SEVERE ACUTE RESPIRATORY SYNDROME DNA VACCINE COMPOSITIONS AND METHODS OF USE

BACKGROUND OF THE INVENTION

[0001] The present invention relates to a novel coronavirus (referred to herein as SARS-CoV) and to SARS-CoV vaccine compositions and methods of treating or preventing SARS-CoV infection and disease in mammals. SARS-CoV was discovered in March of 2003, in association with Severe Acute Respiratory Syndrome (SARS), a newly emerging infectious disease of global importance.

[0002] The recognition of SARS has led to activation of a global response network, with resultant travel restrictions, major quarantine, and closure of health care facilities. As of May 14, 2003, 7628 cases and 587 deaths from SARS have been reported from 29 countries. Initial reports of an atypical pneumonia began to surface in November of 2002 from the Guangdong province of China. This early outbreak reportedly involved 305 people, many of whom were healthcare workers. On February 21, 2003, a healthcare worker from Guangdong traveled to Hong Kong, where his pre-existing cold symptoms escalated and he was hospitalized for acute respiratory distress. From Hong Kong, the illness spread rapidly throughout Southeast Asia and to Canada from this one index case. Seven individuals can be linked to the index case through a stay on the ninth floor of the hotel he occupied during his first night in Hong Kong. Infected persons from three hospitals in the Hong Kong metropolitan area are traceable to this index case as well. The primary mode of transmission has been either person-to-person contact or droplet transmission. Two notable exceptions to this are the hotel in Hong Kong, where direct human contact cannot be established for all those infected, and the Amoy Garden apartment buildings where more than 221 residents have been infected. In the outbreak at the Amoy Garden apartments, an unknown environmental factor is suspected of playing a role in transmission.

[0003] The incubation period ranges on average between two and seven days. Onset of symptoms begins with a high fever associated with chills and rigors. Additional symptoms at onset may include headache, malaise, myalgia, mild

respiratory symptoms and more rarely common cold symptoms such as sore throat and runny nose. After this initial three to seven day period, additional lower respiratory symptoms appear including dry, non-productive cough and dyspnea. Initial chest x-rays reveal small, unilateral, patchy shadowings that progress quickly to bilateral, diffuse infiltrates. *Preliminary. Outbreak news: severe acute respiratory syndrome (SARS). Wkly. Epidemiol. Rec.*, 2003: 81-88 (2003). The median duration of symptoms in a small epidemiologic study was 25.5 days. Tsang, K.W., *et al. A cluster of cases of severe acute respiratory syndrome in Hong Kong, N. Engl. J. Med.* (2003). The severity of illness can range widely from a mild illness to acute respiratory failure resulting in death. Patients with a significant co-morbidity, such as diabetes, or who are older, are more likely to suffer from a severe form of the disease. Questions remain as to why some patients become infected, while others who have intimate contact with infected individuals are spared. It does appear that patients are very contagious at the onset of symptoms. Studies from hospitals in Hong Kong and Hanoi have shown attack rates >56% among healthcare workers caring for SARS patients. It is unclear at this time whether individuals are contagious during the incubation phase.

Important Features of Coronaviruses

[0004] Coronaviruses are large, enveloped, positive-stranded RNA viruses, and they are known to elicit coincident diseases in animals and humans. Mature human coronavirus (HCoV) virions are approximately 100 nm-diameter enveloped particles exposing prominent spike (S), hemagglutinin-esterase (HE) (in some types of coronaviruses), envelope (E) and membrane (M) glycoproteins. Each particle contains an approximately 30 kilobase (kB) RNA genome complexed with an approximately 60 kilodalton (kD) nucleoprotein (N). Fields, B.N. VIROLOGY New York: Lippincott, Williams & Wilkins, (Fields, B.N., ed. 2001). All of the above references are herein incorporated by reference in their entireties.

[0005] The S proteins of HCoV's have two large domains, the variable S1 domain responsible for host cell binding, Breslin, J.J. *et al. J. Virol.* 77: 4435-8 (2003), and the S2 domain containing a heptad coiled-coiled structure reminiscent of those involved in fusion in HIV and influenza. Yoo, D.W. *et al. Virology* 183: 91-8 (1991). The HCoV-229E, group I S protein appears to bind to the human aminopeptidase N glycoprotein, Yeager, C.L., *et al. Nature* 357: 420-2 (1992); Bonavia, A. *et al. J. Virol.* 77: 2530-8 (2003), whereas the HCoV-OC43 strain (HCoV-OC43, group II) may bind via sialic acid moieties. Vlasak, R. *et al. Proc. Natl. Acad. Sci. USA* 85:4526-9 (1988). The genetic variability between strains of coronavirus has not been thoroughly evaluated, although only minor variability has been observed in the S protein in the small number of strains sequenced. Hays, J.P. and Myint, S.H. *J. Virol. Methods* 75: 179-93 (1998); Kunkel, F. and Herrler, G. *Arch. Virol.* 141: 1123-31 (1996). Most coronaviruses are not only species specific, but also somewhat tissue tropic. This tropism is mostly related to changes in the S protein. Sanchez, C.M. *et al. J. Virol.* 73: 7607-18 (1999). Examples of such coronavirus tropism changes are the *in vitro* demonstration that tropism can be experimentally manipulated by genetically replacing a feline S protein with a mouse S protein, and the natural emergence of the porcine respiratory coronavirus (PRCoV) from the transmissible gastroenteritis virus of swine (TGEV) strain merely through a deletion of a region in the S protein. Haijema, B.J. *et al. J. Virol.* 77:4528-38 (2003); Page, K.W. *et al. J. Gen. Virol.* 72:579-87 (1991); Britton, P. *et al. Virus Res.* 21:181-98 (1991). All of the above references are herein incorporated by reference in their entireties.

[0006] The recently discovered novel coronavirus, SARS-CoV, appears to be a new member of the order *Nidovirales*. Concerted efforts by many laboratories worldwide has led to the rapid sequencing of various strains of SARS-CoV, including CUKH-Su10 (GenBank Accession No. AY282752), TOR2 (GenBank Accession No. AY274119 and NC_004781), BJ01 (GenBank Accession No. AY278488), CUHK-W1 (GenBank Accession No. AY278554), Urbani (GenBank Accession No. AY278741) and HKU-39849

(GenBank Accession No. AY278491). The Urbani strain of SARS-CoV, sequenced by the Centers for Disease Control in Atlanta, Georgia, is a 29,727-nucleotide, polyadenylated RNA with a genomic organization that is typical of coronaviruses: 5'-replicase, spike (S), envelope (E), membrane (M)-3'. Rota *et al.*, *Science* 300:1394-1399 (2003), available May 1, 2003 at http://www.sciencexpress.org (hereinafter "Rota et al."). In addition, there are short untranslated regions at both termini, and open reading frames (ORFs) encoding non-structural proteins located between S and E, between M and N, or downstream of N. Rota *et al.* The hemagglutinin-esterase (HE) gene found in group 2 and some group 3 coronaviruses was not found in SARS-CoV. Rota *et al.* Sequencing of the Tor2 SARS-CoV strain by a collaboration of researchers in British Columbia, Canada, yielded a genomic sequence that differed from the Urbani SARS-CoV strain by eight nucleotide bases. Marra *et al.*, *Science* 300:1399-1404(2003), available May 1, 2003 at http://www.sciencexpress.org (hereinafter "Marra et al."). A comparison of the HKU-39849 and CUHK-W1 SARS-CoV strains also differed from the Urbani sequence by 10 or fewer nucleotide bases. Rota *et al.* All of the above references are herein incorporated by reference in their entireties.

[0007] Phylogenetic analyses indicate that, based on the genetic distance between SARS-CoV and other known coronaviruses in all of their genetic regions, no large region of the SARS-CoV genome was derived from other known viruses, and that SARS forms a distinct group within the genus *Cornavirus*. Rota *et al.*; Marra *et al.* The analyses also showed greater sequence conservation among enzymatic proteins of SARS-CoV than among the S, N, M, and E structural proteins; and, while there were regions of amino acid conservation within each protein as between SARS-CoV and other coronaviruses, the overall similarity was low. Rota *et al.* All of the above references are herein incorporated by reference in their entireties.

[0008] A virus, almost identical to the human SARS-CoV virus, has been isolated from rare Chinese masked palm civet cats. This virus is believed to be identical to human SARS-CoV except for a 29 nucleotide deletion in the

region encoding the N protein of the virus. Walgate, R. "Human SARS virus not identical to civet virus" *The Scientist*. May 27, 2003, available at http://www.biomedcentral.com/news/20030527/03/ (visited June 13, 2003), incorporated herein by reference in its entirety.

Coronavirus Vaccine Candidates

[0009] Because SARS-CoV was so recently discovered, there are no vaccines against the virus. The approach to vaccine development can, however, be partially guided by the results of past studies in animals, of which three diseases have received the greatest attention. These are transmissible gastroenteritis virus (TGEV) in swine, feline infectious peritonitis virus (FIPV), and avian infectious bronchitis virus (IBV). Of note, none of the vaccines, most of which have been attenuated vaccines, have proven to be highly efficacious except for inactivated IBV. Enjuanes, L. *et al., Adv. Exp. Med. Biol.* 380: 197-211 (1995). The FIPV vaccine is a live attenuated virus that has provided minimal efficacy in field trials, and the TGEV vaccine has also been problematic. Scott, F. W., *Adv. Vet. Med.* 41:347-58 (1999); Sestak, K. *et al., Vet. Immunol. Immunopathol.* 70:203-21 (1999). All of the above references are herein incorporated by reference in their entireties.

[0010] In the TGEV model, the major focus has been on neutralizing antibody directed at the S glycoprotein. Sestak, K. *et al., Vet. Immunol. Immunopathol.* 70: 203-21 (1999); Tuboly, T. *et al. Vaccine* 18: 2023-8 (2000); Shoup, D.I. *et al. Am. J. Vet. Res.* 58: 242-50 (1997). Protection has also been associated with antibodies in IBV and bovine coronavirus. Mondal, S. P. *et al. Avian. Dis.* 45:1054-9 (2001); Yoo, D.W. *et al. Virology* 180: 395-9 (1991). In fact, in most of the animal models, control of coronavirus infection can be due to antibodies reactive to the N-terminal region of the S protein. Gallagher, T.M. and Buchmeier, M.J. *Virology* 279: 371-4 (2001); Tuboly, T. *et al. Arch. Virol.* 137: 55-67 (1994). In one study of respiratory bovine coronavirus, antibody appearance to the S and N proteins was correlated with recovery.

Lin, X.Q. *et al. Arch. Virol.* 145: 2335-49 (2000); Passive transfer studies have also been successful and demonstrated the value of humoral immune responses. Enjuanes, L. *et al., Adv. Exp. Med. Biol.* 380: 197-211 (1995); Spaan, W.J. *Adv. Exp. Med. Biol.* 276: 201-3 (1990). All of the above references are herein incorporated by reference in their entireties.

[0011] Cell-mediated immune responses have been most clearly detected in coronaviruses against the S, M and N proteins. Spencer, J.S. et al. *Adv. Exp. Med. Biol.* 380: 121-9 (1995); Collisson, E.W. *et al. Dev. Comp. Immunol.* 24: 187-200 (2000); Stohlman, S.A. *et al. Virology* 189: 217-24 (1992). In one study, the use of a DNA vaccine encoding the carboxyl terminus of the N gene of IBV, which induced cytotoxic T cell (CTL) activity, was able to decrease virus titers by 7 logs in target organs. Seo, S.H. *et al. J. Virol.* 71: 7889-94 (1997). Some protection was also noted in a DNA vaccine encoding the N protein in the Mouse Hepatitis Virus (MHV) model. Hayashi, M. *et al. Adv. Exp. Med. Biol.* 440:693-9 (1998). There is also some evidence that CTL may be involved in the control of MHV, and prevent the development of persistent infection and neuropathology. Pewe, L. and Perlman, S. *Virology* 255: 106-16 (1999); Pewe, L. *et al. J. Virol.* 71: 7640-7 (1997). All of the above references are herein incorporated by reference in their entireties.

[0012] A large number of coronavirus challenge studies have been conducted in humans by Tyrrell and colleagues, in which the subjects were inoculated intranasally and followed. Callow, K.A. *et al. Epidemiol. Infect.* 105: 435-46 (1990); Bende, M. *et al. Acta Otolaryngol.* 107: 262-9 (1989). Such challenge studies will clearly be impossible for the much more serious SARS-CoV virus. The presence of antibodies to the challenge strain did not prevent infection or disease, even in the face of rising neutralizing antibody titers. However, a second infection with similar strains led to decreased symptoms, revealing persistence of immunity against homologous challenge. Reed, S.E. *J. Med. Virol.* 13: 179-92 (1984). Also, the 2-4 year cyclical nature of the disease points to some persistence of immune response over time. Reed, S.E. *J. Med. Virol.* 13: 179-92 (1984); Hendley, J.O. *et al. Am. Rev. Respir. Dis.* 105: 805-

11 (1972); Evans, A.S. and Kaslow, R.A. VIRAL INFECTIONS OF HUMANS. 4th ed. New York and London: Plenum Medical Book Company, (Evans, A.S. and Kaslow, R.A., eds., 1997). All of the above references are herein incorporated by reference in their entireties.

[0013] Heterologous "prime boost" strategies have been effective for enhancing immune responses and protection against numerous pathogens. Schneider *et al.*, *Immunol. Rev.* 170:29-38 (1999); Robinson, H.L., *Nat. Rev. Immunol.* 2:239-50 (2002); Gonzalo, R.M. *et al.*, *Vaccine 20*:1226-31 (2002); Tanghe, A., *Infect. Immun. 69*: 3041-7 (2001). Providing antigen in different forms in the prime and the boost injections appears to maximize the immune response to the antigen. DNA vaccine priming followed by boosting with protein in adjuvant or by viral vector delivery of DNA encoding antigen appears to be the most effective way of improving antigen specific antibody and CD4+ T-cell responses or CD8+ T-cell responses respectively. Shiver J.W. *et al.*, *Nature 415*: 331-5 (2002); Gilbert, S.C. *et al.*, *Vaccine* 20:1039-45 (2002); Billaut-Mulot, O. *et al.*, *Vaccine 19*:95-102 (2000); Sin, J.I. *et al.*, DNA Cell Biol. 18:771-9 (1999). Recent data from monkey vaccination studies suggests that adding CRL1005 poloxamer to DNA encoding the HIV gag antigen enhances T-cell responses when monkeys are vaccinated with an HIV gag DNA prime followed by a boost with an adenoviral vector expressing HIV gag (Ad5-gag). The cellular immune responses for a DNA/poloxamer prime followed by an Ad5-gag boost were greater than the responses induced with a DNA (without poloxamer) prime followed by Ad5-gag boost or for Ad5-gag only. Shiver, J.W. *et al. Nature* 415:331-5 (2002). U.S. Patent Appl. Publication No. US 2002/0165172 A1 describes simultaneous administration of a vector construct encoding an immunogenic portion of an antigen and a protein comprising the said immunogenic portion of an antigen such that an immune response is generated. The document is limited to hepatitis B antigens and HIV antigens. Moreover, U.S. Patent No. 6,500,432 is directed to methods of enhancing an immune response of nucleic acid vaccination by simultaneous administration of a polynucleotide and polypeptide of interest.

According to the patent, simultaneous administration means administration of the polynucleotide and the polypeptide during the same immune response, preferably within 0-10 or 3-7 days of each other. The antigens contemplated by the patent include, among others, those of Hepatitis (all forms), HSV, HIV, CMV, EBV, RSV, VZV, HPV, polio, influenza, parasites (*e.g.*, from the genus *Plasmodium*), pathogenic bacteria (including but not limited to *M. tuberculosis*, *M. leprae*, *Chlamydia*, *Shigella*, *B. burgdorferi*, enterotoxigenic *E. coli*, *S. typhosa*, *H. pylori*, *V. cholerae*, *B. pertussis*, etc.). All of the above references are herein incorporated by reference in their entireties.

SUMMARY OF THE INVENTION

[0014] The present invention is directed to compositions and methods for raising a detectable immune response in a vertebrate against the infectious agent transmitting Severe Acute Respiratory Syndrome (SARS), by administering *in vivo*, into a tissue of a vertebrate, at least one polynucleotide comprising one or more nucleic acid fragments, wherein each nucleic acid fragment is a fragment of a coding region operably encoding a polypeptide, or a fragment, variant, or derivative thereof, or a fragment of a codon-optimized coding region operably encoding a polypeptide, or a fragment, variant, or derivative thereof, from a coronavirus which causes SARS (SARS-CoV). The present invention is also directed to administering *in vivo*, into a tissue of the vertebrate the above-described polynucleotide and at least one isolated SARS-CoV polypeptide, or a fragment, variant, or derivative thereof. The isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof can be, for example, a recombinant protein, a purified subunit protein, a protein expressed and carried by a heterologous live or inactivated or attenuated viral vector expressing the protein. According to either method, the polynucleotide is incorporated into the cells of the vertebrate *in vivo*, and an amount of the SARS-CoV protein, or fragment or variant encoded by the polynucleotide sufficient to raise a detectable immune response is produced *in vivo*. The

isolated protein or fragment, variant, or derivative thereof is also administered in an amount sufficient to raise a detectable immune response. The polynucleotide may be administered to the vertebrate either prior to, at the same time (simultaneously), or subsequent to the administration of the isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof.

[0015] Also within the scope of the present invention are combinations of SARS-CoV polypeptides and polynucleotides that encode SARS-CoV polypeptides that assemble into virus-like particles (VLP). One such combination is, but is not limited to a combination of SARS-CoV S, M, and E polypeptides or fragments, variants, or derivatives thereof, and polynucleotides encoding SARS-CoV S, M, and E polypeptides or fragments, variants, or derivatives thereof.

[0016] In a specific embodiment, the invention provides polynucleotide (*e.g.*, DNA) vaccines in which the single formulation comprises a SARS-CoV polypeptide-encoding polynucleotide vaccine as described herein. An alternative embodiment of the invention provides for a multivalent formulation comprising several (*e.g.*, two, three, four, or more) SARS-CoV polypeptide-encoding polynucleotides, as described herein, within a single vaccine composition. The SARS-CoV polypeptide-encoding polynucleotides, fragments or variants thereof may be contained within a single expression vector (*e.g.*, plasmid or viral vector) or may be contained within multiple expression vectors.

[0017] In a specific embodiment, the invention provides combinatorial polynucleotide (*e.g.*, DNA) vaccines which combine both a polynucleotide vaccine and polypeptide (*e.g.*, either a recombinant protein, a purified subunit protein, a viral vector expressing an isolated SARS-CoV polypeptide) vaccine in a single formulation. The single formulation comprises a SARS-CoV polypeptide-encoding polynucleotide vaccine as described herein, and optionally, an effective amount of a desired isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof. The polypeptide may exist in any form, for example, a recombinant protein, a purified subunit protein, or a viral

vector expressing an isolated SARS-CoV polypeptide. The SARS-CoV polypeptide or fragment, variant, or derivative thereof encoded by the polynucleotide vaccine may be identical to the isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof. Alternatively, the SARS-CoV polypeptide or fragment, variant, or derivative thereof encoded by the polynucleotide may be different from the isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof.

[0018] The present invention further provides a method for generating, enhancing, or modulating a protective and/or therapeutic immune response to SARS-CoV in a vertebrate, comprising administering to a vertebrate in need of therapeutic and/or preventative immunity one or more of the compositions described herein.

[0019] The invention also provides for antibodies specifically reactive with SARS Co-V polypeptides which have been produced from an immune response elicited by the administration, to a vertebrate, of polynucleotide and polypeptides of the present invention.

[0020] In one embodiment, purified monoclonal antibodies or polyclonal antibodies containing the variable heavy and light sequences are used as therapeutic and prophylactic agents to treat or prevent SARS-CoV infection by passive antibody therapy. In general, this will comprise administering a therapeutically or prophylactically effective amount of the monoclonal antibodies to a susceptible vertebrate or one exhibiting SARS Co-V infection.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0021] Figure 1 shows the protocol for the preparation of a formulation comprising 0.3 mM BAK, 7.5 mg/ml CRL 1005, and 5 mg/ml of DNA in a final volume of 3.6 ml, through the use of thermal cycling.

[0022] Figure 2 shows the protocol for the preparation of a formulation comprising 0.3 mM BAK, 34 mg/ml or 50 mg/ml CRL 1005, and 2.5 mg/ml DNA in a final volume of 4.0 ml, through the use of thermal cycling.

[0023] Figure 3 shows the protocol for the simplified preparation (without thermal cycling) of a formulation comprising 0.3 mM BAK, 7.5 mg/ml CRL 1005, and 5 mg/ml DNA.

DETAILED DESCRIPTION OF THE INVENTION

[0024] The present invention is directed to compositions and methods for raising a detectable immune response in a vertebrate against the infectious agent transmitting Severe Acute Respiratory Syndrome (SARS), by administering *in vivo*, into a tissue of a vertebrate, at least one polynucleotide comprising one or more nucleic acid fragments, wherein each nucleic acid fragment is a fragment of a coding region operably encoding a polypeptide, or a fragment, variant, or derivative thereof, or a fragment of a codon-optimized coding region operably encoding a polypeptide, or a fragment, variant, or derivative thereof, from a coronavirus which causes SARS (SARS-CoV). The present invention is also directed to administering *in vivo*, into a tissue of the vertebrate the above-described polynucleotide and at least one isolated SARS-CoV polypeptide, or a fragment, variant, or derivative thereof. The isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof can be, for example, a recombinant protein, a purified subunit protein, a protein expressed and carried by a heterologous live or inactivated or attenuated viral vector expressing the protein. According to either method, the polynucleotide is incorporated into the cells of the vertebrate *in vivo*, and an amount of the SARS-CoV protein, or fragment or variant encoded by the polynucleotide sufficient to raise a detectable immune response is produced *in vivo*. The isolated protein or fragment, variant, or derivative thereof is also administered

in an amount sufficient to raise a detectable immune response. The polynucleotide may be administered to the vertebrate either prior to, at the same time (simultaneously), or subsequent to the administration of the isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof.

[0025] In certain embodiments, the present invention provides for methods for raising a detectable immune response to polypeptides from a SARS-CoV virus, comprising administering to a vertebrate a polynucleotide which operably encodes a SARS-CoV polypeptide, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

[0026] The nucleotide and amino acid sequences of several SARS-CoV polypeptides have recently been determined. Several strains of human SARS-CoV (hSARS-CoV) have been sequenced. Sequences available on GenBank include the complete genomic sequences for SARS coronavirus strains CUKH-Su10, TOR2, BJ01, CUHK-W1, Urbani, and HKU-39849. SARS-CoV polypeptides from any of these strains are within the scope of the invention. Non-limiting examples of SARS-CoV polypeptides within the scope of the invention include the Spike (S), Nucleocapsid (N), Envelope (E), and Membrane glycoprotein (M) polypeptides, fragments, derivatives, (*e.g.*, a TPA-S fusion), and variants thereof. As shown in Table 1 below, adapted from Rota *et al.*, the various SARS-CoV strains that have been sequenced differ in various nucleotide base positions, some of which, as shown in Table 2 below, adapted from Marra *et al.*, may result in a different amino acid residue. Thus, also within the scope of the invention are polypeptides that have different amino acids at those positions. The SARS-CoV polypeptide examples described below are from the Urbani strain of SARS-CoV, and are not meant to be limiting in terms of the scope of the invention.

TABLE 1: Comparison of Genomic Sequences of SARS-CoV Strains

Nucleotide Position[d]	Consensus	HKU-39849	CUHK-W1	**Urbani**	TOR2
2,601	T	C	*	*	*
7,746	G	*	T	*	*
7,919	C	*	*	**T**	*
7,930	G	A	*	*	*
8,387	G	C	*	*	*
8,417	G	C	*	*	*
9,404	T	*	C	*	*
9,479	T	*	C	*	*
13,494	G	A	*	*	*
13,495	T	G	*	*	*
16,622	C	*	*	**T**	*
17,564	T	*	G	*	*
17,846	C	*	T	*	*
18,065	G	A	*	*	*
19,064	R	A	G	**G**	A
21,721	G	*	A	*	*
22,222	T	*	C	*	*
23,220	T	*	*	*	G
24,872	T	*	*	**C**	*
25,298	G	*	*	*	A
25,569	T	A	*	*	*
26,600	C	T	*	*	*
26,857	T	*	*	**C**	*
27,827	T	*	C	*	*

TABLE 2: Comparison of Tor2 and Urbani Strains of SARS-CoV and Corresponding Amino Acid Substitutions

Nucleotide Position	**Tor2 Base**	**Corresponding Amino Acid**	**Urbani Base**	**Corresponding Amino Acid**	**Protein**
7,919	C	A	T	V	Rep1A
16,622	C	A	T	A	Rep1B
19,064	A	E	G	E	Rep1B
19,183	T	V	C	A	Rep1B
23,220	G	A	T	S*	Spike (S)
24,872	T	L	C	L	Spike (S)
25,298	A	R	G	G*	ORF 3
26,857	T	S	C	P*	M

*Non-conservative Amino Acid Substitution

[0027] From about nucleotide 21492 to about 25259 of the Urbani strain of the SARS-CoV genome encode the Spike (S) protein. (Bellini *et al.* SARS Coronavirus Urbani, complete genome. GenBank Accession No. AY278741.)

The complete S protein is about 1255 amino acids in length (139.12 kDa) and is predicted, by analogy to other coronaviruses, to be a surface projection glycoprotein precursor. The S protein has several important biologic functions. Monoclonal antibodies against S can neutralize virus infectivity, consistent with the observation that S protein binds to cellular receptors. The S glycoprotein has several important biologic functions. Monoclonal antibodies against S can neutralize virus infectivity, consistent with the observation that S protein binds to cellular receptors. The S protein is encoded by the following polynucleotide sequence in the Urbani strain and is referred to herein as SEQ ID NO:22.

