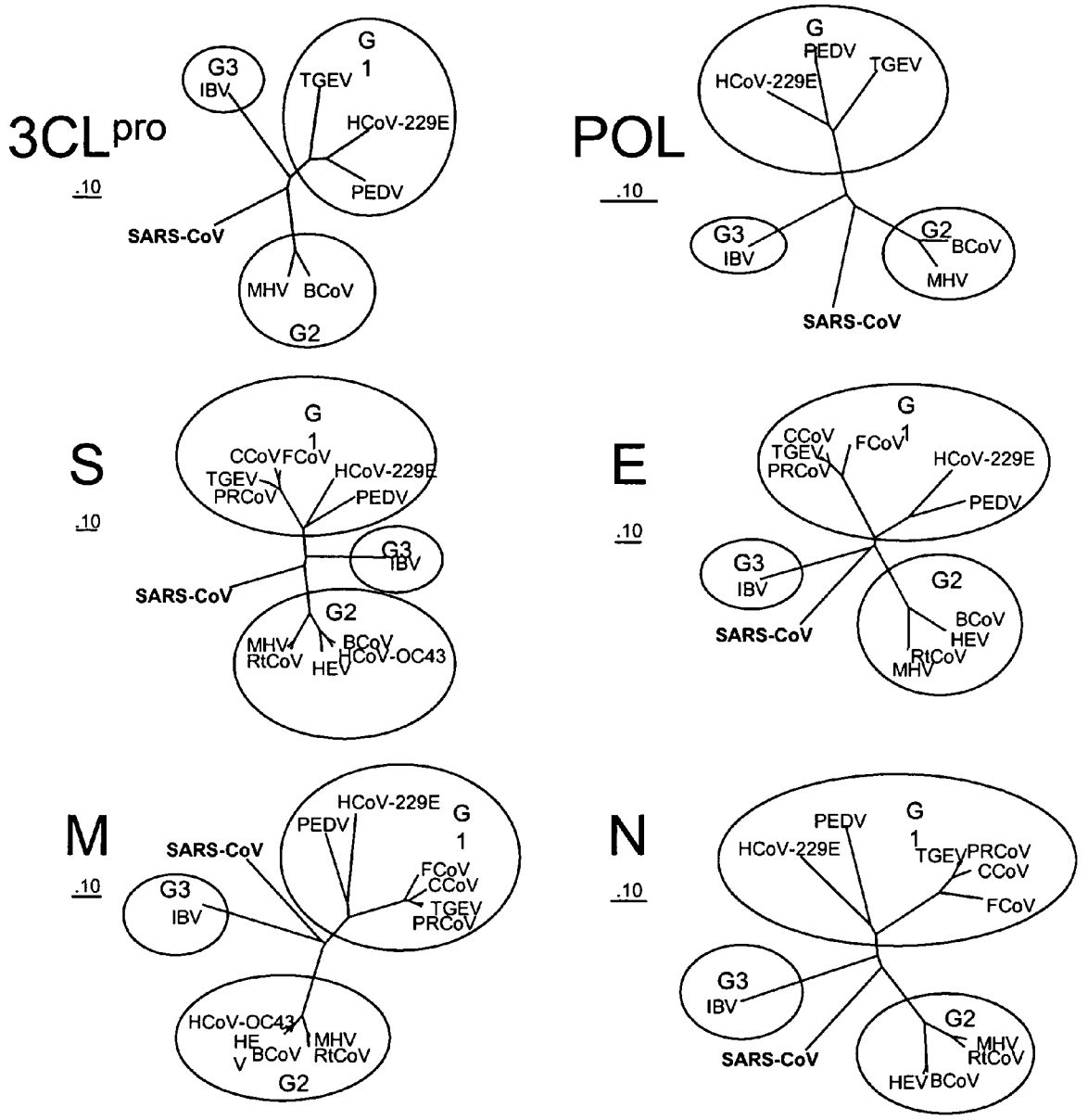

3CLpro
.10
G3
IBV
G
1
TGEV
HCoV-229E
PEDV
SARS-CoV
MHV
BCoV
G2
POL
.10
G
PEDV
TGEV
HCoV-229E
G3
IBV
G2
BCoV
MHV
SARS-CoV
S
.10
G
1
CCoV
FCoV
TGEV
PRCoV
HCoV-229E
PEDV
G3
IBV
SARS-CoV
G2
MHV
RtCoV
BCoV
HEV
HCoV-OC43
E
.10
G
1
CCoV
TGEV
PRCoV
FCoV
HCoV-229E
PEDV
G3
IBV