ATGTTTATTTTCTTATTATTTCTTACTCTCACTAGTGGTAGTGACCTTGACCGGTGCACCACTTTTGATG
ATGTTCAAGCTCCTAATTACACTCAACATACTTCATCTATGAGGGGGGTTTACTATCCTGATGAAATTTT
TAGATCAGACACTCTTTATTTAACTCAGGATTTATTTCTTCCATTTTATTCTAATGTTACAGGGTTTCAT
ACTATTAATCATACGTTTGGCAACCCTGTCATACCTTTTAAGGATGGTATTTATTTTGCTGCCACAGAGA
AATCAAATGTTGTCCGTGGTTGGGTTTTTGGTTCTACCATGAACAACAAGTCACAGTCGGTGATTATTAT
TAACAATTCTACTAATGTTGTTATACGAGCATGTAACTTTGAATTGTGTGACAACCCTTTCTTTGCTGTT
TCTAAACCCATGGGTACACAGACACATACTATGATATTCGATAATGCATTTAATTGCACTTTCGAGTACA
TATCTGATGCCTTTTCGCTTGATGTTTCAGAAAAGTCAGGTAATTTTAAACACTTACGAGAGTTTGTGTT
TAAAAATAAAGATGGGTTTCTCTATGTTTATAAGGGCTATCAACCTATAGATGTAGTTCGTGATCTACCT
TCTGGTTTTAACACTTTGAAACCTATTTTTAAGTTGCCTCTTGGTATTAACATTACAAATTTTAGAGCCA
TTCTTACAGCCTTTTCACCTGCTCAAGACATTTGGGGCACGTCAGCTGCAGCCTATTTTGTTGGCTATTT
AAAGCCAACTACATTTATGCTCAAGTATGATGAAAATGGTACAATCACAGATGCTGTTGATTGTTCTCAA
AATCCACTTGCTGAACTCAAATGCTCTGTTAAGAGCTTTGAGATTGACAAAGGAATTTACCAGACCTCTA
ATTTCAGGGTTGTTCCCTCAGGAGATGTTGTGAGATTCCCTAATATTACAAACTTGTGTCCTTTTGGAGA
GGTTTTTAATGCTACTAAATTCCCTTCTGTCTATGCATGGGAGAGAAAAAAAATTTCTAATTGTGTTGCT
GATTACTCTGTGCTCTACAACTCAACATTTTTTTCAACCTTTAAGTGCTATGGCGTTTCTGCCACTAAGT
TGAATGATCTTTGCTTCTCCAATGTCTATGCAGATTCTTTTGTAGTCAAGGGAGATGATGTAAGACAAAT
AGCGCCAGGACAAACTGGTGTTATTGCTGATTATAATTATAAATTGCCAGATGATTTCATGGGTTGTGTC
CTTGCTTGGAATACTAGGAACATTGATGCTACTTCAACTGGTAATTATAATTATAAATATAGGTATCTTA
GACATGGCAAGCTTAGGCCCTTTGAGAGAGACATATCTAATGTGCCTTTCTCCCCTGATGGCAAACCTTG
CACCCCACCTGCTCTTAATTGTTATTGGCCATTAAATGATTATGGTTTTTACACCACTACTGGCATTGGC
TACCAACCTTACAGAGTTGTAGTACTTTCTTTTGAACTTTTAAATGCACCGGCCACGGTTTGTGGACCAA
AATTATCCACTGACCTTATTAAGAACCAGTGTGTCAATTTTAATTTTAATGGACTCACTGGTACTGGTGT
GTTAACTCCTTCTTCAAAGAGATTTCAACCATTTCAACAATTTGGCCGTGATGTTTCTGATTTCACTGAT
TCCGTTCGAGATCCTAAAACATCTGAAATATTAGACATTTCACCTTGCTCTTTTGGGGGTGTAAGTGTAA
TTACACCTGGAACAAATGCTTCATCTGAAGTTGCTGTTCTATATCAAGATGTTAACTGCACTGATGTTTC
TACAGCAATTCATGCAGATCAACTCACACCAGCTTGGCGCATATATTCTACTGGAAACAATGTATTCCAG
ACTCAAGCAGGCTGTCTTATAGGAGCTGAGCATGTCGACACTTCTTATGAGTGCGACATTCCTATTGGAG
CTGGCATTTGTGCTAGTTACCATACAGTTTCTTTATTACGTAGTACTAGCCAAAAATCTATTGTGGCTTA
TACTATGTCTTTAGGTGCTGATAGTTCAATTGCTTACTCTAATAACACCATTGCTATACCTACTAACTTT
TCAATTAGCATTACTACAGAAGTAATGCCTGTTTCTATGGCTAAAACCTCCGTAGATTGTAATATGTACA
TCTGCGGAGATTCTACTGAATGTGCTAATTTGCTTCTCCAATATGGTAGCTTTTGCACACAACTAAATCG
TGCACTCTCAGGTATTGCTGCTGAACAGGATCGCAACACACGTGAAGTGTTCGCTCAAGTCAAACAAATG
TACAAAACCCCAACTTTGAAATATTTTGGTGGTTTTAATTTTTCACAAATATTACCTGACCCTCTAAAGC
CAACTAAGAGGTCTTTTATTGAGGACTTGCTCTTTAATAAGGTGACACTCGCTGATGCTGGCTTCATGAA
GCAATATGGCGAATGCCTAGGTGATATTAATGCTAGAGATCTCATTTGTGCGCAGAAGTTCAATGGACTT
ACAGTGTTGCCACCTCTGCTCACTGATGATATGATTGCTGCCTACACTGCTGCTCTAGTTAGTGGTACTG
CCACTGCTGGATGGACATTTGGTGCTGGCGCTGCTCTTCAAATACCTTTTGCTATGCAAATGGCATATAG
GTTCAATGGCATTGGAGTTACCCAAAATGTTCTCTATGAGAACCAAAAACAAATCGCCAACCAATTTAAC
AAGGCGATTAGTCAAATTCAAGAATCACTTACAACAACATCAACTGCATTGGGCAAGCTGCAAGACGTTG

TTAACCAGAATGCTCAAGCATTAAACACACTTGTTAAACAACTTAGCTCTAATTTTGGTGCAATTTCAAG
TGTGCTAAATGATATCCTTTCGCGACTTGATAAAGTCGAGGCGGAGGTACAAATTGACAGGTTAATTACA
GGCAGACTTCAAAGCCTTCAAACCTATGTAACACAACAACTAATCAGGGCTGCTGAAATCAGGGCTTCTG
CTAATCTTGCTGCTACTAAAATGTCTGAGTGTGTTCTTGGACAATCAAAAAGAGTTGACTTTTGTGGAAA
GGGCTACCACCTTATGTCCTTCCCACAAGCAGCCCCGCATGGTGTTGTCTTCCTACATGTCACGTATGTG
CCATCCCAGGAGAGGAACTTCACCACAGCGCCAGCAATTTGTCATGAAGGCAAAGCATACTTCCCTCGTG
AAGGTGTTTTTGTGTTTAATGGCACTTCTTGGTTTATTACACAGAGGAACTTCTTTTCTCCACAAATAAT
TACTACAGACAATACATTTGTCTCAGGAAATTGTGATGTCGTTATTGGCATCATTAACAACACAGTTTAT
GATCCTCTGCAACCTGAGCTCGACTCATTCAAAGAAGAGCTGGACAAGTACTTCAAAAATCATACATCAC
CAGATGTTGATCTTGGCGACATTTCAGGCATTAACGCTTCTGTCGTCAACATTCAAAAAGAAATTGACCG
CCTCAATGAGGTCGCTAAAAATTTAAATGAATCACTCATTGACCTTCAAGAATTGGGAAAATATGAGCAA
TATATTAAATGGCCTTGGTATGTTTGGCTCGGCTTCATTGCTGGACTAATTGCCATCGTCATGGTTACAA
TCTTGCTTTGTTGCATGACTAGTTGTTGCAGTTGCCTCAAGGGTGCATGCTCTTGTGGTTCTTGCTGCAA
GTTTGATGAGGATGACTCTGAGCCAGTTCTCAAGGGTGTCAAATTACATTACACATAA

[0028] The S protein has the following amino acid sequence and is referred to herein as SEQ ID NO:23.

MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLF
LPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIIN
NSTNVVIRACNFELCDNPFFAVSKPMGTQTHTMIFDNAFNCTFEYISDAFSLDVSEKS
GNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAIL
TAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVKS
FEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVAD
YSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADY
NYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGK
PCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQ
CVNFNFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCSFGGVS
VITPGTNASSEVAVLYQDVNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAE
HVDTSYECDIPIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNFS
ISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIAAEQDRN
TREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMK
QYGECLGDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGA
ALQIPFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQD
VVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYV
TQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLH
VTYVPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNTFVSG
NCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEID
RLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWLGFIAGLIAIVMVTILLCCMTSCCS
CLKGACSCGSCCKFDEDDSEPVLKGVKLHYT

[0029] The S protein can be divided into three structural domains: a large external domain at the N-terminus, a transmembrane domain and a short carboxyterminal cytoplasmic domain. These domains within the S protein of SARS-CoV Urbani strain have been identified using the program TMHMM2.0. (Sonnhammer *et al. Proc. Of 6th Int. Conf. On Intelligent Systems for Molecular Biology.* AAAI Press:175-182 (1998). Based on this algorithm, amino acids about 1 to about 1195 comprise an extracellular

domain; amino acids about 1196 to about 1218 are part of a transmembrane domain; and amino acids about 1219 to about 1240 comprise the cytoplasmic domain. Removal of residues comprising the transmembrane domain and optionally, the cytoplasmic domain, results in a soluble protein that can be used in the compositions of the invention.

[0030] The large external domain of the S protein is further divided into two sub-domains, S1 and S2. The S1 sub-domain (amino acids about 1 to about 683) includes the N-terminal half of the molecule and forms the globular portion of the spikes. This region contains sequences that are responsible for binding to specific receptors on the membranes of susceptible cells. S1 sequences are variable, containing various degrees of deletion and substitutions in different coronavirus strains or isolates. Mutations in S1 sequences have been associated with altered antigenicity and pathogenicity of the virus. The receptor-binding domain of the S protein of murine hepatitis virus (MHV) is localized within the N-terminal 330 amino acids of the S1 domain. Consequently, the amino acid sequences of the S1 domain may determine the target cell specificity of coronaviruses in animals.

[0031] The S2 sub-domain comprises amino acids about 684 to about 1210 of the S protein. In coronaviruses, the S2 sub-domain of the S protein is usually acylated and contains two heptad repeat motifs. The motifs suggest that this portion of the S protein may assume a coiled-coil structure. The mature S protein forms an oligomer, which is most likely a trimer based on the spike proteins of other coronaviruses. Thus, the S2 subdomain probably constitutes the stalk of the viral spike.

[0032] Non limiting examples of nucleotide sequences encoding the S protein are as follows. It should be noted that S sequences vary between SARS-CoV strains. Virtually any nucleotide sequence encoding a SARS-CoV S protein is suitable for the present invention. In fact, S polynucleotide sequences included in vaccines and therapeutic formulations of the current invention may change from year to year, depending on the prevalent strain or strains of SARS-CoV.

[0033] From about nucleotide 21492 to about 25080 of the Urbani strain of the SARS-CoV genome encode a soluble extracellular portion of the S protein (Bellini *et al.* SARS Coronavirus Urbani, compete genome, Genbank accession number AY278741) and has the following sequence, referred to herein as SEQ ID NO: 1:

ATGTTTATTTTCTTATTATTTCTTACTCTCACTAGTGGTAGTGACCTTGACCGGTGCACC
ACTTTTGATGATGTTCAAGCTCCTAATTACACTCAACATACTTCATCTATGAGGGGGGTT
TACTATCCTGATGAAATTTTAGATCAGACACTCTTTATTTAACTCAGGATTTATTTCTT
CCATTTTATTCTAATGTTACAGGGTTTCATACTATTAATCATACGTTTGGCAACCCTGTC
ATACCTTTTAAGGATGGTATTTATTTTGCTGCCACAGAGAAATCAAATGTTGTCCGTGGT
TGGGTTTTTGGTTCTACCATGAACAACAAGTCACAGTCGGTGATTATTATTAACAATTCT
ACTAATGTTGTTATACGAGCATGTAACTTTGAATTGTGTGACAACCCTTTCTTTGCTGTT
TCTAAACCCATGGGTACACAGACACATACTATGATATTCGATAATGCATTTAATTGCACT
TTCGAGTACATATCTGATGCCTTTTCGCTTGATGTTTCAGAAAAGTCAGGTAATTTTAAA
CACTTACGAGAGTTTGTGTTTAAAAATAAAGATGGGTTTCTCTATGTTTATAAGGGCTAT
CAACCTATAGATGTAGTTCGTGATCTACCTTCTGGTTTTAACACTTTGAAACCTATTTTT
AAGTTGCCTCTTGGTATTAACATTACAAATTTTAGAGCCATTCTTACAGCCTTTTCACCT
GCTCAAGACATTTGGGGCACGTCAGCTGCAGCCTATTTTGTTGGCTATTTAAAGCCAACT
ACATTTATGCTCAAGTATGATGAAAATGGTACAATCACAGATGCTGTTGATTGTTCTCAA
AATCCACTTGCTGAACTCAAATGCTCTGTTAAGAGCTTTGAGATTGACAAAGGAATTTAC
CAGACCTCTAATTTCAGGGTTGTTCCCTCAGGAGATGTTGTGAGATTCCCTAATATTACA
AACTTGTGTCCTTTTGGAGAGGTTTTTAATGCTACTAAATTCCCTTCTGTCTATGCATGG
GAGAGAAAAAAAATTTCTAATTGTGTTGCTGATTACTCTGTGCTCTACAACTCAACATTT
TTTTCAACCTTTAAGTGCTATGGCGTTTCTGCCACTAAGTTGAATGATCTTTGCTTCTCC
AATGTCTATGCAGATTCTTTTGTAGTCAAGGGAGATGATGTAAGACAAATAGCGCCAGGA
CAAACTGGTGTTATTGCTGATTATAATTATAAATTGCCAGATGATTTCATGGGTTGTGTC
CTTGCTTGGAATACTAGGAACATTGATGCTACTTCAACTGGTAATTATAATTATAAATAT
AGGTATCTTAGACATGGCAAGCTTAGGCCCTTTGAGAGAGACATATCTAATGTGCCTTTC
TCCCCTGATGGCAAACCTTGCACCCCACCTGCTCTTAATTGTTATTGGCCATTAAATGAT
TATGGTTTTTACACCACTACTGGCATTGGCTACCAACCTTACAGAGTTGTAGTACTTTCT
TTTGAACTTTTAAATGCACCGGCCACGGTTTGTGGACCAAAATTATCCACTGACCTTATT
AAGAACCAGTGTGTCAATTTTAATTTTAATGGACTCACTGGTACTGGTGTGTTAACTCCT
TCTTCAAAGAGATTTCAACCATTTCAACAATTTGGCCGTGATGTTTCTGATTTCACTGAT
TCCGTTCGAGATCCTAAAACATCTGAAATATTAGACATTTCACCTTGCTCTTTTGGGGGT
GTAAGTGTAATTACACCTGGAACAAATGCTTCATCTGAAGTTGCTGTTCTATATCAAGAT
GTTAACTGCACTGATGTTTCTACAGCAATTCATGCAGATCAACTCACACCAGCTTGGCGC
ATATATTCTACTGGAAACAATGTATTCCAGACTCAAGCAGGCTGTCTTATAGGAGCTGAG
CATGTCGACACTTCTTATGAGTGCGACATTCCTATTGGAGCTGGCATTTGTGCTAGTTAC
CATACAGTTTCTTTATTACGTAGTACTAGCCAAAAATCTATTGTGGCTTATACTATGTCT
TTAGGTGCTGATAGTTCAATTGCTTACTCTAATAACACCATTGCTATACCTACTAACTTT
TCAATTAGCATTACTACAGAAGTAATGCCTGTTTCTATGGCTAAAACCTCCGTAGATTGT
AATATGTACATCTGCGGAGATTCTACTGAATGTGCTAATTTGCTTCTCCAATATGGTAGC
TTTTGCACACAACTAAATCGTGCACTCTCAGGTATTGCTGCTGAACAGGATCGCAACACA
CGTGAAGTGTTCGCTCAAGTCAAACAAATGTACAAAACCCCAACTTTGAAATATTTTGGT
GGTTTTAATTTTTCACAAATATTACCTGACCCTCTAAAGCCAACTAAGAGGTCTTTTATT
GAGGACTTGCTCTTTAATAAGGTGACACTCGCTGATGCTGGCTTCATGAAGCAATATGGC
GAATGCCTAGGTGATATTAATGCTAGAGATCTCATTTGTGCGCAGAAGTTCAATGGACTT
ACAGTGTTGCCACCTCTGCTCACTGATGATATGATTGCTGCCTACACTGCTGCTCTAGTT
AGTGGTACTGCCACTGCTGGATGGACATTTGGTGCTGGCGCTGCTCTTCAAATACCTTTT
GCTATGCAAATGGCATATAGGTTCAATGGCATTGGAGTTACCCAAAATGTTCTCTATGAG
AACCAAAAACAAATCGCCAACCAATTTAACAAGGCGATTAGTCAAATTCAAGAATCACTT
ACAACAACATCAACTGCATTGGGCAAGCTGCAAGACGTTGTTAACCAGAATGCTCAAGCA
TTAAACACACTTGTTAAACAACTTAGCTCTAATTTTGGTGCAATTTCAAGTGTGCTAAAT
GATATCCTTTCGCGACTTGATAAAGTCGAGGCGGAGGTACAAATTGACAGGTTAATTACA
GGCAGACTTCAAAGCCTTCAAACCTATGTAACACAACAACTAATCAGGGCTGCTGAAATC
AGGGCTTCTGCTAATCTTGCTGCTACTAAAATGTCTGAGTGTGTTCTTGGACAATCAAAA
AGAGTTGACTTTTGTGGAAAGGGCTACCACCTTATGTCCTTCCCACAAGCAGCCCCGCAT

GGTGTTGTCTTCCTACATGTCACGTATGTGCCATCCCAGGAGAGGAACTTCACCACAGCG
CCAGCAATTTGTCATGAAGGCAAAGCATACTTCCCTCGTGAAGGTGTTTTTGTGTTTAAT
GGCACTTCTTGGTTTATTACACAGAGGAACTTCTTTTCTCCACAAATAATTACTACAGAC
AATACATTTGTCTCAGGAAATTGTGATGTCGTTATTGGCATCATTAACAACACAGTTTAT
GATCCTCTGCAACCTGAGCTCGACTCATTCAAAGAAGAGCTGGACAAGTACTTCAAAAAT
CATACATCACCAGATGTTGATCTTGGCGACATTTCAGGCATTAACGCTTCTGTCGTCAAC
ATTCAAAAAGAAATTGACCGCCTCAATGAGGTCGCTAAAAATTTAAATGAATCACTCATT
GACCTTCAAGAATTGGGAAAATATGAGCAATATATTAAATGGCCTTGG

[0034] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV S polypeptide, wherein said polynucleotide is 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:1, or a codon-optimized version as described below, and wherein said polynucleotide encodes a polypeptide that elicits a detectable immune response. The present invention is also directed to raising a detectable immune response with or without a wildtype or other secretory leader sequence as described below.

[0035] The amino acid sequence of the soluble S protein encoded by SEQ ID NO:1 has the following sequence shown below and is referred to herein as SEQ ID NO:2:

MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFL
PFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNS
TNVVIRACNFELCDNPFFAVSKPMGTQTHTMIFDNAFNCTFEYISDAFSLDVSEKSGNFK
HLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAILTAFSP
AQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVKSFEIDKGIY
QTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTF
FSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCV
LAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGKPCTPPALNCYWPLND
YGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLTP
SSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCSFGGVSVITPGTNASSEVAVLYQD
VNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDIPIGAGICASY
HTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDC
NMYICGDSTECANLLLQYGSFCTQLNRALSGIAAEQDRNTREVFAQVKQMYKTPTLKYFG
GFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGECLGDINARDLICAQKFNGL
TVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYE
NQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLN
DILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSK
RVDFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQERNFTTAPAICHEGKAYFPREGVFVFN
GTSWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKN
HTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPW

[0036] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV S polypeptide comprising an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:2, wherein

said polypeptide raises a detectable immune response. The present invention is also directed to raising a detectable immune response with or without a wildtype or other secretory leader sequence as described below.

[0037] A conserved protein domain program on the National Center for Biotechnology Information's web site (www.ncbi.nlm.nih.gov) was used to predict domains within the SARS-CoV S protein. Two domains, S1 and S2, were predicted within the soluble portion of the S protein. The S1 domain spans from amino acids about 1 to about 683 of the S protein. The nucleotide sequence encoding the soluble S1 domain from SARS-CoV Urbani strain has the following sequence and is referred to herein as SEQ ID NO:3:

ATGTTTATTTTCTTATTATTTCTTACTCTCACTAGTGGTAGTGACCTTGACCGGTGCACC
ACTTTTGATGATGTTCAAGCTCCTAATTACACTCAACATACTTCATCTATGAGGGGGGTT
TACTATCCTGATGAAATTTTTAGATCAGACACTCTTTATTTAACTCAGGATTTATTTCTT
CCATTTTATTCTAATGTTACAGGGTTTCATACTATTAATCATACGTTTGGCAACCCTGTC
ATACCTTTTAAGGATGGTATTTATTTTGCTGCCACAGAGAAATCAAATGTTGTCCGTGGT
TGGGTTTTTGGTTCTACCATGAACAACAAGTCACAGTCGGTGATTATTATTAACAATTCT
ACTAATGTTGTTATACGAGCATGTAACTTTGAATTGTGTGACAACCCTTTCTTTGCTGTT
TCTAAACCCATGGGTACACAGACACATACTATGATATTCGATAATGCATTTAATTGCACT
TTCGAGTACATATCTGATGCCTTTTCGCTTGATGTTTCAGAAAAGTCAGGTAATTTTAAA
CACTTACGAGAGTTTGTGTTTAAAAATAAAGATGGGTTTCTCTATGTTTATAAGGGCTAT
CAACCTATAGATGTAGTTCGTGATCTACCTTCTGGTTTTAACACTTTGAAACCTATTTTT
AAGTTGCCTCTTGGTATTAACATTACAAATTTTAGAGCCATTCTTACAGCCTTTTCACCT
GCTCAAGACATTTGGGGCACGTCAGCTGCAGCCTATTTTGTTGGCTATTTAAAGCCAACT
ACATTTATGCTCAAGTATGATGAAAATGGTACAATCACAGATGCTGTTGATTGTTCTCAA
AATCCACTTGCTGAACTCAAATGCTCTGTTAAGAGCTTTGAGATTGACAAAGGAATTTAC
CAGACCTCTAATTTCAGGGTTGTTCCCTCAGGAGATGTTGTGAGATTCCCTAATATTACA
AACTTGTGTCCTTTTGGAGAGGTTTTTAATGCTACTAAATTCCCTTCTGTCTATGCATGG
GAGAGAAAAAAAATTTCTAATTGTGTTGCTGATTACTCTGTGCTCTACAACTCAACATTT
TTTTCAACCTTTAAGTGCTATGGCGTTTCTGCCACTAAGTTGAATGATCTTTGCTTCTCC
AATGTCTATGCAGATTCTTTTGTAGTCAAGGGAGATGATGTAAGACAAATAGCGCCAGGA
CAAACTGGTGTTATTGCTGATTATAATTATAAATTGCCAGATGATTTCATGGGTTGTGTC
CTTGCTTGGAATACTAGGAACATTGATGCTACTTCAACTGGTAATTATAATTATAAATAT
AGGTATCTTAGACATGGCAAGCTTAGGCCCTTTGAGAGAGACATATCTAATGTGCCTTTC
TCCCCTGATGGCAAACCTTGCACCCCACCTGCTCTTAATTGTTATTGGCCATTAAATGAT
TATGGTTTTTACACCACTACTGGCATTGGCTACCAACCTTACAGAGTTGTAGTACTTTCT
TTTGAACTTTTAAATGCACCGGCCACGGTTTGTGGACCAAAATTATCCACTGACCTTATT
AAGAACCAGTGTGTCAATTTTAATTTTAATGGACTCACTGGTACTGGTGTGTTAACTCCT
TCTTCAAAGAGATTTCAACCATTTCAACAATTTGGCCGTGATGTTTCTGATTTCACTGAT
TCCGTTCGAGATCCTAAAACATCTGAAATATTAGACATTTCACCTTGCTCTTTTGGGGGT
GTAAGTGTAATTACACCTGGAACAAATGCTTCATCTGAAGTTGCTGTTCTATATCAAGAT
GTTAACTGCACTGATGTTTCTACAGCAATTCATGCAGATCAACTCACACCAGCTTGGCGC
ATATATTCTACTGGAAACAATGTATTCCAGACTCAAGCAGGCTGTCTTATAGGAGCTGAG
CATGTCGACACTTCTTATGAGTGCGACATTCCTATTGGAGCTGGCATTTGTGCTAGTTAC
CATACAGTTTCTTTATTACGTAGTACTAGCCAAAAATCTATTGTGGCTTATACTATGTCT
TTAGGTGCT

[0038] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV S1

polypeptide, wherein said polynucleotide is 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:3, or a codon-optimized version as described below, and wherein said polynucleotide encodes a polypeptide that elicits a detectable immune response. The present invention is also directed to raising a detectable immune response with or without a wildtype or other secretory leader sequence as described below.

[0039] The amino acid sequence of the soluble S1 protein encoded by SEQ ID NO:3 has the following sequence shown below and is referred to herein as SEQ ID NO:4:

MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFL
PFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNS
TNVVIRACNFELCDNPFFAVSKPMGTQTHTMIFDNAFNCTFEYISDAFSLDVSEKSGNFK
HLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAILTAFSP
AQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVKSFEIDKGIY
QTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTF
FSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCV
LAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGKPCTPPALNCYWPLND
YGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLTP
SSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCSFGGVSVITPGTNASSEVAVLYQD
VNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDIPIGAGICASY
HTVSLLRSTSQKSIVAYTMSLGA

[0040] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV S1 polypeptide comprising an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:4, wherein said polypeptide raises a detectable immune response. The present invention is also directed to raising a detectable immune response with or without a wildtype or other secretory leader sequence as described below.

[0041] The S2 domain spans from amino acids about 684 to about 1210 of the S protein. The nucleotide sequence encoding the soluble S2 domain from SARS-CoV Urbani strain has the following sequence and is referred to herein as SEQ ID NO:5:

GATAGTTCAATTGCTTACTCTAATAACACCATTGCTATACCTACTAACTTTTCAATTAGCATTACTACAG
AAGTAATGCCTGTTTCTATGGCTAAAACCTCCGTAGATTGTAATATGTACATCTGCGGAGATTCTACTGA
ATGTGCTAATTTGCTTCTCCAATATGGTAGCTTTTGCACACAACTAAATCGTGCACTCTCAGGTATTGCT
GCTGAACAGGATCGCAACACACGTGAAGTGTTCGCTCAAGTCAAACAAATGTACAAAACCCCAACTTTGA

AATATTTTGGTGGTTTTAATTTTTCACAAATATTACCTGACCCTCTAAAGCCAACTAAGAGGTCTTTTAT
TGAGGACTTGCTCTTTAATAAGGTGACACTCGCTGATGCTGGCTTCATGAAGCAATATGGCGAATGCCTA
GGTGATATTAATGCTAGAGATCTCATTTGTGCGCAGAAGTTCAATGGACTTACAGTGTTGCCACCTCTGC
TCACTGATGATATGATTGCTGCCTACACTGCTGCTCTAGTTAGTGGTACTGCCACTGCTGGATGGACATT
TGGTGCTGGCGCTGCTCTTCAAATACCTTTTGCTATGCAAATGGCATATAGGTTCAATGGCATTGGAGTT
ACCCAAAATGTTCTCTATGAGAACCAAAAACAAATCGCCAACCAATTTAACAAGGCGATTAGTCAAATTC
AAGAATCACTTACAACAACATCAACTGCATTGGGCAAGCTGCAAGACGTTGTTAACCAGAATGCTCAAGC
ATTAAACACACTTGTTAAACAACTTAGCTCTAATTTTGGTGCAATTTCAAGTGTGCTAAATGATATCCTT
TCGCGACTTGATAAAGTCGAGGCGGAGGTACAAATTGACAGGTTAATTACAGGCAGACTTCAAAGCCTTC
AAACCTATGTAACACAACAACTAATCAGGGCTGCTGAAATCAGGGCTTCTGCTAATCTTGCTGCTACTAA
AATGTCTGAGTGTGTTCTTGGACAATCAAAAAGAGTTGACTTTTGTGGAAAGGGCTACCACCTTATGTCC
TTCCCACAAGCAGCCCCGCATGGTGTTGTCTTCCTACATGTCACGTATGTGCCATCCCAGGAGAGGAACT
TCACCACAGCGCCAGCAATTTGTCATGAAGGCAAAGCATACTTCCCTCGTGAAGGTGTTTTTGTGTTTAA
TGGCACTTCTTGGTTTATTACACAGAGGAACTTCTTTTCTCCACAAATAATTACTACAGACAATACATTT
GTCTCAGGAAATTGTGATGTCGTTATTGGCATCATTAACAACACAGTTTATGATCCTCTGCAACCTGAGC
TCGACTCATTCAAAGAAGAGCTGGACAAGTACTTCAAAAATCATACATCACCAGATGTTGATCTTGGCGA
CATTTCAGGCATTAACGCTTCTGTCGTCAACATTCAAAAAGAAATTGACCGCCTCAATGAGGTCGCTAAA
AATTTAAATGAATCACTCATTGACCTTCAAGAATTGGGAAAATATGAGCAATATATTAAATGGCCTTGG

[0042] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV S2 polypeptide, wherein said polynucleotide is 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:5, or a codon-optimized version as described below, and wherein said polynucleotide encodes a polypeptide that elicits a detectable immune response. It should be noted that in order to achieve a polynucleotide "operably encoding" a SARS-CoV S2 polypeptide, at least a methionine codon (ATG) would need to be included, in frame, upstream of the polynucleotide presented herein as SEQ ID NO:5. An example of such a polynucleotide includes, but is not limited to the following, presented herein as SEQ ID NO:54.

ATGGATAGTTCAATTGCTTACTCTAATAACACCATTGCTATACCTACTAACTTTTCAATTAGCATTACTA
CAGAAGTAATGCCTGTTTCTATGGCTAAAACCTCCGTAGATTGTAATATGTACATCTGCGGAGATTCTAC
TGAATGTGCTAATTTGCTTCTCCAATATGGTAGCTTTTGCACACAACTAAATCGTGCACTCTCAGGTATT
GCTGCTGAACAGGATCGCAACACACGTGAAGTGTTCGCTCAAGTCAAACAAATGTACAAAACCCCAACTT
TGAAATATTTTGGTGGTTTTAATTTTTCACAAATATTACCTGACCCTCTAAAGCCAACTAAGAGGTCTTT
TATTGAGGACTTGCTCTTTAATAAGGTGACACTCGCTGATGCTGGCTTCATGAAGCAATATGGCGAATGC
CTAGGTGATATTAATGCTAGAGATCTCATTTGTGCGCAGAAGTTCAATGGACTTACAGTGTTGCCACCTC
TGCTCACTGATGATATGATTGCTGCCTACACTGCTGCTCTAGTTAGTGGTACTGCCACTGCTGGATGGAC
ATTTGGTGCTGGCGCTGCTCTTCAAATACCTTTTGCTATGCAAATGGCATATAGGTTCAATGGCATTGGA
GTTACCCAAAATGTTCTCTATGAGAACCAAAAACAAATCGCCAACCAATTTAACAAGGCGATTAGTCAAA
TTCAAGAATCACTTACAACAACATCAACTGCATTGGGCAAGCTGCAAGACGTTGTTAACCAGAATGCTCA
AGCATTAAACACACTTGTTAAACAACTTAGCTCTAATTTTGGTGCAATTTCAAGTGTGCTAAATGATATC
CTTTCGCGACTTGATAAAGTCGAGGCGGAGGTACAAATTGACAGGTTAATTACAGGCAGACTTCAAAGCC
TTCAAACCTATGTAACACAACAACTAATCAGGGCTGCTGAAATCAGGGCTTCTGCTAATCTTGCTGCTAC
TAAAATGTCTGAGTGTGTTCTTGGACAATCAAAAAGAGTTGACTTTTGTGGAAAGGGCTACCACCTTATG
TCCTTCCCACAAGCAGCCCCGCATGGTGTTGTCTTCCTACATGTCACGTATGTGCCATCCCAGGAGAGGA
ACTTCACCACAGCGCCAGCAATTTGTCATGAAGGCAAAGCATACTTCCCTCGTGAAGGTGTTTTTGTGTT
TAATGGCACTTCTTGGTTTATTACACAGAGGAACTTCTTTTCTCCACAAATAATTACTACAGACAATACA

TTTGTCTCAGGAAATTGTGATGTCGTTATTGGCATCATTAACAACACAGTTTATGATCCTCTGCAACCTG
AGCTCGACTCATTCAAAGAAGAGCTGGACAAGTACTTCAAAAATCATACATCACCAGATGTTGATCTTGG
CGACATTTCAGGCATTAACGCTTCTGTCGTCAACATTCAAAAAGAAATTGACCGCCTCAATGAGGTCGCT
AAAAATTTAAATGAATCACTCATTGACCTTCAAGAATTGGGAAAATATGAGCAATATATTAAATGGCCTT
GG

[0043] The present invention is also directed to raising a detectable immune response with or without a wildtype or other secretory leader sequence as described below.

[0044] The amino acid sequence of the soluble S2 protein encoded by SEQ ID NO:5 has the following sequence shown below and is referred to herein as SEQ ID NO:6

DSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIA
AEQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGECL
GDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGV
TQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDIL
SRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMS
FPQAAPHGVVFLHVTYVPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNTF
VSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAK
NLNESLIDLQELGKYEQYIKWPW

[0045] The amino acid sequence of the soluble S2 protein encoded by SEQ ID NO:54 has the following sequence shown below and is referred to herein as SEQ ID NO:56

MDSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGI
AAEQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGEC
LGDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIG
VTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDI
LSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLM
SFPQAAPHGVVFLHVTYVPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNT
FVSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVA
KNLNESLIDLQELGKYEQYIKWPW

[0046] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV S2 polypeptide comprising an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:6, wherein said polypeptide raises a detectable immune response. The present invention is

also directed to raising a detectable immune response with or without a wildtype or other secretory leader sequence as described below.

[0047] In one embodiment, soluble S, soluble S1 and soluble S2, described herein, are encoded by a polynucleotide which contains the wild-type S secretory leader peptide sequence. The secretory leader peptide of the S protein in SARS-CoV Urbani strain comprises about the first 13 residues of the protein. Marra *et al.* The present invention is also directed to raising a detectable immune response with or without amino acids about 1 to about 10, about 1 to about 11, about 1 to about 12, about 1 to about 13, about 1 to about 14, about 1 to about 15, about 1 to about 16, about 1 to about 17, about 1 to about 18, about 1 to about 19, about 1 to about 20, about 1 to about 21, about 1 to about 22, about 1 to about 23, about 1 to about 24, and about 1 to about 25 of the secretory leader peptide sequence.

[0048] In an alternative embodiment, the secretory leader peptide of soluble S, soluble S1 and soluble S2 can be replaced by the secretory leader peptide of human Tissue Plasminogen Activator (TPA). The polynucleotide sequences encoding the various S polypeptides with the TPA secretory leader peptide are shown below. Soluble TPA-S (SEQ ID NO:7)

ATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTT
TCGCCCAGCGCTAGAGGATCGGGAAGTGACCTTGACCGGTGCACCACTTTTGATGATGTT
CAAGCTCCTAATTACACTCAACATACTTCATCTATGAGGGGGGTTTACTATCCTGATGAA
ATTTTTAGATCAGACACTCTTTATTTAACTCAGGATTTATTTCTTCCATTTTATTCTAAT
GTTACAGGGTTTCATACTATTAATCATACGTTTGGCAACCCTGTCATACCTTTTAAGGAT
GGTATTTATTTTGCTGCCACAGAGAAATCAAATGTTGTCCGTGGTTGGGTTTTTGGTTCT
ACCATGAACAACAAGTCACAGTCGGTGATTATTATTAACAATTCTACTAATGTTGTTATA
CGAGCATGTAACTTTGAATTGTGTGACAACCCTTTCTTTGCTGTTTCTAAACCCATGGGT
ACACAGACACATACTATGATATTCGATAATGCATTTAATTGCACTTTCGAGTACATATCT
GATGCCTTTTCGCTTGATGTTTCAGAAAAGTCAGGTAATTTTAAACACTTACGAGAGTTT
GTGTTTAAAAATAAAGATGGGTTTCTCTATGTTTATAAGGGCTATCAACCTATAGATGTA
GTTCGTGATCTACCTTCTGGTTTTAACACTTTGAAACCTATTTTTAAGTTGCCTCTTGGT
ATTAACATTACAAATTTTAGAGCCATTCTTACAGCCTTTTCACCTGCTCAAGACATTTGG
GGCACGTCAGCTGCAGCCTATTTTGTTGGCTATTTAAAGCCAACTACATTTATGCTCAAG
TATGATGAAAATGGTACAATCACAGATGCTGTTGATTGTTCTCAAAATCCACTTGCTGAA
CTCAAATGCTCTGTTAAGAGCTTTGAGATTGACAAAGGAATTTACCAGACCTCTAATTTC
AGGGTTGTTCCCTCAGGAGATGTTGTGAGATTCCCTAATATTACAAACTTGTGTCCTTTT
GGAGAGGTTTTTAATGCTACTAAATTCCCTTCTGTCTATGCATGGGAGAGAAAAAAAATT
TCTAATTGTGTTGCTGATTACTCTGTGCTCTACAACTCAACATTTTTTTCAACCTTTAAG
TGCTATGGCGTTTCTGCCACTAAGTTGAATGATCTTTGCTTCTCCAATGTCTATGCAGAT
TCTTTTGTAGTCAAGGGAGATGATGTAAGACAAATAGCGCCAGGACAAACTGGTGTTATT
GCTGATTATAATTATAAATTGCCAGATGATTTCATGGGTTGTGTCCTTGCTTGGAATACT
AGGAACATTGATGCTACTTCAACTGGTAATTATAATTATAAATATAGGTATCTTAGACAT
GGCAAGCTTAGGCCCTTTGAGAGAGACATATCTAATGTGCCTTTCTCCCCTGATGGCAAA

CCTTGCACCCCACCTGCTCTTAATTGTTATTGGCCATTAAATGATTATGGTTTTTACACC
ACTACTGGCATTGGCTACCAACCTTACAGAGTTGTAGTACTTTCTTTTGAACTTTTAAAT
GCACCGGCCACGGTTTGTGGACCAAAATTATCCACTGACCTTATTAAGAACCAGTGTGTC
AATTTTAATTTTAATGGACTCACTGGTACTGGTGTGTTAACTCCTTCTTCAAAGAGATTT
CAACCATTTCAACAATTTGGCCGTGATGTTTCTGATTTCACTGATTCCGTTCGAGATCCT
AAAACATCTGAAATATTAGACATTTCACCTTGCTCTTTTGGGGGTGTAAGTGTAATTACA
CCTGGAACAAATGCTTCATCTGAAGTTGCTGTTCTATATCAAGATGTTAACTGCACTGAT
GTTTCTACAGCAATTCATGCAGATCAACTCACACCAGCTTGGCGCATATATTCTACTGGA
AACAATGTATTCCAGACTCAAGCAGGCTGTCTTATAGGAGCTGAGCATGTCGACACTTCT
TATGAGTGCGACATTCCTATTGGAGCTGGCATTTGTGCTAGTTACCATACAGTTTCTTTA
TTACGTAGTACTAGCCAAAAATCTATTGTGGCTTATACTATGTCTTTAGGTGCTGATAGT
TCAATTGCTTACTCTAATAACACCATTGCTATACCTACTAACTTTTCAATTAGCATTACT
ACAGAAGTAATGCCTGTTTCTATGGCTAAAACCTCCGTAGATTGTAATATGTACATCTGC
GGAGATTCTACTGAATGTGCTAATTTGCTTCTCCAATATGGTAGCTTTTGCACACAACTA
AATCGTGCACTCTCAGGTATTGCTGCTGAACAGGATCGCAACACACGTGAAGTGTTCGCT
CAAGTCAAACAAATGTACAAAACCCCAACTTTGAAATATTTTGGTGGTTTTAATTTTTCA
CAAATATTACCTGACCCTCTAAAGCCAACTAAGAGGTCTTTTATTGAGGACTTGCTCTTT
AATAAGGTGACACTCGCTGATGCTGGCTTCATGAAGCAATATGGCGAATGCCTAGGTGAT
ATTAATGCTAGAGATCTCATTTGTGCGCAGAAGTTCAATGGACTTACAGTGTTGCCACCT
CTGCTCACTGATGATATGATTGCTGCCTACACTGCTGCTCTAGTTAGTGGTACTGCCACT
GCTGGATGGACATTTGGTGCTGGCGCTGCTCTTCAAATACCTTTTGCTATGCAAATGGCA
TATAGGTTCAATGGCATTGGAGTTACCCAAAATGTTCTCTATGAGAACCAAAAACAAATC
GCCAACCAATTTAACAAGGCGATTAGTCAAATTCAAGAATCACTTACAACAACATCAACT
GCATTGGGCAAGCTGCAAGACGTTGTTAACCAGAATGCTCAAGCATTAAACACACTTGTT
AAACAACTTAGCTCTAATTTTGGTGCAATTTCAAGTGTGCTAAATGATATCCTTTCGCGA
CTTGATAAAGTCGAGGCGGAGGTACAAATTGACAGGTTAATTACAGGCAGACTTCAAAGC
CTTCAAACCTATGTAACACAACAACTAATCAGGGCTGCTGAAATCAGGGCTTCTGCTAAT
CTTGCTGCTACTAAAATGTCTGAGTGTGTTCTTGGACAATCAAAAAGAGTTGACTTTTGT
GGAAAGGGCTACCACCTTATGTCCTTCCCACAAGCAGCCCCGCATGGTGTTGTCTTCCTA
CATGTCACGTATGTGCCATCCCAGGAGAGGAACTTCACCACAGCGCCAGCAATTTGTCAT
GAAGGCAAAGCATACTTCCCTCGTGAAGGTGTTTTTGTGTTTAATGGCACTTCTTGGTTT
ATTACACAGAGGAACTTCTTTTCTCCACAAATAATTACTACAGACAATACATTTGTCTCA
GGAAATTGTGATGTCGTTATTGGCATCATTAACAACACAGTTTATGATCCTCTGCAACCT
GAGCTCGACTCATTCAAAGAAGAGCTGGACAAGTACTTCAAAAATCATACATCACCAGAT
GTTGATCTTGGCGACATTTCAGGCATTAACGCTTCTGTCGTCAACATTCAAAAAGAAATT
GACCGCCTCAATGAGGTCGCTAAAAATTTAAATGAATCACTCATTGACCTTCAAGAATTG
GGAAAATATGAGCAATATATTAAATGGCCTTGG

Soluble TPA-S1 (SEQ ID NO:9)

ATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTT
TCGCCCAGCGCTAGAGGATCGGGAAGTGACCTTGACCGGTGCACCACTTTTGATGATGTT
CAAGCTCCTAATTACACTCAACATACTTCATCTATGAGGGGGGTTTACTATCCTGATGAA
ATTTTTAGATCAGACACTCTTTATTTAACTCAGGATTTATTTCTTCCATTTTATTCTAAT
GTTACAGGGTTTCATACTATTAATCATACGTTTGGCAACCCTGTCATACCTTTTAAGGAT
GGTATTTATTTTGCTGCCACAGAGAAATCAAATGTTGTCCGTGGTTGGGTTTTTGGTTCT
ACCATGAACAACAAGTCACAGTCGGTGATTATTATTAACAATTCTACTAATGTTGTTATA
CGAGCATGTAACTTTGAATTGTGTGACAACCCTTTCTTTGCTGTTTCTAAACCCATGGGT
ACACAGACACATACTATGATATTCGATAATGCATTTAATTGCACTTTCGAGTACATATCT
GATGCCTTTTCGCTTGATGTTTCAGAAAAGTCAGGTAATTTTAAACACTTACGAGAGTTT
GTGTTTAAAAATAAAGATGGGTTTCTCTATGTTTATAAGGGCTATCAACCTATAGATGTA
GTTCGTGATCTACCTTCTGGTTTTAACACTTTGAAACCTATTTTTAAGTTGCCTCTTGGT
ATTAACATTACAAATTTTAGAGCCATTCTTACAGCCTTTTCACCTGCTCAAGACATTTGG
GGCACGTCAGCTGCAGCCTATTTTGTTGGCTATTTAAAGCCAACTACATTTATGCTCAAG
TATGATGAAAATGGTACAATCACAGATGCTGTTGATTGTTCTCAAAATCCACTTGCTGAA
CTCAAATGCTCTGTTAAGAGCTTTGAGATTGACAAAGGAATTTACCAGACCTCTAATTTC
AGGGTTGTTCCCTCAGGAGATGTTGTGAGATTCCCTAATATTACAAACTTGTGTCCTTTTGGA
GAGGTTTTTAATGCTACTAAATTCCCTTCTGTCTATGCATGGGAGAGAAAAAAAATT
TCTAATTGTGTTGCTGATTACTCTGTGCTCTACAACTCAACATTTTTTTCAACCTTTAAG
TGCTATGGCGTTTCTGCCACTAAGTTGAATGATCTTTGCTTCTCCAATGTCTATGCAGAT
TCTTTTGTAGTCAAGGGAGATGATGTAAGACAAATAGCGCCAGGACAAACTGGTGTTATT
GCTGATTATAATTATAAATTGCCAGATGATTTCATGGGTTGTGTCCTTGCTTGGAATACT

AGGAACATTGATGCTACTTCAACTGGTAATTATAATTATAAATATAGGTATCTTAGACAT
GGCAAGCTTAGGCCCTTTGAGAGAGACATATCTAATGTGCCTTTCTCCCCTGATGGCAAA
CCTTGCACCCCACCTGCTCTTAATTGTTATTGGCCATTAAATGATTATGGTTTTTACACC
ACTACTGGCATTGGCTACCAACCTTACAGAGTTGTAGTACTTTCTTTTGAACTTTTAAAT
GCACCGGCCACGGTTTGTGGACCAAAATTATCCACTGACCTTATTAAGAACCAGTGTGTC
AATTTTAATTTTAATGGACTCACTGGTACTGGTGTGTTAACTCCTTCTTCAAAGAGATTT
CAACCATTTCAACAATTTGGCCGTGATGTTTCTGATTTCACTGATTCCGTTCGAGATCCT
AAAACATCTGAAATATTAGACATTTCACCTTGCTCTTTTGGGGGTGTAAGTGTAATTACA
CCTGGAACAAATGCTTCATCTGAAGTTGCTGTTCTATATCAAGATGTTAACTGCACTGAT
GTTTCTACAGCAATTCATGCAGATCAACTCACACCAGCTTGGCGCATATATTCTACTGGA
AACAATGTATTCCAGACTCAAGCAGGCTGTCTTATAGGAGCTGAGCATGTCGACACTTCT
TATGAGTGCGACATTCCTATTGGAGCTGGCATTTGTGCTAGTTACCATACAGTTTCTTTA
TTACGTAGTACTAGCCAAAAATCTATTGTGGCTTATACTATGTCTTTAGGTGC

Soluble TPA-S2 (SEQ ID NO:11)

ATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTT
TCGCCCAGCGCTAGAGGATCGGGAGATAGTTCAATTGCTTACTCTAATAACACCATTGCT
ATACCTACTAACTTTTCAATTAGCATTACTACAGAAGTAATGCCTGTTTCTATGGCTAAA
ACCTCCGTAGATTGTAATATGTACATCTGCGGAGATTCTACTGAATGTGCTAATTTGCTT
CTCCAATATGGTAGCTTTTGCACACAACTAAATCGTGCACTCTCAGGTATTGCTGCTGAA
CAGGATCGCAACACACGTGAAGTGTTCGCTCAAGTCAAACAAATGTACAAAACCCCAACT
TTGAAATATTTTGGTGGTTTTAATTTTTCACAAATATTACCTGACCCTCTAAAGCCAACT
AAGAGGTCTTTTATTGAGGACTTGCTCTTTAATAAGGTGACACTCGCTGATGCTGGCTTC
ATGAAGCAATATGGCGAATGCCTAGGTGATATTAATGCTAGAGATCTCATTTGTGCGCAG
AAGTTCAATGGACTTACAGTGTTGCCACCTCTGCTCACTGATGATATGATTGCTGCCTAC
ACTGCTGCTCTAGTTAGTGGTACTGCCACTGCTGGATGGACATTTGGTGCTGGCGCTGCT
CTTCAAATACCTTTTGCTATGCAAATGGCATATAGGTTCAATGGCATTGGAGTTACCCAA
AATGTTCTCTATGAGAACCAAAAACAAATCGCCAACCAATTTAACAAGGCGATTAGTCAA
ATTCAAGAATCACTTACAACAACATCAACTGCATTGGGCAAGCTGCAAGACGTTGTTAAC
CAGAATGCTCAAGCATTAAACACACTTGTTAAACAACTTAGCTCTAATTTTGGTGCAATT
TCAAGTGTGCTAAATGATATCCTTTCGCGACTTGATAAAGTCGAGGCGGAGGTACAAATT
GACAGGTTAATTACAGGCAGACTTCAAAGCCTTCAAACCTATGTAACACAACAACTAATC
AGGGCTGCTGAAATCAGGGCTTCTGCTAATCTTGCTGCTACTAAAATGTCTGAGTGTGTT
CTTGGACAATCAAAAAGAGTTGACTTTTGTGGAAAGGGCTACCACCTTATGTCCTTCCCA
CAAGCAGCCCCGCATGGTGTTGTCTTCCTACATGTCACGTATGTGCCATCCCAGGAGAGG
AACTTCACCACAGCGCCAGCAATTTGTCATGAAGGCAAAGCATACTTCCCTCGTGAAGGT
GTTTTTGTGTTTAATGGCACTTCTTGGTTTATTACACAGAGGAACTTCTTTTCTCCACAA
ATAATTACTACAGACAATACATTTGTCTCAGGAAATTGTGATGTCGTTATTGGCATCATT
AACAACACAGTTTATGATCCTCTGCAACCTGAGCTCGACTCATTCAAAGAAGAGCTGGAC
AAGTACTTCAAAAATCATACATCACCAGATGTTGATCTTGGCGACATTTCAGGCATTAAC
GCTTCTGTCGTCAACATTCAAAAAGAAATTGACCGCCTCAATGAGGTCGCTAAAAATTTA
AATGAATCACTCATTGACCTTCAAGAATTGGGAAAATATGAGCAATATATTAAATGGCCT
TGG

[0049] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV S, S1, or S2 polypeptide, wherein said polynucleotide is 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NOs:7, 9, or 11, or a codon-optimized version as described below, and wherein said polynucleotide encodes a polypeptide that elicits a detectable immune response.

[0050] The amino acid sequences of the soluble S protein, S1 and S2 proteins with the TPA secretory leader peptide are shown below. Soluble TPA-S protein (SEQ ID NO:8)

MDAMKRGLCCVLLLCGAVFVSPSARGSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDE
IFRSDTLYLTQDLFLPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGS
TMNNKSQSVIIINNSTNVVIRACNFELCDNPFFAVSKPMGTQTHTMIFDNAFNCTFEYIS
DAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLG
INITNFRAILTAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAE
LKCSVKSFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKI
SNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVI
ADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGK
PCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQCV
NFNFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCSFGGVSVIT
PGTNASSEVAVLYQDVNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTS
YECDIPIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNFSISIT
TEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIAAEQDRNTREVFA
QVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGECLGD
INARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMA
YRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLV
KQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASAN
LAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQERNFTTAPAICH
EGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINNTVYDPLQP
ELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL
GKYEQYIKWPW

Soluble TPA-S1 protein (SEQ ID NO: 10)

MDAMKRGLCCVLLLCGAVFVSPSARGSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDE
IFRSDTLYLTQDLFLPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGS
TMNNKSQSVIIINNSTNVVIRACNFELCDNPFFAVSKPMGTQTHTMIFDNAFNCTFEYIS
DAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLG
INITNFRAILTAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAE
LKCSVKSFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKI
SNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVI
ADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGK
PCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQCV
NFNFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCSFGGVSVIT
PGTNASSEVAVLYQDVNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTS
YECDIPIGAGICASYHTVSLLRSTSQKSIVAYTMSLGA

Soluble TPA-S2 protein (SEQ ID NO:12)

MDAMKRGLCCVLLLCGAVFVSPSARGSGDSSIAYSNNTIAIPTNFSISITTEVMPVSMAK
TSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIAAEQDRNTREVFAQVKQMYKTPT
LKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGECLGDINARDLICAQ
KFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQ
NVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAI
SSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECV
LGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQERNFTTAPAICHEGKAYFPREG
VFVFNGTSWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINNTVYDPLQPELDSFKEELD
KYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWP

[0051] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV S, S1, or S2 polypeptide comprising an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NOs:8, 10, or 12, wherein said polypeptide raises a detectable immune response.