G2
SARS-CoV
BCoV
HEV
RtCoV
MHV
M
.10
HCoV-229E
PEDV
G
1
SARS-CoV
FCoV
CCoV
TGEV
PRCoV
G3
IBV
HCoV-OC43
HE
V
MHV
BCoV
RtCoV
G2
N
.10
PEDV
G
1
HCoV-229E
TGEV
PRCoV
CCoV
FCoV
G3
IBV
SARS-CoV
G2
MHV
RtCoV
HEV
BCoV

FIG. 4

FIG. 5A

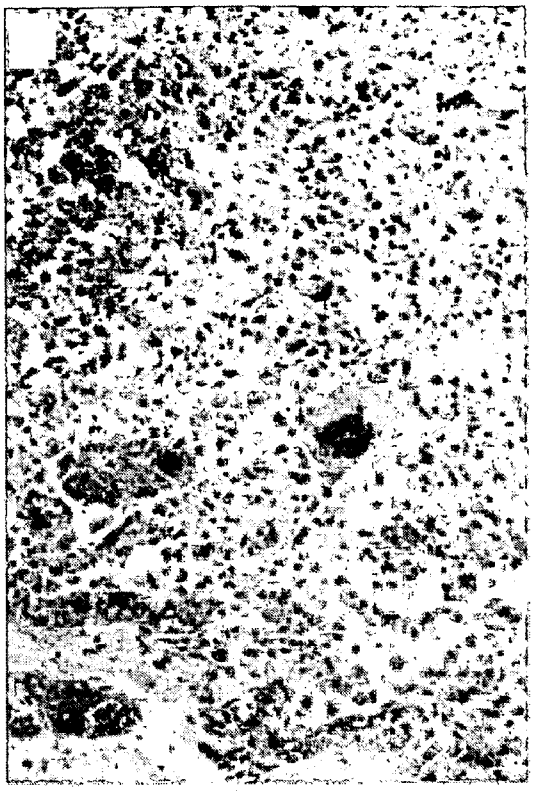
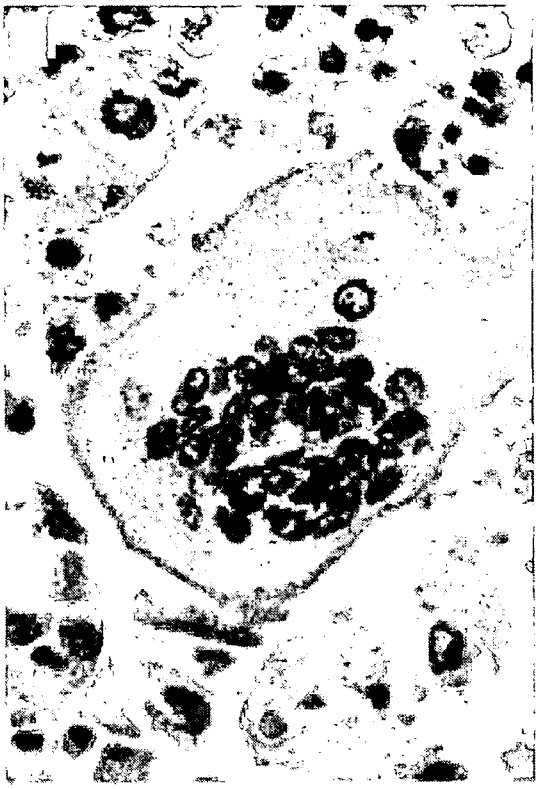