[0052] In a further embodiment, the present invention provides for methods for raising a a detectable immune response to the SARS-CoV polypeptides, comprising administering to a vertebrate a polynucleotide which operably encodes polypeptides, fragments, variants, or derivatives thereof as described above.

[0053] The S protein of some coronaviruses contain an Fcγ-like domain that binds immunoglobulin. Data from the FIPV immunization suggests that high levels of potentially neutralizing antibody may be bound by the Fc-mimicking region of the S protein. Scott, F.W. *Adv. Vet. Med. 41*: 347-58 (1999). Thus, modification or deletion of an Fcγ region of the SARS-CoV S protein may be useful in the compositions of the present invention.

[0054] The nucleocapsid protein (N) is encoded by about nucleotides 28120 through about 29388 of the Urbani strain of SARS-CoV. (Bellini *et al.* SARS Coronavirus Urbani, complete genome. GenBank Accession No. AY278741). The protein is a phosphoprotein of 50 to 60 kd that interacts with viral genomic RNA to form the viral nucleocapsid. N has three relatively conserved structural domains, including an RNA-binding domain in the middle that binds to the leader sequence of viral RNA. N protein in the viral nucleocapsid further interacts with the membrane protein (M), leading to the formation of virus particles. N is also suggested to play a role in viral RNA synthesis, by a study in which an antibody directed against N inhibited an *in vitro* coronavirus RNA polymerase reaction. Marra *et al.* N protein also binds to cellular membranes and phospholipids, a property that may help to facilitate both virus assembly and formation of RNA replication complexes.

[0055] From about nucelotides 28120 to about 29388 of the Urbani strain of the SARS-CoV genome encode the N protein. (Bellini *et al.* SARS Coronavirus Urbani, complete genome. GenBank Accession No. AY278741) and has the following sequence, referred to herein as SEQ ID NO:13:

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ATGTCTGATAATGGACCCCAATCAAACCAACGTAGTGCCCCCCGCATTACATTTGGTGGA
CCCACAGATTCAACTGACAATAACCAGAATGGAGGACGCAATGGGGCAAGGCCAAAACAG
CGCCGACCCCAAGGTTTACCCAATAATACTGCGTCTTGGTTCACAGCTCTCACTCAGCAT
GGCAAGGAGGAACTTAGATTCCCTCGAGGCCAGGGCGTTCCAATCAACACCAATAGTGGT
CCAGATGACCAAATTGGCTACTACCGAAGAGCTACCCGACGAGTTCGTGGTGGTGACGGC
AAAATGAAAGAGCTCAGCCCCAGATGGTACTTCTATTACCTAGGAACTGGCCCAGAAGCT
TCACTTCCCTACGGCGCTAACAAAGAAGGCATCGTATGGGTTGCAACTGAGGGAGCCTTG
AATACACCCAAAGACCACATTGGCACCCGCAATCCTAATAACAATGCTGCCACCGTGCTA
CAACTTCCTCAAGGAACAACATTGCCAAAAGGCTTCTACGCAGAGGGAAGCAGAGGCGGC
AGTCAAGCCTCTTCTCGCTCCTCATCACGTAGTCGCGGTAATTCAAGAAATTCAACTCCT
GGCAGCAGTAGGGGAAATTCTCCTGCTCGAATGGCTAGCGGAGGTGGTGAAACTGCCCTC
GCGCTATTGCTGCTAGACAGATTGAACCAGCTTGAGAGCAAAGTTTCTGGTAAAGGCCAA
CAACAACAAGGCCAAACTGTCACTAAGAAATCTGCTGCTGAGGCATCTAAAAAGCCTCGC
CAAAAACGTACTGCCACAAAACAGTACAACGTCACTCAAGCATTTGGGAGACGTGGTCCA
GAACAAACCCAAGGAAATTTCGGGGACCAAGACCTAATCAGACAAGGAACTGATTACAAA
CATTGGCCGCAAATTGCACAATTTGCTCCAAGTGCCTCTGCATTCTTTGGAATGTCACGC
ATTGGCATGGAAGTCACACCTTCGGGAACATGGCTGACTTATCATGGAGCCATTAAATTG
GATGACAAAGATCCACAATTCAAAGACAACGTCATACTGCTGAACAAGCACATTGACGCA
TACAAAACATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAAAAAGACTGATGAAGCT
CAGCCTTTGCCGCAGAGACAAAAGAAGCAGCCCACTGTGACTCTTCTTCCTGCGGCTGAC
ATGGATGATTTCTCCAGACAACTTCAAAATTCCATGAGTGGAGCTTCTGCTGATTCAACT
CAGGCATAA
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[0056] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV N, polypeptide, wherein said polynucleotide is 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:13, or a codon-optimized version as described below, and wherein said polynucleotide encodes a polypeptide that elicits a detectable immune response.

[0057] The amino acid sequence of the N protein encoded by SEQ ID NO:13 has the following sequence shown below and is referred to herein as SEQ ID NO:14

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MSDNGPQSNQRSAPRITFGGPTDSTDNNQNGGRNGARPKQRRPQGLPNNTASWFTALTQH
GKEELRFPRGQGVPINTNSGPDDQIGYYRRATRRVRGGDGKMKELSPRWYFYYLGTGPEA
SLPYGANKEGIVWVATEGALNTPKDHIGTRNPNNNAATVLQLPQGTTLPKGFYAEGSRGG
SQASSRSSSRSRGNSRNSTPGSSRGNSPARMASGGGETALALLLLDRLNQLESKVSGKGQ
QQQGQTVTKKSAAEASKKPRQKRTATKQYNVTQAFGRRGPEQTQGNFGDQDLIRQGTDYK
HWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYHGAIKLDDKDPQFKDNVILLNKHIDA
```

YKTFPPTEPKKDKKKKTDEAQPLPQRQKKQPTVTLLPAADMDDFSRQLQNSMSGASADST
QA

[0058] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV N polypeptide comprising an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:14, wherein said polypeptide raises a detectable immune response.

[0059] The N protein contains a nuclear localization sequence (NLS) which directs the protein to the nucleus infected cells or cells in which the protein is expressed. The sequence of the NLS is KTFPPTEPKKDKKKKTDEAQ (underlined above) and is referred to herein as SEQ ID NO:17. For purposes of the invention, the NLS may be deleted from the protein to obtain a non-nuclear localized version of the protein. The nucleotide sequence of an N protein lacking the NLS is referred to herein as SEQ ID NO:15 and is shown below.

ATGTCTGATAATGGACCCCAATCAAACCAACGTAGTGCCCCCCGCATTACATTTGGTGGA
CCCACAGATTCAACTGACAATAACCAGAATGGAGGACGCAATGGGGCAAGGCCAAAACAG
CGCCGACCCCAAGGTTTACCCAATAATACTGCGTCTTGGTTCACAGCTCTCACTCAGCAT
GGCAAGGAGGAACTTAGATTCCCTCGAGGCCAGGGCGTTCCAATCAACACCAATAGTGGT
CCAGATGACCAAATTGGCTACTACCGAAGAGCTACCCGACGAGTTCGTGGTGGTGACGGC
AAAATGAAAGAGCTCAGCCCCAGATGGTACTTCTATTACCTAGGAACTGGCCCAGAAGCT
TCACTTCCCTACGGCGCTAACAAAGAAGGCATCGTATGGGTTGCAACTGAGGGAGCCTTG
AATACACCCAAAGACCACATTGGCACCCGCAATCCTAATAACAATGCTGCCACCGTGCTA
CAACTTCCTCAAGGAACAACATTGCCAAAAGGCTTCTACGCAGAGGGAAGCAGAGGCGGC
AGTCAAGCCTCTTCTCGCTCCTCATCACGTAGTCGCGGTAATTCAAGAAATTCAACTCCT
GGCAGCAGTAGGGGAAATTCTCCTGCTCGAATGGCTAGCGGAGGTGGTGAAACTGCCCTC
GCGCTATTGCTGCTAGACAGATTGAACCAGCTTGAGAGCAAAGTTTCTGGTAAAGGCCAA
CAACAACAAGGCCAAACTGTCACTAAGAAATCTGCTGCTGAGGCATCTAAAAAGCCTCGC
CAAAAACGTACTGCCACAAAACAGTACAACGTCACTCAAGCATTTGGGAGACGTGGTCCA
GAACAAACCCAAGGAAATTTCGGGGACCAAGACCTAATCAGACAAGGAACTGATTACAAA
CATTGGCCGCAAATTGCACAATTTGCTCCAAGTGCCTCTGCATTCTTTGGAATGTCACGC
ATTGGCATGGAAGTCACACCTTCGGGAACATGGCTGACTTATCATGGAGCCATTAAATTG
GATGACAAAGATCCACAATTCAAAGACAACGTCATACTGCTGAACAAGCACATTGACGCA
TACCCTTTGCCGCAGAGACAAAAGAAGCAGCCCACTGTGACTCTTCTTCCTGCGGCTGAC
ATGGATGATTTCTCCAGACAACTTCAAAATTCCATGAGTGGAGCTTCTGCTGATTCAACT
CAGGCATAA

[0060] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV N, polypeptide, wherein said polynucleotide is 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:15, or a codon-optimized

version as described below, and wherein said polynucleotide encodes a polypeptide that elicits a detectable immune response.

[0061] The amino acid sequence of the N protein without the NLS sequence is encoded by SEQ ID NO:15 has the following sequence shown below and is referred to herein as SEQ ID NO:16:

```
MSDNGPQSNQRSAPRITFGGPTDSTDNNQNGGRNGARPKQRRPQGLPNNTASWFTALTQHGKEELRFPRG
QGVPINTNSGPDDQIGYYRRATRRVRGGDGKMKELSPRWYFYYLGTGPEASLPYGANKEGIVWVATEGAL
NTPKDHIGTRNPNNNAATVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRGNSRNSTPGSSRGNSPAR
MASGGGETALALLLLDRLNQLESKVSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKQYNVTQAFGRRGP
EQTQGNFGDQDLIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYHGAIKLDDKDPQFKDN
VILLNKHIDAYPLPQRQKKQPTVTLLPAADMDDFSRQLQNSMSGASADSTQA
```

[0062] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV N polypeptide comprising an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:16, wherein said polypeptide raises a detectable immune response.

[0063] The membrane glycoprotein (M) is encoded by about nucleotides 26398 to about 27063 of the Urbani strain of SARS-CoV. (Bellini *et al.* SARS Coronavirus Urbani, complete genome. GenBank Accession No. AY278741). The M protein differs from other coronavirus glycoproteins in that only a short amino terminal domain of M is exposed on the exterior of the viral envelope. This domain is followed by a triple-membrane–spanning domain, an α-helical domain, and a large carboxylterminal domain inside the viral envelope. In some coronaviruses, such as transmissible gastroenteritis coronavirus (TGEV), the carboxylterminus of the M protein is exposed on the virion surface. Glycosylation of the aminoterminal domain is O-linked for MHV and N-linked for infectious bronchitis virus (IBV) and TGEV. Monoclonal antibodies against the external domain of M neutralize viral infectivity, but only in the presence of complement. M proteins of some coronaviruses can induce interferon-α. The M proteins are targeted to the Golgi apparatus and not transported to the plasma membrane. In TGEV and MHV virions, the M glycoprotein is present not only in the viral envelope but

also in the internal core structure. (*Field's Virology*, B.N. Fields, D.M. Knipe, P.M. Howley, R.M. Chanock, J.L. Melnick, T.P. Monath, B. Roizman, and S.E. Straus, eds., 4th Edition. Lippincott-Raven, Philadelphia, PA).

[0064] From about nucelotides 26398 to about 27063 of the Urbani strain of the SARS-CoV genome encode the M protein, Bellini *et al.* SARS Coronavirus Urbani, complete genome, GenBank Accession No. AY27874, and has the following sequence, referred to herein as SEQ ID NO:18:

```
ATGGCAGACAACGGTACTATTACCGTTGAGGAGCTTAAACAACTCCTGGAACAATGGAAC
CTAGTAATAGGTTTCCTATTCCTAGCCTGGATTATGTTACTACAATTTGCCTATTCTAAT
CGGAACAGGTTTTTGTACATAATAAAGCTTGTTTTCCTCTGGCTCTTGTGGCCAGTAACA
CTTGCTTGTTTTGTGCTTGCTGCTGTCTACAGAATTAATTGGGTGACTGGCGGGATTGCG
ATTGCAATGGCTTGTATTGTAGGCTTGATGTGGCTTAGCTACTTCGTTGCTTCCTTCAGG
CTGTTTGCTCGTACCCGCTCAATGTGGTCATTCAACCCAGAAACAAACATTCTTCTCAAT
GTGCCTCTCCGGGGGGACAATTGTGACCAGACCGCTCATGGAAAGTGAACTTGTCATTGGT
GCTGTGATCATTCGTGGTCACTTGCGAATGGCCGGACACCCCCTAGGGCGCTGTGACATT
AAGGACCTGCCAAAAGAGATCACTGTGGCTACATCACGAACGCTTTCTTATTACAAATTA
GGAGCGTCGCAGCGTGTAGGCACTGATTCAGGTTTTGCTGCATACAACCGCTACCGTATT
GGAAACTATAAATTAAATACAGACCACGCCGGTAGCAACGACAATATTGCTTTGCTAGTA
CAGTAA
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[0065] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV M, polypeptide, wherein said polynucleotide is 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:18, or a codon-optimized version as described below, and wherein said polynucleotide encodes a polypeptide that elicits a detectable immune response.

[0066] The amino acid sequence of the M protein encoded by SEQ ID NO:18 has the following sequence shown below and is referred to herein as SEQ ID NO:19:

```
MADNGTITVEELKQLLEQWNLVIGFLFLAWIMLLQFAYSNRNRFLYIIKLVFLWLLWPVT
LACFVLAAVYRINWVTGGIAIAMACIVGLMWLSYFVASFRLFARTRSMWSFNPETNILLN
VPLRGTIVTRPLMESELVIGAVIIRGHLRMAGHPLGRCDIKDLPKEITVATSRTLSYYKL
GASQRVGTDSGFAAYNRYRIGNYKLNTDHAGSNDNIALLVQ
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[0067] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV M polypeptide comprising an amino acid sequence at least 60%, 70%, 80%,

90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:19 wherein said polypeptide raises a detectable immune response.

[0068] The small envelope protein (E) is encoded by about nucleotide 26117 to about 26347 of the Urbani strain of SARS-CoV (Bellini *et al.* SARS Coronavirus Urbani, complete genome, GenBank Accession No. AY278741), and has the following sequence, referred to herein as SEQ ID NO: 20:

```
ATGTACTCATTCGTTTCGGAAGAAACAGGTACGTTAATAGTTAATAGCGTACTTCTTTTT
CTTGCTTTCGTGGTATTCTTGCTAGTCACACTAGCCATCCTTACTGCGCTTCGATTGTGT
GCGTACTGCTGCAATATTGTTAACGTGAGTTTAGTAAAACCAACGGTTTACGTCTACTCG
CGTGTTAAAAATCTGAACTCTTCTGAAGGAGTTCCTGATCTTCTGGTCTAA
```

[0069] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV E, polypeptide, wherein said polynucleotide is 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:20, or a codon-optimized version as described below, and wherein said polynucleotide encodes a polypeptide that elicits a detectable immune response

[0070] Based on protein comparisons with other coronaviruses, the SARS-CoV E protein shares conserved sequences with TGEV and MHV. For some coronaviruses, such as TGEV, the E protein is necessary for replication of the virus, while for others, such as MHV, loss of the E protein merely reduces virus replication without eliminating it completely. Marra *et al.* The protein sequence is shown below and referred to, herein as SEQ ID NO:21.

```
MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPTVYVYS
RVKNLNSSEGVPDLLV
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[0071] In a further embodiment the methods of the present invention provide for administering a polynucleotide which operably encodes a SARS-CoV E polypeptide comprising an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:21 wherein said polypeptide raises a detectable immune response.

[0072] It should be noted that nucleotide sequences encoding various SARS-CoV polypeptides may vary between SARS-CoV strains. Virtually any nucleotide sequence encoding a SARS-CoV protein is suitable for the present invention. In fact, polynucleotide sequences included in vaccines and therapeutic formulations of the current invention may change from year to year, depending on the prevalent strain or strains of SARS-CoV.

[0073] Further examples of SARS-CoV polypeptides within the scope of the invention are multimerized fragments of SARS-CoV polypeptides and polynucleotides that encode multimerized fragments of SARS-CoV polypeptides. The polypeptide fragments of the invention contain at least one antigenic region. The SARS-CoV polypeptide fragments are fused to small assembly polypeptides. Non-limiting examples within the scope of the invention include coiled-coiled structures such as: an amphipathic helix, the yeast CGN4 leucine zipper, the human p53 tetramerization domain, and synthetic coil polypeptides. The SARS-CoV and assembly peptide fusion proteins self-assemble into stable multimers forming dimers, trimers, tetramers, and higher order multimers depending on the interacting amino acid residues. These multimerized SARS-CoV polypeptide fragments have increased local epitope valency which functions to more efficiently activate B lymphocytes, thereby producing a more robust immune response. Also within the scope of the invention are multimerized SARS-CoV polypeptide fragments that maintain conformational neutralizing epitopes.

[0074] Also within the scope of the present invention are combinations of SARS-CoV polypeptides and polynucleotides that encode SARS-CoV polypeptides, where the polypeptides assemble into virus-like particles (VLP). One such combination is, but is not limited to a combination of SARS-CoV S, M, and E polypeptides or fragments, variants, or derivatives thereof, and polynucleotides encoding SARS-CoV S, M, and E polypeptides or fragments, variants, or derivatives thereof. Combinations of SARS-CoV polypeptides that form VLPs may be useful in enhancing immunogenicity of SARS-CoV polypeptides and in eliciting a detectable immune response to the SARS-CoV

virus. Also within the scope of the present invention are methods of producing SARS-CoV VLPs *in vitro* by using protocols that are well known in the art. The production of VLPs may be performed in any tissue culture cell line that can tolerate expression of SARS-CoV polypeptide. Examples of cell lines include, but are not limited to, fungal cells, including yeast cells such as *Saccharomyces* spp. cells; insect cells such as *Drosophila* S2, *Spodoptera* Sf9 or Sf21 cells and *Trichoplusa* High-Five cells; other animal cells (particularly mammalian cells and human cells) such as Vero, MDCK, CV1, 3T3, CPAE, A10, Sp2/0-Ag14, PC12, CHO, COS, HeLa, Bowes melanoma cells, SW-13, NCI-H295, RT4, HT-1376, UM-UC-3, IM-9, KG-1, R54;11, A-172, U-87MG, BT-20, MCF-7, SK-BR-3, ChaGo K-1, CCD-14Br, CaSki, ME-180, FHC, HT-29, Caco-2, SW480, HuTu80, Tera 1, NTERA-2, AN3 CA, KLE, RL95-2, Caki-1, ACHN, 769 P, CCRF-CEM, Hut 78, MOLT 4, HL-60, Hep-3B, HepG2, SK-HEP1, A-549, NCI-H146, NCI-H82, NCI-H82, SK-LU-1, WI-38, MRC-5, HLF-a, CCD-19Lu, C39, Hs294T, SK-MEL5, COLO 829, U266B1, RPMI 2650, BeWo, JEG-3, JAR, SW 1353, MeKam, and SCC-4; and higher plant cells. Appropriate culture media and conditions for the above-described host cells are known in the art.

[0075] De Haan *et al.*, *J. Virol.* 72: 6838-50 (1998), describe the assembly of coronavirus VLPs from the coexpression of mouse hepatitis virus M and E genes in eukaryotic cells. Bos *et al.*, *J. Virol.* 71: 9427-33 describe the role of the S protein in infectivity of coronavirus VLPs produced by coexpression of mouse hepatitis virus S, M, and E proteins. These references are hereby incorporated by reference in their entireties.

[0076] In another embodiment, the VLP comprising SARS-CoV polypeptides S, M, and E provides a method for mimicking a SARS-CoV infection without the use of the actual infectious agent. In addtion, the VLP provides a method for eliciting a detectable immune response to multiple antigens in a confirmation similar to the actual virus particle thereby enhancing the immunogenicity of the SARS-CoV polypeptides.

[0077] The VLP's of the invention can be produced *in vivo* by delivery of S, M or E polynucleotides or polypeptides, described herein, to a vertebrate wherein assembly of the VLPs occurs with the cells of the vertebrate. In an alternative embodiment, VLPs of the invention can be produced *in vitro* in cells that have received the S, M, and E polynucleotides described herein and express said proteins. VLPs are then purified from the cells using techniques known in the art for coronavirus particle purification. These purified particles can then be administered to a vertebrate to elicit a detectable immune response or to study the pathogenesis of the SARS-CoV infection without the need of the actual infectious agent.

[0078] The combination of S, M and E to create virus like particles in the previous examples is not meant to be limiting. Other SARS-CoV polypeptides, which assemble into, or are engineered to assemble into virus like particles, may be used as well.

[0079] The present invention also provides vaccine compositions and methods for delivery of SARS-CoV coding sequences to a vertebrate. In other embodiments, the present invention provides vaccine compositions and methods for delivery of SARS-CoV coding sequences to a vertebrate with optimal expression and safety conferred through codon optimization and/or other manipulations. These vaccine compositions are prepared and administered in such a manner that the encoded gene products are optimally expressed in the vertebrate of interest. As a result, these compositions and methods are useful in stimulating an immune response against SARS-CoV infection. Also included in the invention are expression systems, delivery systems, and codon-optimized SARS-CoV coding regions.

[0080] In a specific embodiment, the invention provides polynucleotide (*e.g.*, DNA) vaccines in which the single formulation comprises a SARS-CoV polypeptide-encoding polynucleotide vaccine as described herein. An alternative embodiment of the invention provides for a multivalent formulation comprising several (*e.g.*, two, three, four, or more) SARS-CoV polypeptide-encoding polynucleotides, as described herein, within a single

vaccine composition. The SARS-CoV polypeptide-encoding polynucleotides, fragments, or variants thereof may be contained within a single expression vector (*e.g.*, plasmid or viral vector) or may be contained within multiple expression vectors.

[0081] In a specific embodiment, the invention provides combinatorial polynucleotide (*e.g.*, DNA) vaccines which combine both a polynucleotide vaccine and polypeptide (*e.g.*, either a recombinant protein, a purified subunit protein, a viral vector expressing an isolated SARS-CoV polypeptide) vaccine in a single formulation. The single formulation comprises a SARS-CoV polypeptide-encoding polynucleotide vaccine as described herein, and optionally, an effective amount of a desired isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof. The polypeptide may exist in any form, for example, a recombinant protein, a purified subunit protein, or a viral vector expressing an isolated SARS-CoV polypeptide. The SARS-CoV polypeptide or fragment, variant, or derivative thereof encoded by the polynucleotide vaccine may be identical to the isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof. Alternatively, the SARS-CoV polypeptide or fragment, variant, or derivative thereof encoded by the polynucleotide may be different from the isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof.

[0082] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a polynucleotide," is understood to represent one or more polynucleotides. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0083] It is to be noted that the term “about” when referring to a polynucleotide, coding region or any nucleotide sequence, for example, is understood to represent plus or minus 1 to 30 nucleotides on either end of the defined coding region, polynucleotide or nucleotide sequence. It is to be noted that when referring to a polypeptide, or polypeptide sequence, that the term “about” is understood to represent plus or minus 1 to 10 amino acids on either end of the defined polypeptide or polypeptide sequence. It should be further

noted that the term "about," when referring to the quantity of a specific codon in a given codon-optimized coding region has a specific meaning, described in more detail below.

[0084] The term "polynucleotide" is intended to encompass a singular nucleic acid or nucleic acid fragment as well as plural nucleic acids or nucleic acid fragments, and refers to an isolated molecule or construct, *e.g.*, a virus genome (*e.g.*, a non-infectious viral genome), messenger RNA (mRNA), plasmid DNA (pDNA), or derivatives of pDNA (*e.g.*, minicircles as described in Darquet, A-M *et al.*, *Gene Therapy* 4:1341-1349 (1997)) comprising a polynucleotide. A nucleic acid or fragment thereof may be provided in linear (*e.g.*, mRNA), circular (*e.g.*, plasmid), or branched form as well as double-stranded or single-stranded forms. A polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)).

[0085] The terms "nucleic acid" or "nucleic acid fragment" refer to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide or construct.

[0086] As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, and the like, are not part of a coding region. Two or more nucleic acids or nucleic acid fragments of the present invention can be present in a single polynucleotide construct, *e.g.*, on a single plasmid, or in separate polynucleotide constructs, *e.g.*, on separate (different) plasmids. Furthermore, any nucleic acid or nucleic acid fragment may encode a single SARS-CoV polypeptide or fragment, derivative, or variant thereof, *e.g.*, or may encode more than one polypeptide, *e.g.*, a nucleic acid may encode two or more polypeptides. In addition, a nucleic acid may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator, or may encode heterologous coding regions fused to

the SARS-CoV coding region, *e.g.*, specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

[0087] The terms "fragment," "variant," "derivative," and "analog," when referring to SARS-CoV polypeptides of the present invention, include any polypeptides which retain at least some of the immunogenicity or antigenicity of the corresponding native polypeptide. Fragments of SARS-CoV polypeptides of the present invention include proteolytic fragments, deletion fragments, and in particular, fragments of SARS-CoV polypeptides which exhibit increased secretion from the cell or higher immunogenicity or reduced pathogenicity when delivered to an animal. Polypeptide fragments further include any portion of the polypeptide which comprises an antigenic or immunogenic epitope of the native polypeptide, including linear as well as three-dimensional epitopes. Variants of SARS-CoV polypeptides of the present invention include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants may occur naturally, such as an allelic variant. By an "allelic variant" is intended alternate forms of a gene occupying a given locus on a chromosome or genome of an organism or virus. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985), which is incorporated herein by reference. Naturally or non-naturally occurring variations such as amino acid deletions, insertions or substitutions may occur. Non-naturally occurring variants may be produced using art-known mutagenesis techniques. Variant polypeptides may comprise conservative or non-conservative amino acid substitutions, deletions or additions. Derivatives of SARS-CoV polypeptides of the present invention, are polypeptides which have been altered so as to exhibit additional features not found on the native polypeptide. Examples include fusion proteins. An analog is another form of a SARS-CoV polypeptide of the present invention. An example is a proprotein which can be activated by cleavage of the proprotein to produce an active mature polypeptide.

[0088] The terms "infectious polynucleotide" or "infectious nucleic acid" are intended to encompass isolated viral polynucleotides and/or nucleic acids which are solely sufficient to mediate the synthesis of complete infectious virus particles upon uptake by permissive cells. Thus, "infectious nucleic acids" do not require pre-synthesized copies of any of the polypeptides it encodes, *e.g.*, viral replicases, in order to initiate its replication cycle in a permissive host cell.

[0089] The terms "non-infectious polynucleotide" or "non-infectious nucleic acid" as defined herein are polynucleotides or nucleic acids which cannot, without additional added materials, *e.g,* polypeptides, mediate the synthesis of complete infectious virus particles upon uptake by permissive cells. An infectious polynucleotide or nucleic acid is not made "non-infectious" simply because it is taken up by a non-permissive cell. For example, an infectious viral polynucleotide from a virus with limited host range is infectious if it is capable of mediating the synthesis of complete infectious virus particles when taken up by cells derived from a permissive host (*i.e.*, a host permissive for the virus itself). The fact that uptake by cells derived from a non-permissive host does not result in the synthesis of complete infectious virus particles does not make the nucleic acid "non-infectious." In other words, the term is not qualified by the nature of the host cell, the tissue type, or the species taking up the polynucleotide or nucleic acid fragment.

[0090] In some cases, an isolated infectious polynucleotide or nucleic acid may produce fully-infectious virus particles in a host cell population which lacks receptors for the virus particles, *i.e.*, is non-permissive for virus entry. Thus viruses produced will not infect surrounding cells. However, if the supernatant containing the virus particles is transferred to cells which are permissive for the virus, infection will take place.

[0091] The terms "replicating polynucleotide" or "replicating nucleic acid" are meant to encompass those polynucleotides and/or nucleic acids which, upon being taken up by a permissive host cell, are capable of producing multiple, *e.g.*, one or more copies of the same polynucleotide or nucleic acid. Infectious

polynucleotides and nucleic acids are a subset of replicating polynucleotides and nucleic acids; the terms are not synonymous. For example, a defective virus genome lacking the genes for virus coat proteins may replicate, *e.g.*, produce multiple copies of itself, but is NOT infectious because it is incapable of mediating the synthesis of complete infectious virus particles unless the coat proteins, or another nucleic acid encoding the coat proteins, are exogenously provided.

[0092] In certain embodiments, the polynucleotide, nucleic acid, or nucleic acid fragment is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid which encodes a polypeptide normally also comprises a promoter and/or other transcription or translation control elements operably associated with the polypeptide-encoding nucleic acid fragment. An operable association is when a nucleic acid fragment encoding a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide-encoding nucleic acid fragment and a promoter associated with the 5' end of the nucleic acid fragment) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the expression regulatory sequences to direct the expression of the gene product, or (3) interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid fragment encoding a polypeptide if the promoter were capable of effecting transcription of that nucleic acid fragment. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific

transcription. Suitable promoters and other transcription control regions are disclosed herein.

[0093] A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit ß-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

[0094] Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, elements from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

[0095] A DNA polynucleotide of the present invention may be a circular or linearized plasmid, or other linear DNA which may also be non-infectious and nonintegrating (*i.e.*, does not integrate into the genome of vertebrate cells). A linearized plasmid is a plasmid that was previously circular but has been linearized, for example, by digestion with a restriction endonuclease. Linear DNA may be advantageous in certain situations as discussed, *e.g.*, in Cherng, J.Y., *et al., J. Control. Release 60*:343-53 (1999), and Chen, Z.Y., *et al. Mol. Ther. 3*:403-10 (2001), both of which are incorporated herein by reference.

[0096] Alternatively, DNA virus genomes may be used to administer DNA polynucleotides into vertebrate cells. In certain embodiments, a DNA virus genome of the present invention is nonreplicative, noninfectious, and/or nonintegrating. Suitable DNA virus genomes include without limitation,

herpesvirus genomes, adenovirus genomes, adeno-associated virus genomes, and poxvirus genomes. References citing methods for the *in vivo* introduction of non-infectious virus genomes to vertebrate tissues are well known to those of ordinary skill in the art, and are cited *supra*.

[0097] In other embodiments, a polynucleotide of the present invention is RNA, for example, in the form of messenger RNA (mRNA). Methods for introducing RNA sequences into vertebrate cells are described in U.S. Patent No. 5,580,859, the disclosure of which is incorporated herein by reference in its entirety.

[0098] Polynucleotides, nucleic acids, and nucleic acid fragments of the present invention may be associated with additional nucleic acids which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a nucleic acid fragment or polynucleotide of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the complete or "full length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native leader sequence is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian leader sequence, or a functional derivative thereof, may be used. For example, the wild-type leader sequence may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse ß-glucuronidase.

[0099] In accordance with one aspect of the present invention, there is provided a polynucleotide construct, for example, a plasmid, comprising a nucleic acid fragment, where the nucleic acid fragment is a fragment of a coding region operably encoding an SARS-CoV-derived polypeptide. In

accordance with another aspect of the present invention, there is provided a polynucleotide construct, for example, a plasmid, comprising a nucleic acid fragment, where the nucleic acid fragment is a fragment of a codon-optimized coding region operably encoding an SARS-CoV-derived polypeptide, where the coding region is optimized for expression in vertebrate cells, of a desired vertebrate species, *e.g.*, humans, to be delivered to a vertebrate to be treated or immunized. Suitable SARS-CoV polypeptides, or fragments, variants, or derivatives thereof may be derived from, but are not limited to, the SARS-CoV S, Soluble S1, Soluble S2, N, E or M proteins. Additional SARS-CoV-derived coding sequences, *e.g.*, coding for S, Soluble S1, Soluble S2, N, E or M, may also be included on the plasmid, or on a separate plasmid, and expressed, either using native SARS-CoV codons or one or more codons optimized for expression in the vertebrate to be treated or immunized. When such a plasmid encoding one or more optimized SARS-CoV sequences and/or one or more optimized SARS-CoV sequences is delivered, *in vivo* to a tissue of the vertebrate to be treated or immunized, one or more of the encoded gene products will be expressed, *i.e.*, transcribed and translated. The level of expression of the gene product(s) will depend to a significant extent on the strength of the associated promoter and the presence and activation of an associated enhancer element, as well as the degree of optimization of the coding region.

[0100] As used herein, the term "plasmid" refers to a construct made up of genetic material (*i.e.*, nucleic acids). Typically a plasmid contains an origin of replication which is functional in bacterial host cells, *e.g.*, *Escherichia coli*, and selectable markers for detecting bacterial host cells comprising the plasmid. Plasmids of the present invention may include genetic elements as described herein arranged such that an inserted coding sequence can be transcribed and translated in eukaryotic cells. Also, the plasmid may include a sequence from a viral nucleic acid. However, such viral sequences normally are not sufficient to direct or allow the incorporation of the plasmid into a viral

particle, and the plasmid is therefore a non-viral vector. In certain embodiments described herein, a plasmid is a closed circular DNA molecule.

[0101] The term "expression" refers to the biological production of a product encoded by a coding sequence. In most cases a DNA sequence, including the coding sequence, is transcribed to form a messenger-RNA (mRNA). The messenger-RNA is then translated to form a polypeptide product which has a relevant biological activity. Also, the process of expression may involve further processing steps to the RNA product of transcription, such as splicing to remove introns, and/or post-translational processing of a polypeptide product.

[0102] As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and comprises any chain or chains of two or more amino acids. Thus, as used herein, terms including, but not limited to “peptide,” “dipeptide,” “tripeptide,” “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids, are included in the definition of a “polypeptide,” and the term “polypeptide” may be used instead of, or interchangeably with any of these terms. The term further includes polypeptides which have undergone post-translational modifications, for example, glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids.

[0103] Also included as polypeptides of the present invention are fragments, derivatives, analogs, or variants of the foregoing polypeptides, and any combination thereof. Polypeptides, and fragments, derivatives, analogs, or variants thereof of the present invention can be antigenic and immunogenic polypeptides related to SARS-CoV polypeptides, which are used to prevent or treat, *i.e.*, cure, ameliorate, lessen the severity of, or prevent or reduce contagion of infectious disease caused by the SARS-CoV.

[0104] As used herein, an antigenic polypeptide or an immunogenic polypeptide is a polypeptide which, when introduced into a vertebrate, reacts

with the vertebrate's immune system molecules, *i.e.*, is antigenic, and/or induces an immune response in the vertebrate, *i.e.*, is immunogenic. It is quite likely that an immunogenic polypeptide will also be antigenic, but an antigenic polypeptide, because of its size or conformation, may not necessarily be immunogenic. Examples of antigenic and immunogenic polypeptides of the present invention include, but are not limited to, *e.g.*, S or fragments, derivatives, or variants thereof; N or fragments, derivatives, or variants thereof; E or fragments, derivatives, or variants thereof; M or fragments, derivatives, or variants thereof; other predicted ORF's within the sequence of the SARS-CoV viruses which may posses antigenic properties, for example, an ORF which may encode for the hemagglutinin-esterase or fragments, derivatives, or variants thereof; or any of the foregoing polypeptides or fragments, derivatives, or variants thereof fused to a heterologous polypeptide, for example, a hepatitis B core antigen. Isolated antigenic and immunogenic polypeptides of the present invention in addition to those encoded by polynucleotides of the invention, may be provided as a recombinant protein, a purified subunit, a viral vector expressing the protein, or may be provided in the form of an inactivated SARS-CoV vaccine, *e.g.*, a live-attenuated virus vaccine, a heat-killed virus vaccine, etc.

[0105] By an "isolated" SARS-CoV polypeptide or a fragment, variant, or derivative thereof is intended a SARS-CoV polypeptide or protein that is not in its natural environment. No particular level of purification is required. For example, an isolated SARS-CoV polypeptide can be removed from its native or natural environment. Recombinantly produced SARS-CoV polypeptides and proteins expressed in host cells are considered isolated for purposed of the invention, as are native or recombinant SARS-CoV polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique, including the separation of SARS-CoV virions from tissue samples or culture cells in which they have been propagated. In addition, an isolated. Thus, isolated SARS-CoV polypeptides and proteins can be provided

as, for example, recombinant SARS-CoV polypeptides, a purified subunit of SARS-CoV, or a viral vector expressing an isolated SARS-CoV polypeptide.

[0106] The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in a vertebrate, for example a human. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an immune response in an animal, as determined by any method known in the art. The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody or T-cell receptor can immunospecifically bind as determined by any method well known in the art. Immunospecific binding excludes non-specific binding but does not exclude cross-reactivity with other antigens. Where all immunogenic epitopes are antigenic, antigenic epitopes need not be immunogenic.

[0107] The term "immunogenic carrier" as used herein refers to a first polypeptide or fragment, variant, or derivative thereof which enhances the immunogenicity of a second polypeptide or fragment, variant, or derivative thereof. Typically, an "immunogenic carrier" is fused to or conjugated to the desired polypeptide or fragment thereof. An example of an "immunogenic carrier" is a recombinant hepatitis B core antigen expressing, as a surface epitope, an immunogenic epitope of interest. See, *e.g.*, European Patent No. EP 0385610 B1, which is incorporated herein by reference in its entirety.

[0108] In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, or between about 8 to about 30 amino acids contained within the amino acid sequence of a SARS-CoV polypeptide of the invention, *e.g.*, an S polypeptide, an N polypeptide, an E polypeptide or an M polypeptide. Certain polypeptides comprising immunogenic or antigenic epitopes are at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Antigenic as well as immunogenic epitopes may be linear, *i.e.*, be comprised of contiguous amino acids in a polypeptide, or may be three dimensional, *i.e.*, where an epitope is comprised of non-contiguous amino acids which come

together due to the secondary or tertiary structure of the polypeptide, thereby forming an epitope.

[0109] As to the selection of peptides or polypeptides bearing an antigenic epitope (*e.g.*, that contain a region of a protein molecule to which an antibody or T cell receptor can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. *See, e.g.*, Sutcliffe, J. G., *et al.*, *Science 219*:660-666 (1983).

[0110] Peptides capable of eliciting an immunogenic response are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins nor to the amino or carboxyl terminals. Peptides that are extremely hydrophobic and those of six or fewer residues generally are ineffective at inducing antibodies that bind to the mimicked protein; longer peptides, especially those containing proline residues, usually are effective. Sutcliffe *et al.*, *supra*, at 661. For instance, 18 of 20 peptides designed according to these guidelines, containing 8-39 residues covering 75% of the sequence of the influenza virus hemagglutinin HA1 polypeptide chain, induced antibodies that reacted with the HA1 protein or intact virus; and 12/12 peptides from the MuLV polymerase and 18/18 from the rabies glycoprotein induced antibodies that precipitated the respective proteins.

Codon Optimization

[0111] "Codon optimization" is defined as modifying a nucleic acid sequence for enhanced expression in the cells of the vertebrate of interest, *e.g.*, human, by replacing at least one, more than one, or a significant number, of codons of the native sequence with codons that are more frequently or most frequently used in the genes of that vertebrate. Various species exhibit particular biases for certain codons of a particular amino acid.

[0112] In one aspect, the present invention relates to polynucleotides comprising nucleic acid fragments of codon-optimized coding regions which encode SARS-CoV polypeptides, or fragments, variants, or derivatives thereof, with the codon usage adapted for optimized expression in the cells of a given vertebrate, *e.g.*, humans. These polynucleotides are prepared by incorporating codons preferred for use in the genes of the vertebrate of interest into the DNA sequence. Also provided are polynucleotide expression constructs, vectors, and host cells comprising nucleic acid fragments of codon-optimized coding regions which encode SARS-CoV polypeptides, and fragments, variants, or derivatives thereof, and various methods of using the polynucleotide expression constructs, vectors, and/or host cells to treat or prevent SARS disease in a vertebrate.

[0113] As used herein the term "codon-optimized coding region" means a nucleic acid coding region that has been adapted for expression in the cells of a given vertebrate by replacing at least one, or more than one, or a significant number, of codons with one or more codons that are more frequently used in the genes of that vertebrate.

[0114] Deviations in the nucleotide sequence that comprise the codons encoding the amino acids of any polypeptide chain allow for variations in the sequence coding for the gene. Since each codon consists of three nucleotides, and the nucleotides comprising DNA are restricted to four specific bases, there are 64 possible combinations of nucleotides, 61 of which encode amino acids (the remaining three codons encode signals ending translation). The "genetic code," which shows which codons encode which amino acids, is reproduced herein as Table 3. As a result, many amino acids are designated by more than one codon. For example, the amino acids alanine and proline are coded for by four triplets, serine and arginine by six triplets, whereas tryptophan and methionine are coded by just one triplet. This degeneracy allows for DNA base composition to vary over a wide range without altering the amino acid sequence of the proteins encoded by the DNA.

TABLE 3: The Standard Genetic Code

	T	C	A	G
T	TTT Phe (F)	TCT Ser (S)	TAT Tyr (Y)	TGT Cys (C)
	TTC "	TCC "	TAC "	TGC
	TTA Leu (L)	TCA "	TAA **Ter**	TGA **Ter**
	TTG "	TCG "	TAG **Ter**	TGG Trp (W)
C	CTT Leu (L)	CCT Pro (P)	CAT His (H)	CGT Arg (R)
	CTC "	CCC "	CAC "	CGC "
	CTA "	CCA "	CAA Gln (Q)	CGA "
	CTG "	CCG "	CAG "	CGG "
A	ATT Ile (I)	ACT Thr (T)	AAT Asn (N)	AGT Ser (S)
	ATC "	ACC "	AAC "	AGC "
	ATA "	ACA "	AAA Lys (K)	AGA Arg (R)
	ATG Met (M)	ACG "	AAG "	AGG "
G	GTT Val (V)	GCT Ala (A)	GAT Asp (D)	GGT Gly (G)
	GTC "	GCC "	GAC "	GGC "
	GTA "	GCA "	GAA Glu (E)	GGA "
	GTG "	GCG "	GAG "	GGG "

[0115] Many organisms display a bias for use of particular codons to code for insertion of a particular amino acid in a growing peptide chain. Codon preference or codon bias, differences in codon usage between organisms, is afforded by degeneracy of the genetic code, and is well documented among many organisms. Codon bias often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, *inter alia*, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization.

[0116] Given the large number of gene sequences available for a wide variety of animal, plant and microbial species, it is possible to calculate the relative

frequencies of codon usage. Codon usage tables are readily available, for example, at the "Codon Usage Database," available at http://www.kazusa.or.jp/codon/ (visited July 9, 2002), and these tables can be adapted in a number of ways. *See* Nakamura, Y., *et al.* "Codon usage tabulated from the international DNA sequence databases: status for the year 2000" *Nucl. Acids Res.* 28:292 (2000). As examples, the codon usage tables for human, mouse, domestic cat, and cow, calculated from GenBank Release 128.0 (15 February 2002), are reproduced below as Tables 4-7. These tables use mRNA nomenclature, and so instead of thymine (T) which is found in DNA, the tables use uracil (U) which is found in RNA. The tables have been adapted so that frequencies are calculated for each amino acid, rather than for all 64 codons.

TABLE 4: Codon Usage Table for Human Genes (*Homo sapiens*)

Amino Acid	Codon	Number	Frequency
Phe	UUU	326146	0.4525
Phe	UUC	394680	0.5475
Total		720826	
Leu	UUA	139249	0.0728
Leu	UUG	242151	0.1266
Leu	CUU	246206	0.1287
Leu	CUC	374262	0.1956
Leu	CUA	133980	0.0700
Leu	CUG	777077	0.4062
Total		1912925	
Ile	AUU	303721	0.3554
Ile	AUC	414483	0.4850
Ile	AUA	136399	0.1596
Total		854603	
Met	AUG	430946	1.0000
Total		430946	
Val	GUU	210423	0.1773
Val	GUC	282445	0.2380

Amino Acid	Codon	Number	Frequency
Val	GUA	134991	0.1137
Val	GUG	559044	0.4710
Total		1186903	
Ser	UCU	282407	0.1840
Ser	UCC	336349	0.2191
Ser	UCA	225963	0.1472
Ser	UCG	86761	0.0565
Ser	AGU	230047	0.1499
Ser	AGC	373362	0.2433
Total		1534889	
Pro	CCU	333705	0.2834
Pro	CCC	386462	0.3281
Pro	CCA	322220	0.2736
Pro	CCG	135317	0.1149
Total		1177704	
Thr	ACU	247913	0.2419
Thr	ACC	371420	0.3624
Thr	ACA	285655	0.2787
Thr	ACG	120022	0.1171
Total		1025010	
Ala	GCU	360146	0.2637
Ala	GCC	551452	0.4037
Ala	GCA	308034	0.2255
Ala	GCG	146233	0.1071
Total		1365865	
Tyr	UAU	232240	0.4347
Tyr	UAC	301978	0.5653
Total		534218	
His	CAU	201389	0.4113
His	CAC	288200	0.5887
Total		489589	
Gln	CAA	227742	0.2541
Gln	CAG	668391	0.7459
Total		896133	
Asn	AAU	322271	0.4614
Asn	AAC	376210	0.5386
Total		698481	

Amino Acid	Codon	Number	Frequency
Lys	AAA	462660	0.4212
Lys	AAG	635755	0.5788
Total		1098415	
Asp	GAU	430744	0.4613
Asp	GAC	502940	0.5387
Total		933684	
Glu	GAA	561277	0.4161
Glu	GAG	787712	0.5839
Total		1348989	
Cys	UGU	190962	0.4468
Cys	UGC	236400	0.5532
Total		427362	
Trp	UGG	248083	1.0000
Total		248083	
Arg	CGU	90899	0.0830
Arg	CGC	210931	0.1927
Arg	CGA	122555	0.1120
Arg	CGG	228970	0.2092
Arg	AGA	221221	0.2021
Arg	AGG	220119	0.2011
Total		1094695	
Gly	GGU	209450	0.1632
Gly	GGC	441320	0.3438
Gly	GGA	315726	0.2459
Gly	GGG	317263	0.2471
Total		1283759	
Stop	UAA	13963	
Stop	UAG	10631	
Stop	UGA	24607	

TABLE 5: Codon Usage Table for Mouse Genes (*Mus musculus*)

Amino Acid	Codon	Number	Frequency
Phe	UUU	150467	0.4321
Phe	UUC	197795	0.5679
Total		348262	

Amino Acid	Codon	Number	Frequency
Leu	UUA	55635	0.0625
Leu	UUG	116210	0.1306
Leu	CUU	114699	0.1289
Leu	CUC	179248	0.2015
Leu	CUA	69237	0.0778
Leu	CUG	354743	0.3987
Total		889772	
Ile	AUU	137513	0.3367
Ile	AUC	208533	0.5106
Ile	AUA	62349	0.1527
Total		408395	
Met	AUG	204546	1.0000
Total		204546	
Val	GUU	93754	0.1673
Val	GUC	140762	0.2513
Val	GUA	64417	0.1150
Val	GUG	261308	0.4664
Total		560241	
Ser	UCU	139576	0.1936
Ser	UCC	160313	0.2224
Ser	UCA	100524	0.1394
Ser	UCG	38632	0.0536
Ser	AGU	108413	0.1504
Ser	AGC	173518	0.2407
Total		720976	
Pro	CCU	162613	0.3036
Pro	CCC	164796	0.3077
Pro	CCA	151091	0.2821
Pro	CCG	57032	0.1065
Total		535532	
Thr	ACU	119832	0.2472
Thr	ACC	172415	0.3556
Thr	ACA	140420	0.2896
Thr	ACG	52142	0.1076
Total		484809	
Ala	GCU	178593	0.2905
Ala	GCC	236018	0.3839
Ala	GCA	139697	0.2272
Ala	GCG	60444	0.0983
Total		614752	

Amino Acid	Codon	Number	Frequency
Tyr	UAU	108556	0.4219
Tyr	UAC	148772	0.5781
Total		257328	
His	CAU	88786	0.3973
His	CAC	134705	0.6027
Total		223491	
Gln	CAA	101783	0.2520
Gln	CAG	302064	0.7480
Total		403847	
Asn	AAU	138868	0.4254
Asn	AAC	187541	0.5746
Total		326409	
Lys	AAA	188707	0.3839
Lys	AAG	302799	0.6161
Total		491506	
Asp	GAU	189372	0.4414
Asp	GAC	239670	0.5586
Total		429042	
Glu	GAA	235842	0.4015
Glu	GAG	351582	0.5985
Total		587424	
Cys	UGU	97385	0.4716
Cys	UGC	109130	0.5284
Total		206515	
Trp	UGG	112588	1.0000
Total		112588	
Arg	CGU	41703	0.0863
Arg	CGC	86351	0.1787
Arg	CGA	58928	0.1220
Arg	CGG	92277	0.1910
Arg	AGA	101029	0.2091
Arg	AGG	102859	0.2129
Total		483147	
Gly	GGU	103673	0.1750
Gly	GGC	198604	0.3352
Gly	GGA	151497	0.2557
Gly	GGG	138700	0.2341
Total		592474	

Amino Acid	Codon	Number	Frequency
Stop	UAA	5499	
Stop	UAG	4661	
Stop	UGA	10356	

TABLE 6: Codon Usage Table for Domestic Cat Genes (*Felis cattus*)

Amino Acid	Codon	Number	Frequency of usage
Phe	UUU	1204.00	0.4039
Phe	UUC	1777.00	0.5961
Total		2981	
Leu	UUA	404.00	0.0570
Leu	UUG	857.00	0.1209
Leu	CUU	791.00	0.1116
Leu	CUC	1513.00	0.2135
Leu	CUA	488.00	0.0688
Leu	CUG	3035.00	0.4282
Total		7088	
Ile	AUU	1018.00	0.2984
Ile	AUC	1835.00	0.5380
Ile	AUA	558.00	0.1636
Total		3411	
Met	AUG	1553.00	0.0036
Total		1553	
Val	GUU	696.00	0.1512
Val	GUC	1279.00	0.2779
Val	GUA	463.00	0.1006
Val	GUG	2164.00	0.4702
Total		4602	
Ser	UCU	940.00	0.1875
Ser	UCC	1260.00	0.2513
Ser	UCA	608.00	0.1213
Ser	UCG	332.00	0.0662
Ser	AGU	672.00	0.1340
Ser	AGC	1202.00	0.2397
Total		5014	
Pro	CCU	958.00	0.2626
Pro	CCC	1375.00	0.3769
Pro	CCA	850.00	0.2330
Pro	CCG	465.00	0.1275
Total		3648	
Thr	ACU	822.00	0.2127

Amino Acid	Codon	Number	Frequency of usage
Thr	ACC	1574.00	0.4072
Thr	ACA	903.00	0.2336
Thr	ACG	566.00	0.1464
Total		3865	
Ala	GCU	1129.00	0.2496
Ala	GCC	1951.00	0.4313
Ala	GCA	883.00	0.1952
Ala	GCG	561.00	0.1240
Total		4524	
Tyr	UAU	837.00	0.3779
Tyr	UAC	1378.00	0.6221
Total		2215	
His	CAU	594.00	0.3738
His	CAC	995.00	0.6262
Total		1589	
Gln	CAA	747.00	0.2783
Gln	CAG	1937.00	0.7217
Total		2684	
Asn	AAU	1109.00	0.3949
Asn	AAC	1699.00	0.6051
Total		2808	
Lys	AAA	1445.00	0.4088
Lys	AAG	2090.00	0.5912
Total		3535	
Asp	GAU	1255.00	0.4055
Asp	GAC	1840.00	0.5945
Total		3095	
Glu	GAA	1637.00	0.4164
Glu	GAG	2294.00	0.5836
Total		3931	
Cys	UGU	719.00	0.4425
Cys	UGC	906.00	0.5575
Total		1625	
Trp	UGG	1073.00	1.0000
Total		1073	
Arg	CGU	236.00	0.0700
Arg	CGC	629.00	0.1865

Amino Acid	Codon	Number	Frequency of usage
Arg	CGA	354.00	0.1050
Arg	CGG	662.00	0.1963
Arg	AGA	712.00	0.2112
Arg	AGG	779.00	0.2310
Total		3372	
Gly	GGU	648.00	0.1498
Gly	GGC	1536.00	0.3551
Gly	GGA	1065.00	0.2462
Gly	GGG	1077.00	0.2490
Total		4326	
Stop	UAA	55	
Stop	UAG	36	
Stop	UGA	110	

TABLE 7: Codon Usage Table for Cow Genes (*Bos taurus*)

Amino Acid	Codon	Number	Frequency of usage
Phe	UUU	13002	0.4112
Phe	UUC	18614	0.5888
Total		31616	
Leu	UUA	4467	0.0590
Leu	UUG	9024	0.1192
Leu	CUU	9069	0.1198
Leu	CUC	16003	0.2114
Leu	CUA	4608	0.0609
Leu	CUG	32536	0.4298
Total		75707	
Ile	AUU	12474	0.3313
Ile	AUC	19800	0.5258
Ile	AUA	5381	0.1429
Total		37655	
Met	AUG	17770	1.0000
Total		17770	
Val	GUU	8212	0.1635
Val	GUC	12846	0.2558
Val	GUA	4932	0.0982
Val	GUG	24222	0.4824
Total		50212	
Ser	UCU	10287	0.1804
Ser	UCC	13258	0.2325
Ser	UCA	7678	0.1347

Amino Acid	Codon	Number	Frequency of usage
Ser	UCG	3470	0.0609
Ser	AGU	8040	0.1410
Ser	AGC	14279	0.2505
Total		57012	
Pro	CCU	11695	0.2684
Pro	CCC	15221	0.3493
Pro	CCA	11039	0.2533
Pro	CCG	5621	0.1290
Total		43576	
Thr	ACU	9372	0.2203
Thr	ACC	16574	0.3895
Thr	ACA	10892	0.2560
Thr	ACG	5712	0.1342
Total		42550	
Ala	GCU	13923	0.2592
Ala	GCC	23073	0.4295
Ala	GCA	10704	0.1992
Ala	GCG	6025	0.1121
Total		53725	
Tyr	UAU	9441	0.3882
Tyr	UAC	14882	0.6118
Total		24323	
His	CAU	6528	0.3649
His	CAC	11363	0.6351
Total		17891	
Gln	CAA	8060	0.2430
Gln	CAG	25108	0.7570
Total		33168	
Asn	AAU	12491	0.4088
Asn	AAC	18063	0.5912
Total		30554	
Lys	AAA	17244	0.3897
Lys	AAG	27000	0.6103
Total		44244	
Asp	GAU	16615	0.4239
Asp	GAC	22580	0.5761
Total		39195	

Amino Acid	Codon	Number	Frequency of usage
Glu	GAA	21102	0.4007
Glu	GAG	31555	0.5993
Total		52657	
Cys	UGU	7556	0.4200
Cys	UGC	10436	0.5800
Total		17992	
Trp	UGG	10706	1.0000
Total		10706	
Arg	CGU	3391	0.0824
Arg	CGC	7998	0.1943
Arg	CGA	4558	0.1108
Arg	CGG	8300	0.2017
Arg	AGA	8237	0.2001
Arg	AGG	8671	0.2107
Total		41155	
Gly	GGU	8508	0.1616
Gly	GGC	18517	0.3518
Gly	GGA	12838	0.2439
Gly	GGG	12772	0.2427
Total		52635	
Stop	UAA	555	
Stop	UAG	394	
Stop	UGA	392	

[0117] By utilizing these or similar tables, one of ordinary skill in the art can apply the frequencies to any given polypeptide sequence, and produce a nucleic acid fragment of a codon-optimized coding region which encodes the polypeptide, but which uses codons more optimal for a given species. Codon-optimized coding regions can be designed by various different methods.