FIG. 5B



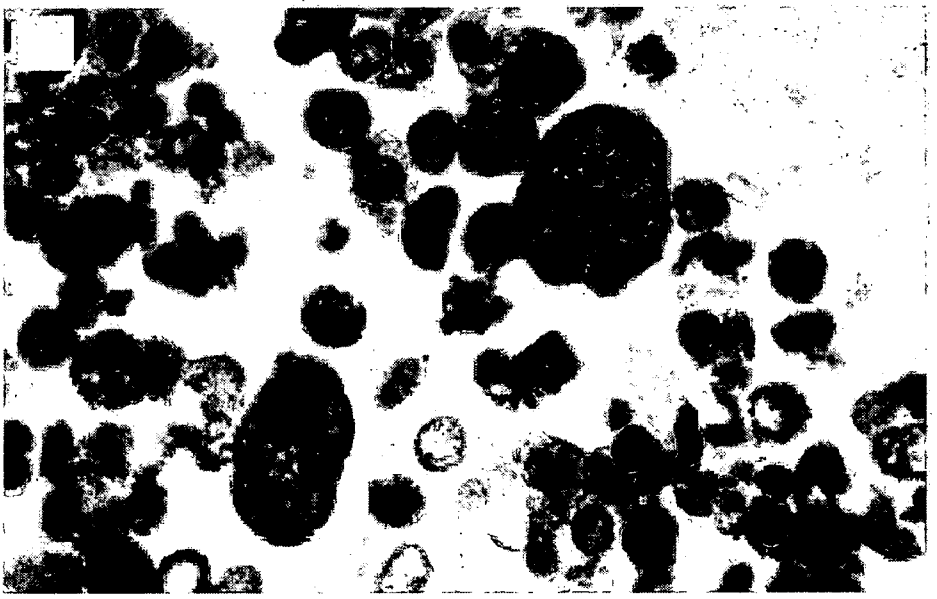


FIG. 5C

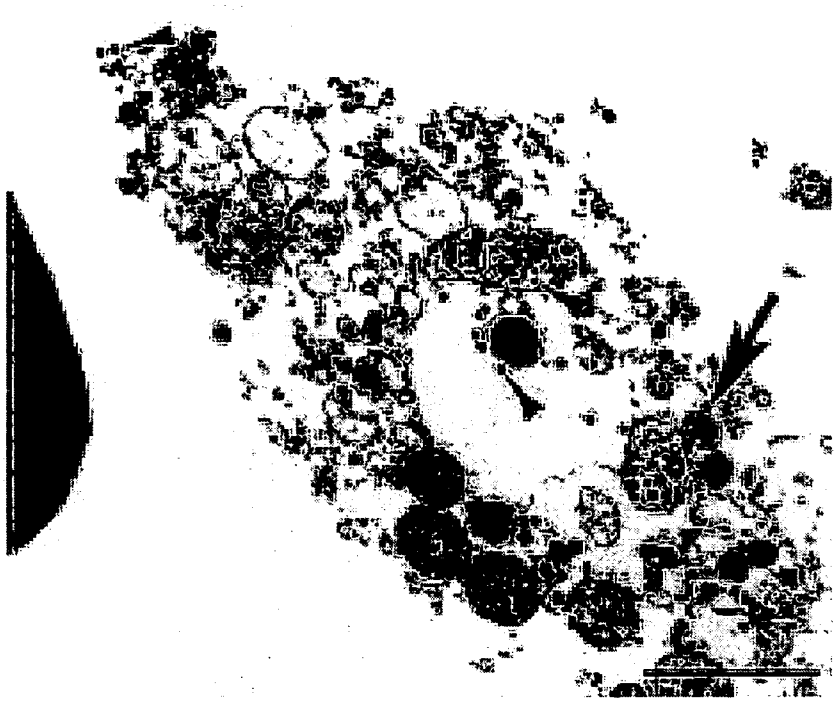


FIG. 6A

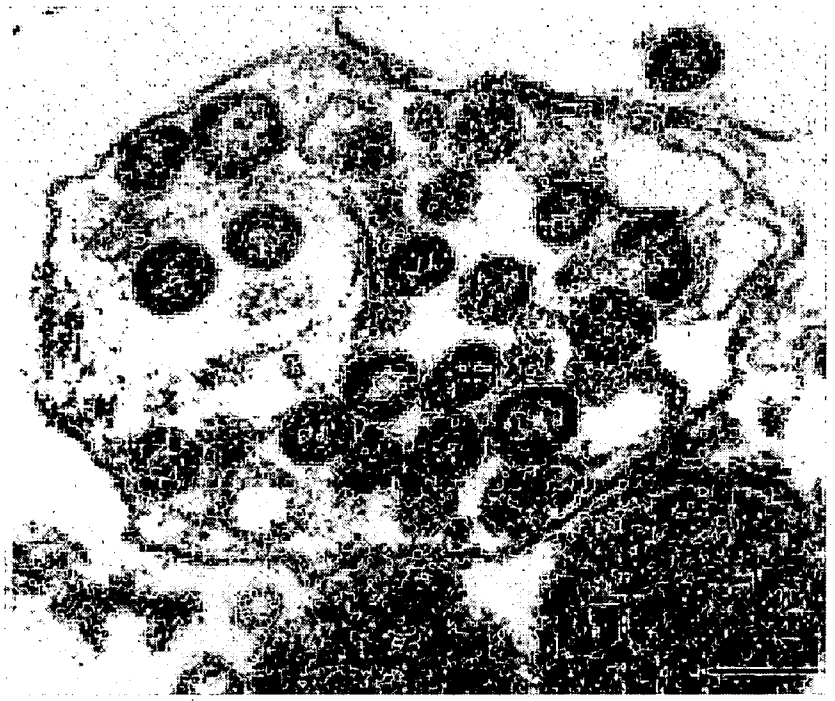


FIG. 6B

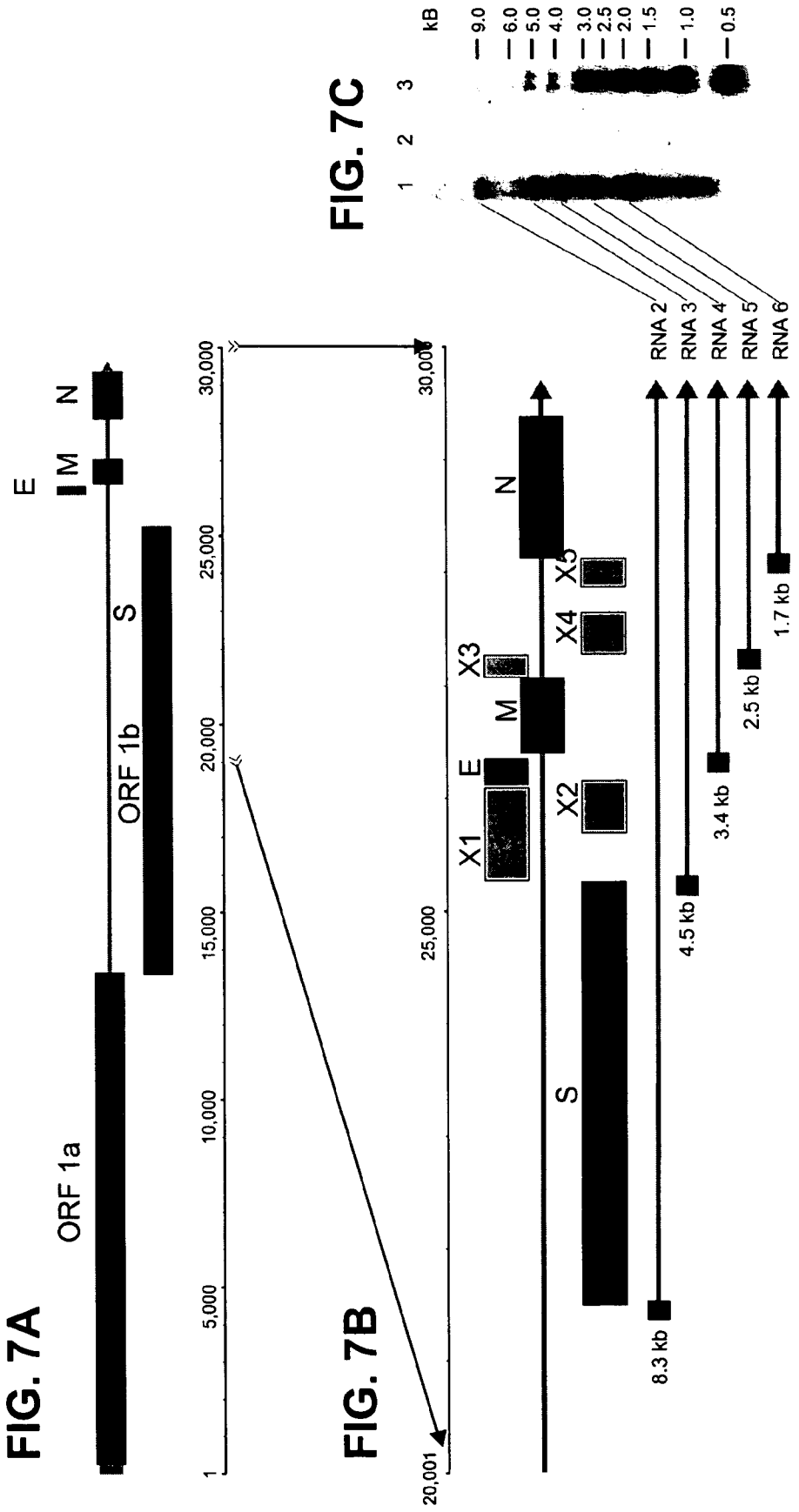


FIG. 7A
ORF 1a
ORF 1b
E
M
N
S
1
5,000
10,000
15,000
20,000
25,000
30,000
FIG. 7B
20,001
25,000
30,000
X1
X2
X3
X4
X5
8.3 kb
4.5 kb
3.4 kb
2.5 kb
1.7 kb
FIG. 7C
kB
9.0
6.0
5.0
4.0
3.0
2.5
2.0
1.5
1.0
0.5
1
2
3
RNA 2
RNA 3
RNA 4
RNA 5
RNA 6

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-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 el., *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. **1**A–B are photomicrographs illustrating typical early cytopathic effects seen with coronavirus isolates and serum from SARS patients. FIG. **1**A is a photomicrograph of Vero E6 cells inoculated with an oropharyngeal specimen from a SARS patient (×40). FIG. **1**B 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. **2**A–B are electronmicrographs illustrating ultrastructural characteristics of the SARS-associated coronavirus (SARS-CoV). FIG. **2**A 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. **2**B 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 generated by using Clustalx 1.83 with the Gonnet protein comparison matrix. The resulting trees were adjusted for final output using treetool 2.0.1.

FIGS. **5**A–C are photomicrographs illustrating diffuse alveolar damage in a patient with SARS (FIGS. **5**A–B), and immunohistochemical staining of SARS-CoV-infected Vero E6 cells (FIG. **5**C). FIG. **5**A 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. **5**B is a higher magnification photomicrograph of lung tissue from the same SARS patient (×250). Syncytial cells show no conspicuous viral inclusions. FIG. **5**C 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. **6**A–B are electronmicrographs illustrating ultrastructural characteristics of a coronavirus-infected cell in bronchoalveolar lavage (BAL) from a SARS patient. FIG. **6**A is an electronmicrograph of a coronavirus-infected cell. Numerous intracellular and extracellular particles are present; virions are indicated by the arrowheads. FIG. **6**B is a higher magnification electronmicrograph of the area seen at the arrow in FIG. **6**A (rotated clockwise approximately 90°). Bars: FIG. **6**A, 1 μm; FIG. **6**B, 100 nm.

FIGS. **7**A–C illustrate the organization of the SARS-CoV genome. FIG. **7**A 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. **7**B 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. **7**C 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:

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 polyprotein 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 polyprotein 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 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 to 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

(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/polyprotein.