[0118] In one method, termed "uniform optimization," a codon usage table is used to find the single most frequent codon used for any given amino acid, and that codon is used each time that particular amino acid appears in the polypeptide sequence. For example, referring to Table 4 above, the most frequent codon for leucine in humans is CUG, which is used 41% of the time. Thus, all of the leucine residues in a given amino acid sequence would be assigned the codon CUG. A coding region for SARS-CoV soluble S protein

(SEQ ID NO:1) optimized by the "uniform optimization" method is presented herein as SEQ ID NO:25.

[0119] In another method, termed "full-optimization," the actual frequencies of the codons are distributed randomly throughout the coding region. Thus, using this method for optimization, if a hypothetical polypeptide sequence had 100 leucine residues, referring to Table 4 for frequency of usage in humans, about 7, or 7% of the leucine codons would be UUA, about 13, or 13% of the leucine codons would be UUG, about 13, or 13% of the leucine codons would be CUU, about 20, or 20% of the leucine codons would be CUC, about 7, or 7% of the leucine codons would be CUA, and about 41, or 41% of the leucine codons would be CUG. These frequencies would be distributed randomly throughout the leucine codons in the coding region encoding the hypothetical polypeptide. As will be understood by those of ordinary skill in the art, the distribution of codons in the sequence can vary significantly using this method, however, the sequence always encodes the same polypeptide.

[0120] As an example, a nucleotide sequence for soluble S (SEQ ID NO:1) fully optimized for human codon usage, is shown as SEQ ID NO:24.

[0121] In using the "full-optimization" method, an entire polypeptide sequence may be codon-optimized as described above. With respect to various desired fragments, variants, or derivatives of the complete polypeptide, the fragment, variant, or derivative may first be designed, and is then codon-optimized individually. Alternatively, a full-length polypeptide sequence is codon-optimized for a given species, resulting in a codon-optimized coding region encoding the entire polypeptide; then nucleic acid fragments of the codon-optimized coding region, which encode fragments, variants, and derivatives of the polypeptide, are made from the original codon-optimized coding region. As will be well understood by those of ordinary skill in the art, if codons have been randomly assigned to the full-length coding region based on their frequency of use in a given species, nucleic acid fragments encoding fragments, variants, and derivatives would not necessarily be fully codon-optimized for the given species. However, such sequences are

still much closer to the codon usage of the desired species than the native codon usage. The advantage of this approach is that synthesizing codon-optimized nucleic acid fragments encoding each fragment, variant, and derivative of a given polypeptide, although routine, would be time consuming and would result in significant expense.

[0122] When using the "full-optimization" method, the term "about" is used precisely to account for fractional percentages of codon frequencies for a given amino acid. As used herein, "about" is defined as one amino acid more or one amino acid less than the value given. The whole number value of amino acids is rounded up if the fractional frequency of usage is 0.50 or greater, and is rounded down if the fractional frequency of use is 0.49 or less. Using again the example of the frequency of usage of leucine in human genes, for a hypothetical polypeptide having 62 leucine residues, the fractional frequency of codon usage would be calculated by multiplying 62 by the frequencies for the various codons. Thus, 7.28 percent of 62 equals 4.51 UUA codons, or "about 5," *i.e.*, 4, 5, or 6 UUA codons, 12.66 percent of 62 equals 7.85 UUG codons or "about 8," *i.e.*, 7, 8, or 9 UUG codons, 12.87 percent of 62 equals 7.98 CUU codons, or "about 8," *i.e.*, 7, 8, or 9 CUU codons, 19.56 percent of 62 equals 12.13 CUC codons or "about 12," *i.e.*, 11, 12, or 13 CUC codons, 7.00 percent of 62 equals 4.34 CUA codons or "about 4," *i.e.*, 3, 4, or 5 CUA codons, and 40.62 percent of 62 equals 25.19 CUG codons, or "about 25," *i.e.*, 24, 25, or 26 CUG codons.

[0123] In a third method termed "minimal optimization," coding regions are only partially optimized. For example, the invention includes a nucleic acid fragment of a codon-optimized coding region encoding a polypeptide in which at least about 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the codon positions have been codon-optimized for a given species. That is, they contain a codon that is preferentially used in the genes of a desired species, *e.g.*, a vertebrate species, *e.g.*, humans, in place of a codon that is normally used in the native nucleic acid sequence. Codons that are rarely

found in the genes of the vertebrate of interest are changed to codons more commonly utilized in the coding regions of the vertebrate of interest.

[0124] Thus, those codons which are used more frequently in the SARS-CoV gene of interest than in genes of the vertebrate of interest are substituted with more frequently-used codons. The difference in frequency at which the SARS-CoV codons are substituted may vary based on a number factors as discussed below. For example, codons used at least twice more per thousand in SARS-CoV genes as compared to genes of the vertebrate of interest are substituted with the most frequently used codon for that amino acid in the vertebrate of interest. This ratio may be adjusted higher or lower depending on various factors such as those discussed below. Accordingly, a codon in a SARS-CoV native coding region would be substituted with a codon used more frequently for that amino acid in coding regions of the vertebrate of interest if the codon is used 1.1 times, 1.2 times, 1.3 times, 1.4 times, 1.5 times, 1.6 times, 1.7 times, 1.8 times, 1.9 times, 2.0 times, 2.1 times, 2.2 times, 2.3 times, 2.4 times, 2.5 times, 2.6 times, 2.7 times, 2.8 times, 2.9 times, 3.0 times, 3.1 times, 3.2 times, 3.3. times, 3.4 times, 3.5 times, 3.6 times. 3.7 times, 3.8 times, 3.9 times, 4.0 times, 4.1 times, 4.2 times, 4.3 times, 4.4 times, 4.5 times, 4.6 times, 4.7 times, 4.8 times, 4.9 times, 5.0 times, 5.5 times, 6.0 times, 6.5 times, 7.0 times, 7.5 times, 8.0 times, 8.5 times, 9.0 times, 9.5 times, 10.0 times, 10.5 times, 11.0 times, 11.5 times, 12.0 times, 12.5 times, 13.0 times, 13.5 times, 14.0 times, 14.5 times, 15.0 times, 15.5 times, 16.0 times, 16.5 times, 17.0 times, 17.5 times, 18.0 times, 18.5 times, 19.0 times, 19.5 times, 20 times, 21 times, 22 times, 23 times, 24 times, 25 times, or greater more frequently in SARS-CoV coding regions than in coding regions of the vertebrate of interest.

[0125] This minimal human codon optimization for highly variant codons has several advantages, which include but are not limited to the following examples. Since fewer changes are made to the nucleotide sequence of the gene of interest, fewer manipulations are required, which leads to reduced risk of introducing unwanted mutations and lower cost, as well as allowing the use

of commercially available site-directed mutagenesis kits, and reducing the need for expensive oligonucleotide synthesis. Further, decreasing the number of changes in the nucleotide sequence decreases the potential of altering the secondary structure of the sequence, which can have a significant impact on gene expression in certain host cells. The introduction of undesirable restriction sites is also reduced, facilitating the subcloning of the genes of interest into the plasmid expression vector.

[0126] In a fourth method, termed "standardized optimization," a Codon Usage Table (CUT) for the sequence to be optimized is generated and compared to the CUT for human genomic DNA (see, *e.g.*, Table 8 below). Codons are identified for which there is a difference of at least 10 percentage points in codon usage between human and query DNA. When such a codon is found, all of the wild type codons for that amino acid are modified to conform to predominant human codon.

[0127] The codon usage frequencies for all established SARS-CoV open reading frames (ORFs) is compared to the codon usage frequencies for humans in Table 8 below.

TABLE 8

SARS CoV Urbani Codon Frequencies using all established ORFs

Amino Acid	Codon	Urbani Number	Urbani Frequency of usage	Human Number	Human Frequency of usage
Phe	UUU	272	0.6154	326146	0.4525
Phe	UUC	170	0.3846	394680	0.5475
Total		442		720826	
Leu	UUA	150	0.1777	139249	0.0728
Leu	UUG	150	0.1777	242151	0.1266
Leu	CUU	254	0.3009	246206	0.1287
Leu	CUC	119	0.1410	374262	0.1956
Leu	CUA	90	0.1066	133980	0.0700
Leu	CUG	81	0.0960	777077	0.4062
Total		844		1912925	

Ile	AUU	262	0.5784	303721	0.3554
Ile	AUC	98	0.2163	414483	0.4850
Ile	AUA	93	0.2053	136399	0.1596
Total		453		854603	
Met	AUG	212	0.0005	430946	1.0000
Total		212		430946	
Val	GUU	299	0.4194	210423	0.1773
Val	GUC	126	0.1767	282445	0.2380
Val	GUA	152	0.2132	134991	0.1137
Val	GUG	136	0.1907	559044	0.4710
Total		713		1186903	
Ser	UCU	202	0.3328	282407	0.1840
Ser	UCC	41	0.0675	336349	0.2191
Ser	UCA	176	0.2900	225963	0.1472
Ser	UCG	20	0.0329	86761	0.0565
Ser	AGU	118	0.1944	230047	0.1499
Ser	AGC	50	0.0824	373362	0.2433
Total		607		1534889	
Pro	CCU	163	0.4405	333705	0.2834
Pro	CCC	38	0.1027	386462	0.3281
Pro	CCA	156	0.4216	322220	0.2736
Pro	CCG	13	0.0351	135317	0.1149
Total		370		1177704	
Thr	ACU	275	0.4264	247913	0.2419
Thr	ACC	86	0.1333	371420	0.3624
Thr	ACA	257	0.3985	285655	0.2787
Thr	ACG	27	0.0419	120022	0.1171
Total		645		1025010	

[0128] The present invention provides isolated polynucleotides comprising codon-optimized coding regions of SARS-CoV polypeptides, *e.g.*, S, S1, S2 N, E, or M, or fragments, variants, or derivatives thereof.

[0129] Additionally, a minimally codon-optimized nucleotide sequence can be designed by changing only certain codons found more frequently in SARS-

CoV genes than in human genes. For example, if it is desired to substitute more frequently used codons in humans for those codons that occur at least 2 times more frequently in SARS-CoV genes.

[0130] In another form of minimal optimization, a Codon Usage Table (CUT) for the specific SARS-CoV sequence in question is generated and compared to the CUT for human genomic DNA. Amino acids are identified for which there is a difference of at least 10 percentage points in codon usage between human and SARS-CoV DNA (either more or less). Then, the wild type SARS-CoV codon is modified to conform to the predominant human codon for each such amino acid. Furthermore, the remainder of codons for that amino acid are also modified such that they conform to the predominant human codon for each such amino acid.

[0131] In certain embodiments described herein, a codon-optimized coding region encoding SEQ ID NO:2 is optimized according to codon usage in humans (*Homo sapiens*). Alternatively, a codon-optimized coding region encoding SEQ ID NO:2 may be optimized according to codon usage in any plant, animal, or microbial species. Codon-optimized coding regions encoding SEQ ID NO:2, optimized according to codon usage in humans are designed as follows. The amino acid composition of SEQ ID NO:2 is shown in Table 9.

TABLE 9

AMINO ACID		Number in SEQ ID NO:2
A	Ala	81
R	Arg	39
C	Cys	30
G	Gly	74
H	His	14
I	Ile	74
L	Leu	92
K	Lys	56
M	Met	18
F	Phe	81
P	Pro	56
S	Ser	91
T	Thr	96
W	Trp	10

Y	Tyr	52
V	Val	86
N	Asn	81
D	Asp	70
Q	Gln	55
E	Glu	40

[0132] Using the amino acid composition shown in Table 9, a human codon-optimized coding region which encodes SEQ ID NO:2 can be designed by any of the methods discussed herein. For "uniform" optimization, each amino acid is assigned the most frequent codon used in the human genome for that amino acid. According to this method, codons are assigned to the coding region encoding SEQ ID NO:2 as follows: the 81 phenylalanine codons are TTC, the 92 leucine codons are CTG, the 74 isoleucine codons are ATC, the 18 methionine codons are ATG, the 86 valine codons are GTG, the 91 serine codons are AGC, the 56 proline codons are CCC, the 96 threonine codons are ACC, the 81 alanine codons are GCC, the 52 tyrosine codons are TAC, the 14 histidine codons are CAC, the 55 glutamine codons are CAG, the 81 asparagine codons are AAC, the 56 lysine codons are AAG, the 70 aspartic acid codons are GAC, the 40 glutamic acid codons are GAG, the 30 cysteine codons are TGC, the 10 tryptophan codon is TGG, the 39 arginine codons are CGG, AGA, or AGG (the frequencies of usage of these three codons in the human genome are not significantly different), and the 74 glycine codons are GGC. The codon-optimized coding region designed by this method is presented herein as SEQ ID NO:25.

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ATGTTCATCTTCCTGCTGTTCCTGACCCTG
ACCAGCGGCAGCGACCTGGACCGGTGCACC
ACCTTCGACGACGTGCAGGCCCCCAACTAC
ACCCAGCACACCAGCAGCATGCGGGGCGTG
TACTACCCCGACGAGATCTTCCGGAGCGAC
ACCCTGTACCTGACCCAGGACCTGTTCCTG
CCCTTCTACAGCAACGTGACCGGCTTCCAC
ACCATCAACCACACCTTCGGCAACCCCGTG
ATCCCCTTCAAGGACGGCATCTACTTCGCC
GCCACCGAGAAGAGCAACGTGGTGCGGGGC
TGGGTGTTCGGCAGCACCATGAACAACAAG
AGCCAGAGCGTGATCATCATCAACAACAGC
ACCAACGTGGTGATCCGGGCCTGCAACTTC
GAGCTGTGCGACAACCCCTTCTTCGCCGTG
AGCAAGCCCATGGGCACCCAGACCCACACC
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ATGATCTTCGACAACGCCTTCAACTGCACC
TTCGAGTACATCAGCGACGCCTTCAGCCTG
GACGTGAGCGAGAAGAGCGGCAACTTCAAG
CACCTGCGGGAGTTCGTGTTCAAGAACAAG
GACGGCTTCCTGTACGTGTACAAGGGCTAC
CAGCCCATCGACGTGGTGCGGGACCTGCCC
AGCGGCTTCAACACCCTGAAGCCCATCTTC
AAGCTGCCCCTGGGCATCAACATCACCAAC
TTCCGGGCCATCCTGACCGCCTTCAGCCCC
GCCCAGGACATCTGGGGCACCAGCGCCGCC
GCCTACTTCGTGGGCTACCTGAAGCCCACC
ACCTTCATGCTGAAGTACGACGAGAACGGC
ACCATCACCGACGCCGTGGACTGCAGCCAG
AACCCCCTGGCCGAGCTGAAGTGCAGCGTG
AAGAGCTTCGAGATCGACAAGGGCATCTAC
CAGACCAGCAACTTCCGGGTGGTGCCCAGC
GGCGACGTGGTGCGGTTCCCCAACATCACC
AACCTGTGCCCCTTCGGCGAGGTGTTCAAC
GCCACCAAGTTCCCCAGCGTGTACGCCTGG
GAGCGGAAGAAGATCAGCAACTGCGTGGCC
GACTACAGCGTGCTGTACAACAGCACCTTC
TTCAGCACCTTCAAGTGCTACGGCGTGAGC
GCCACCAAGCTGAACGACCTGTGCTTCAGC
AACGTGTACGCCGACAGCTTCGTGGTGAAG
GGCGACGACGTGCGGCAGATCGCCCCCGGC
CAGACCGGCGTGATCGCCGACTACAACTAC
AAGCTGCCCGACGACTTCATGGGCTGCGTG
CTGGCCTGGAACACCCGGAACATCGACGCC
ACCAGCACCGGCAACTACAACTACAAGTAC
CGGTACCTGCGGCACGGCAAGCTGCGGCCC
TTCGAGCGGGACATCAGCAACGTGCCCTTC
AGCCCCGACGGCAAGCCCTGCACCCCCCCC
GCCCTGAACTGCTACTGGCCCCTGAACGAC
TACGGCTTCTACACCACCACCGGCATCGGC
TACCAGCCCTACCGGGTGGTGGTGCTGAGC
TTCGAGCTGCTGAACGCCCCCGCCACCGTG
TGCGGCCCCAAGCTGAGCACCGACCTGATC
AAGAACCAGTGCGTGAACTTCAACTTCAAC
GGCCTGACCGGCACCGGCGTGCTGACCCCC
AGCAGCAAGCGGTTCCAGCCCTTCCAGCAG
TTCGGCCGGGACGTGAGCGACTTCACCGAC
AGCGTGCGGGACCCCAAGACCAGCGAGATC
CTGGACATCAGCCCCTGCAGCTTCGGCGGC
GTGAGCGTGATCACCCCCGGCACCAACGCC
AGCAGCGAGGTGGCCGTGCTGTACCAGGAC
GTGAACTGCACCGACGTGAGCACCGCCATC
CACGCCGACCAGCTGACCCCCGCCTGGCGG
ATCTACAGCACCGGCAACAACGTGTTCCAG
ACCCAGGCCGGCTGCCTGATCGGCGCCGAG
CACGTGGACACCAGCTACGAGTGCGACATC
CCCATCGGCGCCGGCATCTGCGCCAGCTAC
CACACCGTGAGCCTGCTGCGGAGCACCAGC
CAGAAGAGCATCGTGGCCTACACCATGAGC
CTGGGCGCCGACAGCAGCATCGCCTACAGC
AACAACACCATCGCCATCCCCACCAACTTC
AGCATCAGCATCACCACCGAGGTGATGCCC
GTGAGCATGGCCAAGACCAGCGTGGACTGC
AACATGTACATCTGCGGCGACAGCACCGAG
TGCGCCAACCTGCTGCTGCAGTACGGCAGC
TTCTGCACCCAGCTGAACCGGGCCCTGAGC
GGCATCGCCGCCGAGCAGGACCGGAACACC
CGGGAGGTGTTCGCCCAGGTGAAGCAGATG
TACAAGACCCCCACCCTGAAGTACTTCGGC

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GGCTTCAACTTCAGCCAGATCCTGCCCGAC
CCCCTGAAGCCCACCAAGCGGAGCTTCATC
GAGGACCTGCTGTTCAACAAGGTGACCCTG
GCCGACGCCGGCTTCATGAAGCAGTACGGC
GAGTGCCTGGGCGACATCAACGCCCGGGAC
CTGATCTGCGCCCAGAAGTTCAACGGCCTG
ACCGTGCTGCCCCCCCTGCTGACCGACGAC
ATGATCGCCGCCTACACCGCCGCCCTGGTG
AGCGGCACCGCCACCGCCGGCTGGACCTTC
GGCGCCGGCGCCGCCCTGCAGATCCCCTTC
GCCATGCAGATGGCCTACCGGTTCAACGGC
ATCGGCGTGACCCAGAACGTGCTGTACGAG
AACCAGAAGCAGATCGCCAACCAGTTCAAC
AAGGCCATCAGCCAGATCCAGGAGAGCCTG
ACCACCACCAGCACCGCCCTGGGCAAGCTG
CAGGACGTGGTGAACCAGAACGCCCAGGCC
CTGAACACCCTGGTGAAGCAGCTGAGCAGC
AACTTCGGCGCCATCAGCAGCGTGCTGAAC
GACATCCTGAGCCGGCTGGACAAGGTGGAG
GCCGAGGTGCAGATCGACCGGCTGATCACC
GGCCGGCTGCAGAGCCTGCAGACCTACGTG
ACCCAGCAGCTGATCCGGGCCGCCGAGATC
CGGGCCAGCGCCAACCTGGCCGCCACCAAG
ATGAGCGAGTGCGTGCTGGGCCAGAGCAAG
CGGGTGGACTTCTGCGGCAAGGGCTACCAC
CTGATGAGCTTCCCCCAGGCCGCCCCCCAC
GGCGTGGTGTTCCTGCACGTGACCTACGTG
CCCAGCCAGGAGCGGAACTTCACCACCGCC
CCCGCCATCTGCCACGAGGGCAAGGCCTAC
TTCCCCCGGGAGGGCGTGTTCGTGTTCAAC
GGCACCAGCTGGTTCATCACCCAGCGGAAC
TTCTTCAGCCCCCAGATCATCACCACCGAC
AACACCTTCGTGAGCGGCAACTGCGACGTG
GTGATCGGCATCATCAACAACACCGTGTAC
GACCCCCTGCAGCCCGAGCTGGACAGCTTC
AAGGAGGAGCTGGACAAGTACTTCAAGAAC
CACACCAGCCCCGACGTGGACCTGGGCGAC
ATCAGCGGCATCAACGCCAGCGTGGTGAAC
ATCCAGAAGGAGATCGACCGGCTGAACGAG
GTGGCCAAGAACCTGAACGAGAGCCTGATC
GACCTGCAGGAGCTGGGCAAGTACGAGCAG
TACATCAAGTGGCCCTGG
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[0133] Alternatively, a human codon-optimized coding region which encodes SEQ ID NO:2 can be designed by the "full optimization" method, where each amino acid is assigned codons based on the frequency of usage in the human genome. These frequencies are shown in Table 4 above. Using this latter method, codons are assigned to the coding region encoding SEQ ID NO:2 as follows: about 37 of the 81 phenylalanine codons are TTT, and about 44 of the phenylalanine codons are TTC; about 7 of the 92 leucine codons are TTA, about 12 of the leucine codons are TTG, about 12 of the leucine codons are CTT, about 18 of the leucine codons are CTC, about 7 of the leucine codons

are CTA, and about 36 of the leucine codons are CTG; about 26 of the 74 isoleucine codons are ATT, about 35 of the isoleucine codons are ATC, and about 13 of the isoleucine codons are ATA; the 18 methionine codons are ATG; about 15 of the 86 valine codons are GTT, about 40 of the valine codons are GTG, about 10 of the valine codons are GTA, and about 21 of the valine codons are GTC; about 17 of the 91 serine codons are TCT, about 20 of the serine codons are TCC, about 14 of the serine codons are TCA, about 5 of the serine codons are TCG, about 13 of the serine codons are AGT, and about 22 of the serine codons are AGC; about 16 of the 56 proline codons are CCT, about 18 of the proline codons are CCC, about 16 of the proline codons are CCA, and about 6 of the proline codons are CCG; about 23 of the 96 threonine codons are ACT, about 35 of the threonine codons are ACC, about 27 of the threonine codons are ACA, and about 11 of the threonine codons are ACG; about 21 of the 81 alanine codons are GCT, about 33 of the alanine codons are GCC, about 18 of the alanine codons are GCA, and about 9 of the alanine codons are GCG; about 23 of the 52 tyrosine codons are TAT and about 29 of the tyrosine codons are TAC; about 6 of the 14 histidine codons are CAT and about 8 of the histidine codons are CAC; about 14 of the 55 glutamine codons are CAA and about 41 of the glutamine codons are CAG; about 37 of the 81 asparagine codons are AAT and about 44 of the asparagine codons are AAC; about 24 of the 56 lysine codons are AAA and about 32 of the lysine codons are AAG; about 32 of the 70 aspartic acid codons are GAT and about 38 of the aspartic acid codons are GAC; about 17 of the 40 glutamic acid codons are GAA and about 23 of the glutamic acid codons are GAG; about 14 of the 30 cysteine codons are TGT and about 16 of the cysteine codons are TGC; the 10 tryptophan codons are TGG; about 3 of the 39 arginine codons are CGT, about 7 of the arginine codons are CGC, about 4 of the arginine codons are CGA, about 8 of the arginine codons are CGG, about 9 of the arginine codons are AGA, and about 8 of the arginine codons are AGG; and about 12 of the 74 glycine codons are GGT, about 25 of the glycine codons are GGC, about 19 of the glycine codons are GGA, and about 18 of the glycine codons are GGG.