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 is 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 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,* 2^{nd} ed., vol. 1–3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989 and Ausubel et al. *Short Protocols in Molecular Biology,* 4^{th} 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,* 2^{nd} 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 "polyprotein" 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.

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 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 ed., 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 μM). 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, radiolabeled, 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 know 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 Blackhole 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,* 4^{th} 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 know 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 ehtylenimines).

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	Hospital-ization	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	Hospital-ization	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. **1**A). 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-cm^2 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. **2**A) (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. **2**B). 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'GCCTCTCTTGTTCTTGCTCGC3' (SEQ ID NO: 14), and Cor-p-R1 (–) 5'CAGGTAAGCGTAAAACTCATC3 (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 μl reaction mixture containing 500 ng of RNA, 200 U of Superscript™ 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 μl of 5× 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 μl PCR reaction containing 67 mM Tris-HCl (pH 8.8), 1 mM each primer, 17 mM ammonium sulfate, 6 mM EDTA, 2 mM $MgCl_2$, 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 Superscript™ 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× 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 subgenomic 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 bronchitis 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 antibodies, 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-

fected 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. **5**A). 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. **5**B), 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. **5**C). 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. **5**C 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. **6**A–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.

	Immunohistochemical reactivity of antiserum with coronavirus-infected culture cells		
Antiserum	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. **1**B). 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 (Hanoi)	9.1	5	<100	<25
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 hybrididization 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 cm^2 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'-coterminal 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

	Genome Location			Predicted Size	
ORF	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]
X5	27,778	27,864	28,115	84	2,021[d]
N	28,111	28,120	29,385	422	1,688[c]

[a]The location is the 3'-most nucleotide in the consensus TRS, AAAC-GAAC.
[b]Not including poly(A). Predicted size is based on the position of the conserved TRS.
[c]Corresponding mRNA detected by Northern blot analysis (FIG. 7C)
[d]No 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 guanidine 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	TTAAACCAGGTGGAACATCATCCGGTG (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	CAAACATTGGCCGCAAATT (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	CTCTTTCATTTTGCCGTCACCACCAC (SEQ ID NO: 27)	
3'-NTR	F	AGCTCTCCCTAGCATTATTCACTG (SEQ ID NO: 28)	3'-NTR
	R	CACCACATTTTCATCGAGGC (SEQ ID NO: 29)	
	P	TACCCTCGATCGTACTCCGCGT (SEQ ID NO: 30)	
M	F	TGTAGGCACTGATTCAGGTTTTG (SEQ ID NO: 31)	M protein
	R	CGGCGTGGTCTGTATTTAATTTA (SEQ ID NO: 32)	
	P	CTGCATACAACCGCTACCGTATTGGAA (SEQ ID NO: 33)	

[a]RT-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 following 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 ($C_T \leqq 45$ 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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Glu Thr Pro Ile Ala Tyr Arg Asn Val Leu Leu Arg Lys Asn Gly Asn
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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 Leu Leu Glu Ile Lys Asp Lys Glu Gln Tyr Cys
785                 790                 795                 800

Ala Leu Ser Pro Gly Leu Leu Ala Thr Asn Asn Val Phe Arg Leu Lys
                805                 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 Leu  Trp Ser Thr Lys Pro  Val Asp Thr
    1985                1990                1995

Ser Asn  Ser Phe Glu Val Leu  Ala Val Glu Asp Thr  Gln Gly Met
    2000                2005                2010

Asp Asn  Leu Ala Cys Glu Ser  Gln Gln Pro Thr Ser  Glu Glu Val
    2015                2020                2025

Val Glu  Asn Pro Thr Ile Gln  Lys Glu Val Ile Glu  Cys Asp Val
    2030                2035                2040

Lys Thr  Thr Glu Val Val Gly  Asn Val Ile Leu Lys  Pro Ser Asp
    2045                2050                2055

Glu Gly  Val Lys Val Thr Gln  Glu Leu Gly His Glu  Asp Leu Met
    2060                2065                2070

Ala Ala  Tyr Val Glu Asn Thr  Ser Ile Thr Ile Lys  Lys Pro Asn
    2075                2080                2085

Glu Leu  Ser Leu Ala Leu Gly  Leu Lys Thr Ile Ala  Thr His Gly
    2090                2095                2100

Ile Ala  Ala Ile Asn Ser Val  Pro Trp Ser Lys Ile  Leu Ala Tyr
    2105                2110                2115

Val Lys  Pro Phe Leu Gly Gln  Ala Ala Ile Thr Thr  Ser Asn Cys
    2120                2125                2130

Ala Lys  Arg Leu Ala Gln Arg  Val Phe Asn Asn Tyr  Met Pro Tyr
    2135                2140                2145

Val Phe  Thr Leu Leu Phe Gln  Leu Cys Thr Phe Thr  Lys Ser Thr
    2150                2155                2160

Asn Ser  Arg Ile Arg Ala Ser  Leu Pro Thr Thr Ile  Ala Lys Asn
    2165                2170                2175

Ser Val  Lys Ser Val Ala Lys  Leu Cys Leu Asp Ala  Gly Ile Asn
    2180                2185                2190

Tyr Val  Lys Ser Pro Lys Phe  Ser Lys Leu Phe Thr  Ile Ala Met
    2195                2200                2205

Trp Leu  Leu Leu Leu Ser Ile  Cys Leu Gly Ser Leu  Ile Cys Val
    2210                2215                2220

Thr Ala  Ala Phe Gly Val Leu  Leu Ser Asn Phe Gly  Ala Pro Ser
    2225                2230                2235

Tyr Cys  Asn Gly Val Arg Glu  Leu Tyr Leu Asn Ser  Ser Asn Val
    2240                2245                2250

Thr Thr  Met Asp Phe Cys Glu  Gly Ser Phe Pro Cys  Ser Ile Cys
    2255                2260                2265

Leu Ser  Gly Leu Asp Ser Leu  Asp Ser Tyr Pro Ala  Leu Glu Thr
    2270                2275                2280

Ile Gln  Val Thr Ile Ser Ser  Tyr Lys Leu Asp Leu  Thr Ile Leu
    2285                2290                2295

Gly Leu  Ala Ala Glu Trp Val  Leu Ala Tyr Met Leu  Phe Thr Lys
    2300                2305                2310

Phe Phe  Tyr Leu Leu Gly Leu  Ser Ala Ile Met Gln  Val Phe Phe
    2315                2320                2325

Gly Tyr  Phe Ala Ser His Phe  Ile Ser Asn Ser Trp  Leu Met Trp
    2330                2335                2340

Phe Ile  Ile Ser Ile Val Gln  Met Ala Pro Val Ser  Ala Met Val
    2345                2350                2355

Arg Met  Tyr Ile Phe Phe Ala  Ser Phe Tyr Tyr Ile  Trp Lys Ser
    2360                2365                2370
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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  Cys Ala Thr Thr Arg  Gln Val Val Asn Val  Ile Thr Thr
    2720                2725                2730

Lys Ile  Ser Leu Lys Gly Gly  Lys Ile Val Ser Thr  Cys Phe Lys
    2735                2740                2745

Leu Met  Leu Lys Ala Thr Leu  Leu Cys Val Leu Ala  Ala Leu Val
    2750                2755                2760
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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 Leu  Leu Pro Leu Thr Gln  Tyr Asn Arg
    3170                3175                3180

Tyr Leu  Ala Leu Tyr Asn Lys  Tyr Lys Tyr Phe Ser  Gly Ala Leu
    3185                3190                3195

Asp Thr  Thr Ser Tyr Arg Glu  Ala Ala Cys Cys His  Leu Ala Lys
    3200                3205                3210

Ala Leu  Asn Asp Phe Ser Asn  Ser Gly Ala Asp Val  Leu Tyr Gln
    3215                3220                3225

Pro Pro  Gln Thr Ser Ile Thr  Ser Ala Val Leu Gln  Ser Gly Phe
    3230                3235                3240

Arg Lys  Met Ala Phe Pro Ser  Gly Lys Val Glu Gly  Cys Met Val
    3245                3250                3255