[0134] As described above, the term "about" means that the number of amino acids encoded by a certain codon may be one more or one less than the number given. It would be understood by those of ordinary skill in the art that the total number of any amino acid in the polypeptide sequence must remain constant, therefore, if there is one "more" of one codon encoding a give amino acid, there would have to be one "less" of another codon encoding that same amino acid.

[0135] A representative "fully optimized" codon-optimized coding region encoding SEQ ID NO:2, optimized according to codon usage in humans is presented herein as SEQ ID NO:24.

```
ATG TTT ATC TTC CTC CTC TTC CTG ACG CTC
ACT AGC GGA TCC GAC TTA GAT CGG TGT ACC
ACT TTC GAC GAC GTC CAG GCC CCT AAC TAT
ACT CAA CAT ACC TCC AGT ATG CGC GGG GTG
TAC TAT CCA GAT GAG ATT TTT CGG AGC GAC
ACT CTG TAC TTA ACA CAG GAC CTG TTT CTA
CCG TTT TAT TCA AAT GTA ACC GGC TTC CAC
ACC ATT AAC CAT ACA TTT GGC AAT CCC GTG
ATA CCA TTC AAA GAC GGC ATT TAC TTC GCC
GCA ACA GAA AAG AGC AAT GTT GTG AGG GGG
TGG GTC TTC GGC TCC ACA ATG AAC AAT AAA
TCT CAG TCT GTC ATC ATC ATC AAT AAC AGC
ACT AAC GTG GTA ATC CGT GCC TGC AAT TTC
GAG CTT TGT GAC AAC CCA TTC TTC GCC GTG
TCT AAG CCT ATG GGC ACC CAG ACT CAC ACA
ATG ATC TTT GAC AAT GCT TTC AAC TGC ACC
TTC GAA TAC ATA TCA GAT GCA TTC TCT TTG
GAT GTC AGT GAA AAG TCT GGA AAC TTT AAA
CAT CTG AGA GAG TTT GTC TTC AAA AAC AAG
GAC GGC TTT CTC TAC GTT TAC AAG GGT TAT
CAG CCC ATT GAT GTG GTG CGG GAC CTC CCT
TCA GGG TTT AAC ACA TTG AAA CCA ATA TTC
AAA CTG CCC CTG GGT ATC AAT ATT ACT AAC
TTT CGA GCC ATC TTG ACC GCC TTT TCC CCC
GCG CAA GAC ATA TGG GGA ACC AGC GCG GCA
GCC TAT TTC GTC GGT TAT CTG AAG CCC ACT
ACA TTT ATG CTG AAG TAC GAC GAG AAC GGA
ACC ATT ACC GAT GCT GTC GAT TGT TCA CAG
AAT CCA CTG GCT GAA TTG AAA TGC TCC GTG
AAG AGC TTT GAG ATC GAT AAG GGG ATT TAC
CAG ACG TCT AAT TTT CGA GTG GTT CCC TCA
GGA GAT GTG GTT AGA TTC CCC AAT ATC ACA
AAT TTG TGC CCC TTC GGT GAA GTG TTC AAT
GCC ACA AAG TTC CCG TCT GTC TAC GCT TGG
GAG CGG AAA AAG ATA AGC AAC TGT GTC GCG
GAT TAC AGT GTC CTA TAT AAC TCG ACC TTT
TTT AGC ACG TTC AAG TGT TAC GGG GTG AGT
GCT ACT AAA CTG AAT GAT TTA TGT TTT AGT
AAC GTT TAT GCA GAC TCC TTT GTT GTA AAG
GGT GAT GAC GTG CGC CAA ATT GCA CCT GGG
CAG ACC GGA GTG ATC GCA GAT TAT AAC TAC
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AAA CTT CCA GAC GAC TTT ATG GGA TGC GTG
CTC GCC TGG AAC ACT CGC AAC ATC GAC GCA
ACC AGC ACC GGG AAC TAT AAT TAC AAA TAC
AGA TAC CTC AGG CAC GGC AAG CTG CGG CCT
TTT GAG CGG GAT ATC TCA AAC GTC CCA TTT
AGC CCG GAC GGC AAG CCC TGT ACT CCT CCC
GCA CTT AAC TGT TAC TGG CCA CTG AAC GAT
TAT GGC TTT TAT ACC ACA ACC GGC ATC GGC
TAC CAG CCC TAC CGG GTG GTG GTG CTA TCT
TTC GAG CTG CTG AAC GCG CCT GCC ACC GTA
TGT GGG CCC AAG CTT TCG ACA GAT CTC ATC
AAG AAC CAA TGC GTA AAT TTC AAT TTC AAT
GGC CTT ACA GGA ACC GGT GTG CTG ACA CCC
TCC TCC AAG AGG TTT CAA CCT TTC CAG CAG
TTT GGA CGT GAC GTC TCA GAC TTT ACT GAC
AGT GTG AGG GAT CCT AAG ACC TCT GAA ATC
CTG GAT ATA TCT CCC TGT TCC TTC GGT GGG
GTT AGT GTG ATA ACC CCT GGG ACA AAT GCT
AGT TCC GAA GTG GCC GTA CTC TAT CAA GAC
GTG AAC TGC ACA GAC GTG TCA ACC GCC ATC
CAC GCT GAT CAA CTC ACA CCG GCT TGG CGG
ATC TAT AGC ACT GGC AAT AAC GTG TTC CAA
ACG CAG GCC GGC TGC CTT ATA GGG GCA GAG
CAT GTC GAC ACT TCT TAC GAG TGT GAT ATA
CCA ATC GGA GCC GGC ATC TGC GCC TCA TAC
CAC ACG GTG AGC TTG CTG CGC TCC ACC AGT
CAG AAG AGT ATT GTC GCA TAC ACC ATG TCA
CTC GGC GCA GAT TCA AGT ATC GCC TAC AGC
AAT AAC ACT ATC GCT ATT CCT ACC AAC TTT
TCC ATT TCC ATC ACA ACT GAG GTT ATG CCT
GTC TCC ATG GCT AAG ACT TCC GTG GAC TGC
AAT ATG TAC ATT TGT GGG GAC TCT ACC GAG
TGC GCT AAC CTT TTA CTG CAG TAT GGC TCC
TTC TGC ACA CAG CTG AAT AGA GCC CTG AGC
GGA ATT GCC GCT GAG CAG GAT AGA AAT ACG
AGA GAA GTG TTT GCC CAG GTG AAA CAG ATG
TAT AAG ACT CCA ACC TTG AAG TAT TTC GGA
GGG TTC AAT TTT AGC CAG ATC CTT CCT GAC
CCC TTG AAG CCG ACC AAA AGG AGC TTC ATC
GAA GAT CTT CTG TTC AAC AAA GTT ACT TTA
GCG GAC GCC GGG TTC ATG AAA CAG TAT GGC
GAG TGT CTC GGG GAT ATT AAT GCC CGC GAT
CTC ATC TGT GCT CAG AAA TTC AAC GGC CTC
ACA GTG CTC CCC CCA CTT CTG ACG GAT GAT
ATG ATC GCC GCT TAC ACA GCC GCA CTC GTG
AGC GGC ACC GCC ACA GCC GGT TGG ACA TTC
GGA GCT GGA GCC GCA TTA CAG ATT CCA TTC
GCT ATG CAG ATG GCG TAC AGG TTC AAC GGA
ATA GGC GTG ACC CAG AAC GTG TTG TAT GAA
AAT CAG AAG CAG ATT GCG AAC CAG TTC AAC
AAA GCC ATT TCT CAA ATC CAG GAG TCC CTG
ACC ACC ACA AGC ACG GCA CTG GGA AAG CTG
CAA GAC GTG GTC AAC CAG AAC GCC CAA GCC
CTA AAT ACC CTG GTT AAG CAG CTG TCT AGC
AAT TTT GGA GCG ATT TCA TCT GTC CTT AAC
GAT ATA CTA TCA AGA CTG GAC AAA GTG GAG
GCA GAG GTC CAA ATC GAC CGC CTG ATT ACG
GGC CGC CTC CAG AGC CTT CAG ACG TAT GTG
ACA CAG CAG CTG ATA AGA GCT GCT GAA ATA
CGA GCC TCG GCT AAT CTG GCC GCA ACC AAA
ATG TCC GAA TGC GTC CTG GGG CAG TCC AAA
CGT GTC GAT TTC TGC GGC AAA GGT TAC CAT
TTG ATG TCA TTT CCA CAG GCG GCT CCT CAC

GGC GTA GTG TTT CTG CAC GTG ACT TAT GTA
CCT TCG CAG GAA AGG AAC TTC ACA ACT GCC
CCA GCC ATC TGC CAT GAG GGA AAA GCA TAT
TTC CCC CGA GAA GGT GTT TTC GTT TTC AAC
GGG ACA AGC TGG TTC ATT ACT CAA AGG AAT
TTT TTT TCG CCA CAG ATC ATT ACC ACT GAT
AAC ACA TTT GTA TCT GGT AAC TGC GAC GTA
GTT ATC GGG ATT ATC AAT AAT ACG GTC TAT
GAC CCC TTG CAA CCT GAG CTG GAT AGC TTT
AAG GAA GAG CTG GAC AAG TAC TTT AAG AAT
CAC ACC TCT CCA GAC GTG GAC CTG GGA GAC
ATC TCC GGC ATT AAT GCA AGT GTT GTG AAT
ATT CAG AAA GAG ATT GAT AGA CTA AAC GAA
GTT GCT AAG AAC TTG AAT GAG AGT TTA ATT
GAC CTA CAG GAG CTC GGT AAG TAC GAA CAG
TAC ATC AAA TGG CCG TGG

[0136] Another representative codon-optimized coding region encoding SEQ ID NO:2 is presented herein as SEQ ID NO: 44.

ATG TTT ATC TTC CTG CTG TTT CTG ACA CTG
ACA AGC GGC AGT GAC CTG GAT AGA TGC ACA
ACG TTT GAC GAC GTG CAG GCC CCC AAC TAC
ACC CAG CAT ACA TCC AGC ATG AGG GGC GTT
TAC TAC CCC GAT GAG ATC TTT AGA AGT GAT
ACT CTG TAT CTG ACT CAG GAC CTG TTT CTG
CCC TTC TAT TCT AAC GTT ACT GGC TTC CAT
ACA ATC AAC CAC ACC TTC GGC AAC CCC GTA
ATA CCC TTT AAG GAT GGC ATC TAC TTT GCC
GCC ACC GAG AAG TCT AAC GTA GTG AGA GGC
TGG GTG TTC GGC AGT ACT ATG AAC AAC AAG
TCT CAG TCT GTG ATA ATA ATC AAC AAC TCC
ACT AAC GTC GTC ATC AGA GCC TGT AAC TTC
GAG CTG TGC GAT AAC CCC TTC TTC GCC GTT
TCG AAG CCC ATG GGC ACT CAG ACC CAT ACA
ATG ATC TTT GAT AAC GCC TTC AAC TGC ACC
TTT GAG TAT ATC TCT GAT GCC TTC AGT CTG
GAT GTG TCC GAG AAG TCA GGC AAC TTC AAG
CAT CTG AGA GAG TTT GTG TTC AAG AAC AAG
GAT GGC TTT CTG TAC GTC TAC AAG GGC TAC
CAG CCC ATA GAT GTG GTA CGT GAC CTG CCC
AGC GGC TTC AAC ACT CTG AAG CCC ATA TTC
AAG CTG CCC CTG GGC ATA AAC ATT ACC AAC
TTT AGA GCC ATT CTG ACG GCC TTC TCC CCC
GCC CAG GAT ATC TGG GGC ACA AGT GCC GCC
GCC TAC TTC GTG GGC TAC CTG AAG CCC ACA
ACT TTT ATG CTG AAG TAC GAC GAG AAC GGC
ACC ATA ACA GAT GCC GTG GAC TGT TCT CAG
AAC CCC CTG GCC GAG CTG AAG TGC TCA GTT
AAG AGT TTT GAG ATA GAT AAG GGC ATC TAT
CAG ACA AGC AAC TTC CGC GTG GTC CCC AGC
GGC GAT GTG GTG AGG TTT CCC AAC ATT ACC
AAC CTG TGC CCC TTC GGC GAG GTA TTC AAC
GCC ACA AAG TTC CCC TCC GTT TAC GCC TGG
GAG AGG AAG AAG ATT TCA AAC TGC GTG GCC
GAC TAC TCG GTG CTG TAT AAC TCT ACT TTC
TTC AGT ACC TTT AAG TGC TAC GGC GTG TCT
GCC ACA AAG CTG AAC GAT CTG TGC TTT AGC
AAC GTG TAT GCC GAT AGC TTC GTC GTC AAG

GGC GAC GAC GTC AGA CAG ATC GCC CCC GGC
CAG ACA GGC GTC ATC GCC GAC TAC AAC TAC
AAG CTG CCC GAC GAT TTC ATG GGC TGC GTG
CTG GCC TGG AAC ACG AGG AAC ATA GAT GCC
ACC AGC ACT GGC AAC TAC AAC TAC AAG TAC
AGA TAT CTG CGG CAC GGC AAG CTG AGG CCC
TTC GAG AGA GAC ATC TCT AAC GTT CCC TTT
TCC CCC GAT GGC AAG CCC TGC ACT CCC CCC
GCC CTG AAC TGC TAC TGG CCC CTG AAC GAC
TAT GGC TTC TAC ACC ACA ACT GGC ATC GGC
TAT CAG CCC TAC CGC GTA GTC GTG CTG TCG
TTC GAG CTG CTG AAC GCC CCC GCC ACA GTC
TGC GGC CCC AAG CTG TCC ACT GAC CTG ATT
AAG AAC CAG TGT GTG AAC TTC AAC TTT AAC
GGC CTG ACT GGC ACC GGC GTG CTG ACA CCC
AGC AGC AAG CGG TTC CAG CCC TTC CAG CAG
TTT GGC AGA GAC GTG TCT GAT TTC ACA GAT
TCC GTG AGA GAT CCC AAG ACT TCC GAG ATA
CTG GAT ATC AGT CCC TGC TCC TTC GGC GGC
GTG TCA GTT ATT ACA CCC GGC ACT AAC GCC
TCG TCC GAG GTA GCC GTT CTG TAT CAG GAC
GTG AAC TGC ACT GAT GTG AGT ACA GCC ATC
CAC GCC GAC CAG CTG ACC CCC GCC TGG CGG
ATT TAT AGT ACG GGC AAC AAC GTC TTT CAG
ACT CAG GCC GGC TGC CTG ATC GGC GCC GAG
CAT GTA GAT ACG TCT TAT GAG TGC GAC ATC
CCC ATC GGC GCC GGC ATC TGC GCC AGC TAT
CAC ACC GTT TCT CTG CTG CGA AGT ACT TCT
CAG AAG TCT ATA GTG GCC TAC ACC ATG TCT
CTG GGC GCC GAT AGC TCT ATC GCC TAT AGC
AAC AAC ACT ATA GCC ATC CCC ACA AAC TTC
TCT ATT TCT ATC ACT ACA GAG GTG ATG CCC
GTC TCC ATG GCC AAG ACC AGC GTT GAT TGC
AAC ATG TAC ATC TGC GGC GAT AGT ACA GAG
TGC GCC AAC CTG CTG CTG CAG TAT GGC AGC
TTC TGC ACC CAG CTG AAC AGA GCC CTG TCT
GGC ATC GCC GCC GAG CAG GAT AGG AAC ACA
AGA GAG GTT TTC GCC CAG GTT AAG CAG ATG
TAC AAG ACT CCC ACT CTG AAG TAC TTT GGC
GGC TTT AAC TTT TCT CAG ATT CTG CCC GAT
CCC CTG AAG CCC ACT AAG AGG AGT TTC ATA
GAG GAC CTG CTG TTC AAC AAG GTG ACT CTG
GCC GAC GCC GGC TTT ATG AAG CAG TAC GGC
GAG TGC CTG GGC GAT ATC AAC GCC AGA GAC
CTG ATC TGT GCC CAG AAG TTT AAC GGC CTG
ACA GTA CTG CCC CCC CTG CTG ACT GAT GAC
ATG ATT GCC GCC TAT ACG GCC GCC CTG GTG
TCT GGC ACT GCC ACC GCC GGC TGG ACC TTT
GGC GCC GGC GCC GCC CTG CAG ATA CCC TTT
GCC ATG CAG ATG GCC TAC CGA TTC AAC GGC
ATA GGC GTA ACC CAG AAC GTT CTG TAT GAG
AAC CAG AAG CAG ATA GCC AAC CAG TTC AAC
AAG GCC ATC TCT CAG ATT CAG GAG TCT CTG
ACC ACT ACA TCT ACT GCC CTG GGC AAG CTG
CAG GAC GTA GTG AAC CAG AAC GCC CAG GCC
CTG AAC ACC CTG GTT AAG CAG CTG TCA AGT
AAC TTC GGC GCC ATC TCT AGC GTT CTG AAC
GAT ATA CTG AGT CGG CTG GAT AAG GTG GAG
GCC GAG GTG CAG ATT GAC AGA CTG ATC ACA
GGC AGA CTG CAG TCT CTG CAG ACA TAT GTT
ACT CAG CAG CTG ATA AGG GCC GCC GAG ATT
AGA GCC AGT GCC AAC CTG GCC GCC ACT AAG
ATG TCC GAG TGC GTC CTG GGC CAG AGT AAG

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AGG GTA GAC TTT TGT GGC AAG GGC TAT CAC
CTG ATG TCC TTC CCC CAG GCC GCC CCC CAC
GGC GTC GTG TTT CTG CAT GTC ACT TAT GTT
CCC TCA CAG GAG AGG AAC TTC ACG ACC GCC
CCC GCC ATC TGC CAC GAG GGC AAG GCC TAT
TTC CCC AGG GAG GGC GTC TTC GTA TTC AAC
GGC ACG AGT TGG TTC ATC ACC CAG CGA AAC
TTC TTT TCG CCC CAG ATA ATT ACA ACG GAC
AAC ACT TTT GTA AGT GGC AAC TGC GAT GTC
GTC ATC GGC ATA ATC AAC AAC ACG GTT TAC
GAC CCC CTG CAG CCC GAG CTG GAT TCA TTC
AAG GAG GAG CTG GAC AAG TAC TTC AAG AAC
CAT ACT AGC CCC GAC GTT GAT CTG GGC GAC
ATA AGC GGC ATC AAC GCC AGT GTA GTC AAC
ATA CAG AAG GAG ATC GAT AGA CTG AAC GAG
GTG GCC AAG AAC CTG AAC GAG TCT CTG ATA
GAC CTG CAG GAG CTG GGC AAG TAC GAG CAG
TAC ATC AAG TGG CCC TGG
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[0137] A representative codon-optimized coding region encoding SEQ ID NO:2 according to the "standardized optimization" method is presented herein as SEQ ID NO: 67.

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ATG TTC ATC TTC CTG CTG TTC CTG ACC CTG
ACC AGC GGC AGC GAC CTG GAT CGC TGC ACC
ACC TTC GAT GAC GTG CAG GCC CCC AAC TAC
ACC CAG CAT ACC AGC AGC ATG CGC GGC GTG
TAC TAC CCC GAT GAG ATC TTC CGC AGC GAC
ACC CTG TAC CTG ACC CAG GAC CTG TTC CTG
CCC TTC TAC AGC AAC GTG ACC GGC TTC CAC
ACC ATC AAC CAT ACC TTC GGC AAC CCC GTG
ATC CCC TTC AAG GAC GGC ATC TAC TTC GCC
GCC ACC GAG AAG AGC AAC GTG GTG CGC GGC
TGG GTG TTC GGC AGC ACC ATG AAC AAC AAG
AGC CAG AGC GTG ATC ATC ATC AAC AAC AGC
ACC AAC GTG GTG ATC CGC GCC TGC AAC TTC
GAG CTG TGC GAC AAC CCC TTC TTC GCC GTG
AGC AAG CCC ATG GGC ACC CAG ACC CAT ACC
ATG ATC TTC GAT AAC GCC TTC AAC TGC ACC
TTC GAG TAC ATC AGC GAC GCC TTC AGC CTG
GAC GTG AGC GAG AAG AGC GGC AAC TTC AAG
CAT CTG CGC GAG TTC GTG TTC AAG AAC AAG
GAT GGC TTC CTG TAC GTG TAC AAG GGC TAC
CAG CCC ATC GAC GTG GTG CGC GAT CTG CCC
AGC GGC TTC AAC ACC CTG AAG CCC ATC TTC
AAG CTG CCC CTG GGC ATC AAC ATC ACC AAC
TTC CGC GCC ATC CTG ACC GCC TTC AGC CCC
GCC CAG GAC ATC TGG GGC ACC AGC GCC GCC
GCC TAC TTC GTG GGC TAC CTG AAG CCC ACC
ACC TTC ATG CTG AAG TAC GAT GAG AAC GGC
ACC ATC ACC GAC GCC GTG GAC TGC AGC CAG
AAC CCC CTG GCC GAG CTG AAG TGC AGC GTG
AAG AGC TTC GAG ATC GAT AAG GGC ATC TAC
CAG ACC AGC AAC TTC CGC GTG GTG CCC AGC
GGC GAC GTG GTG CGC TTC CCC AAC ATC ACC
AAC CTG TGT CCC TTC GGC GAG GTG TTC AAC
GCC ACC AAG TTC CCC AGC GTG TAC GCC TGG
GAG CGC AAG AAG ATC AGC AAC TGC GTG GCC
GAC TAC AGC GTG CTG TAC AAC AGC ACC TTC
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TTC AGC ACC TTC AAG TGC TAC GGC GTG AGC
GCC ACC AAG CTG AAC GAT CTG TGC TTC AGC
AAC GTG TAC GCC GAC AGC TTC GTG GTG AAG
GGC GAT GAT GTG CGC CAG ATC GCC CCC GGC
CAG ACC GGC GTG ATC GCC GAT TAC AAC TAC
AAG CTG CCC GAC GAC TTC ATG GGC TGC GTG
CTG GCC TGG AAC ACC CGC AAC ATC GAC GCC
ACC AGC ACC GGC AAC TAC AAC TAC AAG TAC
CGC TAC CTG CGC CAT GGC AAG CTG CGC CCC
TTC GAG CGC GAT ATC AGC AAC GTG CCC TTC
AGC CCC GAT GGC AAG CCC TGC ACC CCC CCC
GCC CTG AAC TGT TAC TGG CCC CTG AAC GAC
TAC GGC TTC TAC ACC ACC ACC GGC ATC GGC
TAC CAG CCC TAC CGC GTG GTG GTG CTG AGC
TTC GAG CTG CTG AAC GCC CCC GCC ACC GTG
TGC GGC CCC AAG CTG AGC ACC GAC CTG ATC
AAG AAC CAG TGC GTG AAC TTC AAC TTC AAC
GGC CTG ACC GGC ACC GGC GTG CTG ACC CCC
AGC AGC AAG CGC TTC CAG CCC TTC CAG CAG
TTC GGC CGC GAT GTG AGC GAC TTC ACC GAT
AGC GTG CGC GAC CCC AAG ACC AGC GAG ATC
CTG GAT ATC AGC CCC TGC AGC TTC GGC GGC
GTG AGC GTG ATC ACC CCC GGC ACC AAC GCC
AGC AGC GAG GTG GCC GTG CTG TAC CAG GAT
GTG AAC TGT ACC GAT GTG AGC ACC GCC ATC
CAC GCC GAT CAG CTG ACC CCC GCC TGG CGC
ATC TAC AGC ACC GGC AAC AAC GTG TTC CAG
ACC CAG GCC GGC TGC CTG ATC GGC GCC GAG
CAT GTG GAC ACC AGC TAC GAG TGT GAC ATC
CCC ATC GGC GCC GGC ATC TGT GCC AGC TAC
CAC ACC GTG AGC CTG CTG CGC AGC ACC AGC
CAG AAG AGC ATC GTG GCC TAC ACC ATG AGC
CTG GGC GCC GAT AGC AGC ATC GCC TAC AGC
AAC AAC ACC ATC GCC ATC CCC ACC AAC TTC
AGC ATC AGC ATC ACC ACC GAG GTG ATG CCC
GTG AGC ATG GCC AAG ACC AGC GTG GAC TGC
AAC ATG TAC ATC TGC GGC GAT AGC ACC GAG
TGC GCC AAC CTG CTG CTG CAG TAC GGC AGC
TTC TGC ACC CAG CTG AAC CGC GCC CTG AGC
GGC ATC GCC GCC GAG CAG GAT CGC AAC ACC
CGC GAG GTG TTC GCC CAG GTG AAG CAG ATG
TAC AAG ACC CCC ACC CTG AAG TAC TTC GGC
GGC TTC AAC TTC AGC CAG ATC CTG CCC GAT
CCC CTG AAG CCC ACC AAG CGC AGC TTC ATC
GAG GAT CTG CTG TTC AAC AAG GTG ACC CTG
GCC GAT GCC GGC TTC ATG AAG CAG TAC GGC
GAG TGC CTG GGC GAT ATC AAC GCC CGC GAT
CTG ATC TGC GCC CAG AAG TTC AAC GGC CTG
ACC GTG CTG CCC CCC CTG CTG ACC GAC GAC
ATG ATC GCC GCC TAC ACC GCC GCC CTG GTG
AGC GGC ACC GCC ACC GCC GGC TGG ACC TTC
GGC GCC GGC GCC GCC CTG CAG ATC CCC TTC
GCC ATG CAG ATG GCC TAC CGC TTC AAC GGC
ATC GGC GTG ACC CAG AAC GTG CTG TAC GAG
AAC CAG AAG CAG ATC GCC AAC CAG TTC AAC
AAG GCC ATC AGC CAG ATC CAG GAG AGC CTG
ACC ACC ACC AGC ACC GCC CTG GGC AAG CTG
CAG GAC GTG GTG AAC CAG AAC GCC CAG GCC
CTG AAC ACC CTG GTG AAG CAG CTG AGC AGC
AAC TTC GGC GCC ATC AGC AGC GTG CTG AAC
GAC ATC CTG AGC CGC CTG GAT AAG GTG GAG
GCC GAG GTG CAG ATC GAT CGC CTG ATC ACC
GGC CGC CTG CAG AGC CTG CAG ACC TAC GTG

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ACC CAG CAG CTG ATC CGC GCC GCC GAG ATC
CGC GCC AGC GCC AAC CTG GCC GCC ACC AAG
ATG AGC GAG TGC GTG CTG GGC CAG AGC AAG
CGC GTG GAT TTC TGC GGC AAG GGC TAC CAC
CTG ATG AGC TTC CCC CAG GCC GCC CCC CAT
GGC GTG GTG TTC CTG CAC GTG ACC TAC GTG
CCC AGC CAG GAG CGC AAC TTC ACC ACC GCC
CCC GCC ATC TGC CAC GAG GGC AAG GCC TAC
TTC CCC CGC GAG GGC GTG TTC GTG TTC AAC
GGC ACC AGC TGG TTC ATC ACC CAG CGC AAC
TTC TTC AGC CCC CAG ATC ATC ACC ACC GAT
AAC ACC TTC GTG AGC GGC AAC TGC GAT GTG
GTG ATC GGC ATC ATC AAC AAC ACC GTG TAC
GAT CCC CTG CAG CCC GAG CTG GAC AGC TTC
AAG GAG GAG CTG GAT AAG TAC TTC AAG AAC
CAC ACC AGC CCC GAC GTG GAT CTG GGC GAT
ATC AGC GGC ATC AAC GCC AGC GTG GTG AAC
ATC CAG AAG GAG ATC GAT CGC CTG AAC GAG
GTG GCC AAG AAC CTG AAC GAG AGC CTG ATC
GAC CTG CAG GAG CTG GGC AAG TAC GAG CAG
TAC ATC AAG TGG CCC TGG
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[0138] In certain embodiments described herein, a codon-optimized coding region encoding SEQ ID NO:4 is optimized according to codon usage in humans (*Homo sapiens*). Alternatively, a codon-optimized coding region encoding SEQ ID NO:4 may be optimized according to codon usage in any plant, animal, or microbial species. Codon-optimized coding regions encoding SEQ ID NO:4, optimized according to codon usage in humans are designed as follows. The amino acid composition of SEQ ID NO:4 is shown in Table 10.