Gln Val  Thr Cys Gly Thr Thr  Thr Leu Asn Gly Leu  Trp Leu Asp
    3260                3265                3270

Asp Thr  Val Tyr Cys Pro Arg  His Val Ile Cys Thr  Ala Glu Asp
    3275                3280                3285

Met Leu  Asn Pro Asn Tyr Glu  Asp Leu Leu Ile Arg  Lys Ser Asn
    3290                3295                3300

His Ser  Phe Leu Val Gln Ala  Gly Asn Val Gln Leu  Arg Val Ile
    3305                3310                3315

Gly His  Ser Met Gln Asn Cys  Leu Leu Arg Leu Lys  Val Asp Thr
    3320                3325                3330

Ser Asn  Pro Lys Thr Pro Lys  Tyr Lys Phe Val Arg  Ile Gln Pro
    3335                3340                3345

Gly Gln  Thr Phe Ser Val Leu  Ala Cys Tyr Asn Gly  Ser Pro Ser
    3350                3355                3360

Gly Val  Tyr Gln Cys Ala Met  Arg Pro Asn His Thr  Ile Lys Gly
    3365                3370                3375

Ser Phe  Leu Asn Gly Ser Cys  Gly Ser Val Gly Phe  Asn Ile Asp
    3380                3385                3390

Tyr Asp  Cys Val Ser Phe Cys  Tyr Met His His Met  Glu Leu Pro
    3395                3400                3405

Thr Gly  Val His Ala Gly Thr  Asp Leu Glu Gly Lys  Phe Tyr Gly
    3410                3415                3420

Pro Phe  Val Asp Arg Gln Thr  Ala Gln Ala Ala Gly  Thr Asp Thr
    3425                3430                3435

Thr Ile  Thr Leu Asn Val Leu  Ala Trp Leu Tyr Ala  Ala Val Ile
    3440                3445                3450

Asn Gly  Asp Arg Trp Phe Leu  Asn Arg Phe Thr Thr  Thr Leu Asn
    3455                3460                3465

Asp Phe  Asn Leu Val Ala Met  Lys Tyr Asn Tyr Glu  Pro Leu Thr
    3470                3475                3480

Gln Asp  His Val Asp Ile Leu  Gly Pro Leu Ser Ala  Gln Thr Gly
    3485                3490                3495

Ile Ala  Val Leu Asp Met Cys  Ala Ala Leu Lys Glu  Leu Leu Gln
    3500                3505                3510

Asn Gly  Met Asn Gly Arg Thr  Ile Leu Gly Ser Thr  Ile Leu Glu
    3515                3520                3525

Asp Glu  Phe Thr Pro Phe Asp  Val Val Arg Gln Cys  Ser Gly Val
    3530                3535                3540

Thr Phe  Gln Gly Lys Phe Lys  Lys Ile Val Lys Gly  Thr His His
    3545                3550                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
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    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                  80

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                 160

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                 240

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                 320

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                 400

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
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Asp 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 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
    930                 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 Ile Lys Trp Pro  Trp Tyr Val Trp Leu  Gly Phe Ile
    1190                1195                1200

Ala Gly  Leu Ile Ala Ile Val  Met Val Thr Ile Leu  Leu Cys Cys
    1205                1210                1215

Met Thr  Ser Cys Cys Ser Cys  Leu Lys Gly Ala Cys  Ser Cys Gly
    1220                1225                1230

Ser Cys  Cys Lys Phe Asp Glu  Asp Asp Ser Glu Pro  Val Leu Lys
    1235                1240                1245

Gly Val  Lys Leu His Tyr Thr
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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


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

<400> SEQUENCE: 7

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


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

<400> SEQUENCE: 8

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


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

<400> SEQUENCE: 9

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


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

<400> SEQUENCE: 10

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


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

<400> SEQUENCE: 11

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 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 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
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<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
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<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 gattcaggtt ttg 23

<210> SEQ ID NO 32
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide.

<400> SEQUENCE: 32

cggcgtggtc 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 nucleotide sequence as set forth in SEQ ID NO: 1.

* * * * *