TABLE 10

AMINO ACID		Number in SEQ ID NO:4
A	Ala	38
R	Arg	23
C	Cys	20
G	Gly	44
H	His	9
I	Ile	38
L	Leu	46
K	Lys	31
M	Met	8
F	Phe	53
P	Pro	37
S	Ser	56
T	Thr	58

W	Trp	6
Y	Tyr	35
V	Val	53
N	Asn	46
D	Asp	44
Q	Gln	21
E	Glu	17

[0139] Using the amino acid composition shown in Table 10, a human codon-optimized coding region which encodes SEQ ID NO:4 can be designed by any of the methods discussed herein. For "uniform" optimization, each amino acid is assigned the most frequent codon used in the human genome for that amino acid. According to this method, codons are assigned to the coding region encoding SEQ ID NO:4 as follows: the 53 phenylalanine codons are TTC, the 46 leucine codons are CTG, the 38 isoleucine codons are ATC, the 8 methionine codons are ATG, the 53 valine codons are GTG, the 56 serine codons are AGC, the 37 proline codons are CCC, the 58 threonine codons are ACC, the 38 alanine codons are GCC, the 35 tyrosine codons are TAC, the 9 histidine codons are CAC, the 21 glutamine codons are CAG, the 46 asparagine codons are AAC, the 31 lysine codons are AAG, the 44 aspartic acid codons are GAC, the 17 glutamic acid codons are GAG, the 20 cysteine codons are TGC; the 6 tryptophan codons are TGG, the 23 arginine codons are CGG, AGA, or AGG (the frequencies of usage of these three codons in the human genome are not significantly different), and the 44 glycine codons are GGC. The codon-optimized S1 coding region designed by this method is presented herein as SEQ ID NO:27.

ATGTTCATCTTCCTGCTGTTCCTGACCCTGACCAGCGGCAGCGACCTGGA
CAGATGCACCACCTTCGACGACGTGCAGGCCCCCAACTACACCCAGCACA
CCAGCAGCATGAGAGGCGTGTACTACCCCGACGAGATCTTCAGAAGCGAC
ACCCTGTACCTGACCCAGGACCTGTTCCTGCCCTTCTACAGCAACGTGAC
CGGCTTCCACACCATCAACCACACCTTCGGCAACCCCGTGATCCCCTTCA
AGGACGGCATCTACTTCGCCGCCACCGAGAAGAGCAACGTGGTGAGAGGC
TGGGTGTTCGGCAGCACCATGAACAACAAGAGCCAGAGCGTGATCATCAT
CAACAACAGCACCAACGTGGTGATCAGAGCCTGCAACTTCGAGCTGTGCG
ACAACCCCTTCTTCGCCGTGAGCAAGCCCATGGGCACCCAGACCCACACC
ATGATCTTCGACAACGCCTTCAACTGCACCTTCGAGTACATCAGCGACGC
CTTCAGCCTGGACGTGAGCGAGAAGAGCGGCAACTTCAAGCACCTGAGAG
AGTTCGTGTTCAAGAACAAGGACGGCTTCCTGTACGTGTACAAGGGCTAC

CAGCCCATCGACGTGGTGAGAGACCTGCCCAGCGGCTTCAACACCCTGAA
GCCCATCTTCAAGCTGCCCCTGGGCATCAACATCACCAACTTCAGAGCCA
TCCTGACCGCCTTCAGCCCCGCCCAGGACATCTGGGGCACCAGCGCCGCC
GCCTACTTCGTGGGCTACCTGAAGCCCACCACCTTCATGCTGAAGTACGA
CGAGAACGGCACCATCACCGACGCCGTGGACTGCAGCCAGAACCCCCTGG
CCGAGCTGAAGTGCAGCGTGAAGAGCTTCGAGATCGACAAGGGCATCTAC
CAGACCAGCAACTTCAGAGTGGTGCCCAGCGGCGACGTGGTGAGATTCCC
CAACATCACCAACCTGTGCCCCTTCGGCGAGGTGTTCAACGCCACCAAGT
TCCCCAGCGTGTACGCCTGGGAGAGAAAGAAGATCAGCAACTGCGTGGCC
GACTACAGCGTGCTGTACAACAGCACCTTCTTCAGCACCTTCAAGTGCTA
CGGCGTGAGCGCCACCAAGCTGAACGACCTGTGCTTCAGCAACGTGTACG
CCGACAGCTTCGTGGTGAAGGGCGACGACGTGAGACAGATCGCCCCCGGC
CAGACCGGCGTGATCGCCGACTACAACTACAAGCTGCCCGACGACTTCAT
GGGCTGCGTGCTGGCCTGGAACACCAGAAACATCGACGCCACCAGCACCG
GCAACTACAACTACAAGTACAGATACCTGAGACACGGCAAGCTGAGACCC
TTCGAGAGAGACATCAGCAACGTGCCCTTCAGCCCCGACGGCAAGCCCTG
CACCCCCCCCGCCCTGAACTGCTACTGGCCCCTGAACGACTACGGCTTCT
ACACCACCACCGGCATCGGCTACCAGCCCTACAGAGTGGTGGTGCTGAGC
TTCGAGCTGCTGAACGCCCCCGCCACCGTGTGCGGCCCCAAGCTGAGCAC
CGACCTGATCAAGAACCAGTGCGTGAACTTCAACTTCAACGGCCTGACCG
GCACCGGCGTGCTGACCCCCAGCAGCAAGAGATTCCAGCCCTTCCAGCAG
TTCGGCAGAGACGTGAGCGACTTCACCGACAGCGTGAGAGACCCCAAGAC
CAGCGAGATCCTGGACATCAGCCCCTGCAGCTTCGGCGGCGTGAGCGTGA
TCACCCCCGGCACCAACGCCAGCAGCGAGGTGGCCGTGCTGTACCAGGAC
GTGAACTGCACCGACGTGAGCACCGCCATCCACGCCGACCAGCTGACCCC
CGCCTGGAGAATCTACAGCACCGGCAACAACGTGTTCCAGACCCAGGCCG
GCTGCCTGATCGGCGCCGAGCACGTGGACACCAGCTACGAGTGCGACATC
CCCATCGGCGCCGGCATCTGCGCCAGCTACCACACCGTGAGCCTGCTGAG
AAGCACCAGCCAGAAGAGCATCGTGGCCTACACCATGAGCCTGGGCGCC

[0140] Alternatively, a human codon-optimized coding region which encodes SEQ ID NO:4 can be designed by the "full optimization" method, where each amino acid is assigned codons based on the frequency of usage in the human genome. These frequencies are shown in Table 4 above. Using this latter method, codons are assigned to the coding region encoding SEQ ID NO:4 as follows: about 24 of the 53 phenylalanine codons are TTT, and about 29 of the phenylalanine codons are TTC; about 3 of the 46 leucine codons are TTA, about 6 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 9 of the leucine codons are CTC, about 4 of the leucine codons are CTA, and about 18 of the leucine codons are CTG; about 13 of the 38 isoleucine codons are ATT, about 18 of the isoleucine codons are ATC, and about 7 of the isoleucine codons are ATA; the 8 methionine codons are ATG; about 10 of the 53 valine codons are GTT, about 13 of the valine codons are GTC, about 5 of the valine codons are GTA, and about 25 of the valine codons

are GTG; about 10 of the 56 serine codons are TCT, about 12 of the serine codons are TCC, about 8 of the serine codons are TCA, about 3 of the serine codons are TCG, about 9 of the serine codons are AGT, and about 14 of the serine codons are AGC; about 10 of the 37 proline codons are CCT, about 12 of the proline codons are CCC, about 11 of the proline codons are CCA, and about 4 of the proline codons are CCG; about 14 of the 58 threonine codons are ACT, about 21 of the threonine codons are ACC, about 16 of the threonine codons are ACA, and about 7 of the threonine codons are ACG; about 10 of the 38 alanine codons are GCT, about 15 of the alanine codons are GCC, about 9 of the alanine codons are GCA, and about 4 of the alanine codons are GCG; about 15 of the 35 tyrosine codons are TAT and about 20 of the tyrosine codons are TAC; about 4 of the 9 histidine codons are CAT and about 5 of the histidine codons are CAC; about 5 of the 21 glutamine codons are CAA and about 16 of the glutamine codons are CAG; about 21 of the 46 asparagine codons are AAT and about 25 of the asparagine codons are AAC; about 13 of the 31 lysine codons are AAA and about 18 of the lysine codons are AAG; about 20 of the 44 aspartic acid codons are GAT and about 24 of the aspartic acid codons are GAC; about 7 of the 17 glutamic acid codons are GAA and about 10 of the glutamic acid codons are GAG; about 9 of the 20 cysteine codons are TGT and about 11 of the cysteine codons are TGC; the 6 tryptophan codons are TGG; about 2 of the 23 arginine codons are CGT, about 4 of the arginine codons are CGC, about 3 of the arginine codons are CGA, about 5 of the arginine codons are CGG, about 4 of the arginine codons are AGA, and about 5 of the arginine codons are AGG; and about 7 of the 44 glycine codons are GGT, about 15 of the glycine codons are GGC, about 11 of the glycine codons are GGA, and about 11 of the glycine codons are GGG.

[0141] As described above, the term "about" means that the number of amino acids encoded by a certain codon may be one more or one less than the number given. It would be understood by those of ordinary skill in the art that the total number of any amino acid in the polypeptide sequence must remain constant, therefore, if there is one "more" of one codon encoding a give amino

acid, there would have to be one "less" of another codon encoding that same amino acid.

[0142] A representative "fully optimized" codon-optimized coding region encoding SEQ ID NO:4, optimized according to codon usage in humans is presented herein as SEQ ID NO:26.

ATG TTT ATC TTT TTG CTG TTT CTC ACA TTA
ACT TCG GGG TCT GAC CTG GAC CGG TGC ACC
ACA TTC GAT GAC GTC CAA GCC CCC AAC TAC
ACT CAG CAT ACA TCT AGC ATG CGC GGC GTG
TAC TAC CCA GAT GAG ATC TTT AGG TCC GAC
ACC CTT TAT CTG ACC CAG GAC CTT TTT CTT
CCT TTC TAC TCT AAT GTA ACT GGG TTC CAT
ACC ATC AAC CAT ACC TTT GGC AAC CCA GTG
ATT CCA TTT AAG GAT GGT ATT TAC TTC GCC
GCG ACC GAG AAA TCA AAT GTT GTG CGC GGC
TGG GTT TTC GGC TCC ACC ATG AAC AAT AAG
AGT CAG TCC GTA ATT ATC ATT AAC AAT AGT
ACA AAC GTG GTG ATC AGG GCA TGT AAT TTT
GAA TTG TGC GAC AAC CCT TTC TTC GCT GTA
AGC AAA CCC ATG GGG ACG CAG ACT CAC ACG
ATG ATC TTC GAT AAC GCT TTC AAT TGC ACG
TTT GAG TAC ATA TCC GAT GCC TTT TCT CTA
GAT GTG TCC GAA AAA TCA GGG AAT TTT AAG
CAC CTG AGA GAG TTC GTC TTT AAG AAC AAG
GAC GGT TTC TTG TAC GTG TAC AAG GGA TAC
CAG CCG ATC GAC GTG GTG CGG GAC CTA CCC
AGC GGA TTC AAC ACC CTC AAG CCC ATT TTT
AAG CTC CCA CTG GGT ATC AAT ATA ACT AAC
TTC AGA GCC ATT CTC ACA GCT TTC TCT CCA
GCT CAG GAT ATT TGG GGG ACT AGT GCG GCA
GCT TAT TTC GTG GGA TAC CTT AAG CCC ACA
ACC TTC ATG TTG AAA TAC GAT GAG AAC GGA
ACC ATA ACT GAC GCA GTT GAC TGC TCA CAG
AAC CCC CTC GCA GAG TTG AAA TGC TCA GTT
AAA TCC TTT GAG ATC GAC AAG GGT ATT TAC
CAG ACC AGT AAC TTT AGA GTC GTG CCG TCA
GGC GAC GTC GTG AGG TTT CCT AAC ATC ACA
AAT CTA TGT CCT TTC GGA GAA GTG TTC AAT
GCC ACA AAG TTC CCC AGC GTG TAC GCC TGG
GAG CGA AAA AAG ATA TCT AAC TGC GTC GCA
GAC TAC AGC GTA CTG TAT AAC AGC ACT TTT
TTC AGC ACC TTT AAG TGT TAT GGG GTG TCA
GCA ACA AAA CTG AAC GAT CTC TGC TTT TCA
AAC GTT TAT GCC GAT TCC TTC GTT GTC AAG
GGA GAC GAT GTC CGT CAA ATT GCT CCC GGG
CAA ACT GGC GTT ATC GCT GAC TAT AAC TAT
AAA CTG CCA GAC GAT TTT ATG GGG TGT GTC
CTC GCA TGG AAT ACG CGC AAC ATC GAT GCG
ACC TCT ACC GGA AAC TAC AAC TAT AAA TAT
AGG TAT CTT CGG CAC GGG AAA TTA CGG CCG

TTC GAG CGA GAT ATT TCG AAC GTG CCT TTC
AGT CCC GAT GGA AAA CCA TGT ACT CCT CCA
GCC CTC AAT TGT TAC TGG CCA TTG AAT GAC
TAC GGG TTC TAC ACG ACA ACT GGA ATA GGC
TAT CAG CCT TAT CGT GTC GTC GTT CTT TCT
TTC GAA CTG CTG AAT GCT CCC GCC ACG GTG
TGC GGT CCA AAA CTC AGC ACC GAC CTG ATC
AAG AAT CAG TGC GTG AAT TTC AAT TTC AAC
GGC CTG ACA GGC ACA GGC GTT CTG ACC CCA
AGC TCC AAG CGC TTC CAG CCC TTC CAG CAA
TTT GGC AGG GAT GTG TCC GAC TTT ACC GAT
TCA GTG CGA GAT CCC AAG ACC AGT GAA ATA
CTA GAC ATT TCT CCG TGT AGC TTT GGC GGC
GTG TCT GTC ATT ACT CCT GGG ACG AAT GCC
TCG AGC GAG GTG GCG GTG TTA TAT CAG GAC
GTT AAT TGT ACA GAC GTC AGT ACC GCC ATA
CAT GCT GAT CAG CTG ACT CCT GCA TGG AGA
ATC TAC TCC ACA GGA AAT AAT GTG TTT CAG
ACA CAA GCA GGT TGC CTG ATC GGA GCC GAA
CAC GTC GAC ACC AGC TAC GAA TGT GAT ATC
CCT ATC GGT GCC GGC ATC TGC GCT AGT TAT
CAC ACA GTA AGC CTG CTG CGG AGC ACC AGT
CAG AAG TCC ATT GTG GCC TAT ACT ATG TCC
CTG GGC GCC

[0143] Another representative codon-optimized coding region encoding SEQ ID NO:4 is presented herein as SEQ ID NO:45.

ATG TTC ATC TTC CTG CTG TTT CTG ACA CTG
ACT TCT GGC TCA GAT CTG GAT AGA TGC ACT
ACC TTT GAC GAT GTA CAG GCC CCC AAC TAC
ACT CAG CAC ACA TCG TCC ATG CGA GGC GTG
TAT TAC CCC GAC GAG ATC TTC AGA AGT GAC
ACT CTG TAC CTG ACA CAG GAC CTG TTC CTG
CCC TTT TAC TCT AAC GTG ACT GGC TTT CAC
ACT ATC AAC CAT ACC TTC GGC AAC CCC GTA
ATC CCC TTC AAG GAT GGC ATC TAT TTT GCC
GCC ACC GAG AAG TCC AAC GTG GTG AGG GGC
TGG GTC TTC GGC AGT ACG ATG AAC AAC AAG
TCT CAG TCC GTG ATA ATC ATA AAC AAC AGT
ACT AAC GTG GTT ATA AGA GCC TGC AAC TTC
GAG CTG TGC GAC AAC CCC TTC TTC GCC GTG
TCC AAG CCC ATG GGC ACA CAG ACC CAC ACC
ATG ATA TTC GAC AAC GCC TTT AAC TGT ACT
TTC GAG TAT ATA AGC GAT GCC TTC AGT CTG
GAT GTT TCT GAG AAG TCA GGC AAC TTT AAG
CAT CTG AGA GAG TTC GTA TTC AAG AAC AAG
GAC GGC TTT CTG TAT GTT TAT AAG GGC TAC
CAG CCC ATA GAT GTC GTG CGG GAT CTG CCC
AGC GGC TTC AAC ACA CTG AAG CCC ATT TTT
AAG CTG CCC CTG GGC ATC AAC ATA ACC AAC
TTT AGA GCC ATC CTG ACT GCC TTT AGC CCC
GCC CAG GAT ATA TGG GGC ACT AGC GCC GCC
GCC TAT TTC GTC GGC TAC CTG AAG CCC ACC
ACA TTC ATG CTG AAG TAC GAT GAG AAC GGC

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ACA ATT ACG GAT GCC GTA GAT TGC AGT CAG
AAC CCC CTG GCC GAG CTG AAG TGC AGT GTG
AAG TCT TTC GAG ATC GAC AAG GGC ATA TAC
CAG ACT TCT AAC TTT CGG GTG GTT CCC AGC
GGC GAC GTT GTT AGG TTT CCC AAC ATC ACC
AAC CTG TGC CCC TTC GGC GAG GTG TTT AAC
GCC ACA AAG TTC CCC TCC GTA TAT GCC TGG
GAG AGG AAG AAG ATT TCG AAC TGC GTG GCC
GAC TAT AGC GTC CTG TAC AAC TCT ACA TTC
TTT TCT ACA TTC AAG TGC TAC GGC GTC AGT
GCC ACT AAG CTG AAC GAC CTG TGC TTC AGC
AAC GTG TAT GCC GAC TCA TTT GTA GTT AAG
GGC GAT GAT GTG AGA CAG ATT GCC CCC GGC
CAG ACA GGC GTG ATC GCC GAT TAT AAC TAT
AAG CTG CCC GAC GAT TTC ATG GGC TGC GTT
CTG GCC TGG AAC ACA AGG AAC ATC GAT GCC
ACT AGC ACT GGC AAC TAC AAC TAC AAG TAC
AGG TAT CTG AGA CAC GGC AAG CTG AGG CCC
TTC GAG CGA GAT ATC AGT AAC GTA CCC TTC
AGT CCC GAC GGC AAG CCC TGC ACT CCC CCC
GCC CTG AAC TGC TAT TGG CCC CTG AAC GAC
TAC GGC TTT TAT ACC ACT ACA GGC ATC GGC
TAC CAG CCC TAC AGG GTT GTG GTG CTG AGC
TTC GAG CTG CTG AAC GCC CCC GCC ACT GTT
TGC GGC CCC AAG CTG TCA ACG GAT CTG ATC
AAG AAC CAG TGC GTA AAC TTT AAC TTT AAC
GGC CTG ACA GGC ACA GGC GTC CTG ACT CCC
TCT AGT AAG AGA TTC CAG CCC TTT CAG CAG
TTC GGC CGC GAC GTC AGC GAT TTT ACG GAT
AGT GTG AGA GAT CCC AAG ACC AGC GAG ATC
CTG GAC ATT AGT CCC TGT TCT TTC GGC GGC
GTG TCT GTC ATA ACG CCC GGC ACG AAC GCC
TCT TCT GAG GTC GCC GTT CTG TAC CAG GAC
GTC AAC TGT ACA GAC GTC TCC ACA GCC ATA
CAC GCC GAT CAG CTG ACT CCC GCC TGG AGA
ATT TAC TCT ACC GGC AAC AAC GTC TTC CAG
ACC CAG GCC GGC TGC CTG ATC GGC GCC GAG
CAT GTG GAT ACT TCC TAC GAG TGC GAC ATA
CCC ATC GGC GCC GGC ATT TGC GCC TCG TAC
CAT ACC GTG TCT CTG CTG AGA TCT ACC TCT
CAG AAG AGT ATC GTT GCC TAC ACT ATG TCC
CTG GGC GCC
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[0144] A representative codon-optimized coding region encoding SEQ ID NO:4 according to the "standardized optimization" method is presented herein as SEQ ID NO: 68.

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ATG TTC ATC TTC CTG CTG TTC CTG ACC CTG
ACC AGC GGC AGC GAT CTG GAC CGC TGC ACC
ACC TTC GAC GAT GTG CAG GCC CCC AAC TAC
ACC CAG CAC ACC AGC AGC ATG CGC GGC GTG
TAC TAC CCC GAT GAG ATC TTC CGC AGC GAT
ACC CTG TAC CTG ACC CAG GAT CTG TTC CTG
CCC TTC TAC AGC AAC GTG ACC GGC TTC CAT
ACC ATC AAC CAC ACC TTC GGC AAC CCC GTG
ATC CCC TTC AAG GAT GGC ATC TAC TTC GCC
GCC ACC GAG AAG AGC AAC GTG GTG CGC GGC
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TGG GTG TTC GGC AGC ACC ATG AAC AAC AAG
AGC CAG AGC GTG ATC ATC ATC AAC AAC AGC
ACC AAC GTG GTG ATC CGC GCC TGC AAC TTC
GAG CTG TGC GAC AAC CCC TTC TTC GCC GTG
AGC AAG CCC ATG GGC ACC CAG ACC CAC ACC
ATG ATC TTC GAC AAC GCC TTC AAC TGC ACC
TTC GAG TAC ATC AGC GAT GCC TTC AGC CTG
GAC GTG AGC GAG AAG AGC GGC AAC TTC AAG
CAT CTG CGC GAG TTC GTG TTC AAG AAC AAG
GAT GGC TTC CTG TAC GTG TAC AAG GGC TAC
CAG CCC ATC GAC GTG GTG CGC GAC CTG CCC
AGC GGC TTC AAC ACC CTG AAG CCC ATC TTC
AAG CTG CCC CTG GGC ATC AAC ATC ACC AAC
TTC CGC GCC ATC CTG ACC GCC TTC AGC CCC
GCC CAG GAT ATC TGG GGC ACC AGC GCC GCC
GCC TAC TTC GTG GGC TAC CTG AAG CCC ACC
ACC TTC ATG CTG AAG TAC GAC GAG AAC GGC
ACC ATC ACC GAT GCC GTG GAT TGC AGC CAG
AAC CCC CTG GCC GAG CTG AAG TGC AGC GTG
AAG AGC TTC GAG ATC GAT AAG GGC ATC TAC
CAG ACC AGC AAC TTC CGC GTG GTG CCC AGC
GGC GAC GTG GTG CGC TTC CCC AAC ATC ACC
AAC CTG TGC CCC TTC GGC GAG GTG TTC AAC
GCC ACC AAG TTC CCC AGC GTG TAC GCC TGG
GAG CGC AAG AAG ATC AGC AAC TGC GTG GCC
GAT TAC AGC GTG CTG TAC AAC AGC ACC TTC
TTC AGC ACC TTC AAG TGC TAC GGC GTG AGC
GCC ACC AAG CTG AAC GAC CTG TGC TTC AGC
AAC GTG TAC GCC GAC AGC TTC GTG GTG AAG
GGC GAC GAC GTG CGC CAG ATC GCC CCC GGC
CAG ACC GGC GTG ATC GCC GAT TAC AAC TAC
AAG CTG CCC GAT GAC TTC ATG GGC TGC GTG
CTG GCC TGG AAC ACC CGC AAC ATC GAT GCC
ACC AGC ACC GGC AAC TAC AAC TAC AAG TAC
CGC TAC CTG CGC CAC GGC AAG CTG CGC CCC
TTC GAG CGC GAT ATC AGC AAC GTG CCC TTC
AGC CCC GAT GGC AAG CCC TGC ACC CCC CCC
GCC CTG AAC TGT TAC TGG CCC CTG AAC GAT
TAC GGC TTC TAC ACC ACC ACC GGC ATC GGC
TAC CAG CCC TAC CGC GTG GTG GTG CTG AGC
TTC GAG CTG CTG AAC GCC CCC GCC ACC GTG
TGC GGC CCC AAG CTG AGC ACC GAC CTG ATC
AAG AAC CAG TGC GTG AAC TTC AAC TTC AAC
GGC CTG ACC GGC ACC GGC GTG CTG ACC CCC
AGC AGC AAG CGC TTC CAG CCC TTC CAG CAG
TTC GGC CGC GAC GTG AGC GAC TTC ACC GAC
AGC GTG CGC GAT CCC AAG ACC AGC GAG ATC
CTG GAT ATC AGC CCC TGC AGC TTC GGC GGC
GTG AGC GTG ATC ACC CCC GGC ACC AAC GCC
AGC AGC GAG GTG GCC GTG CTG TAC CAG GAC
GTG AAC TGC ACC GAT GTG AGC ACC GCC ATC
CAC GCC GAT CAG CTG ACC CCC GCC TGG CGC
ATC TAC AGC ACC GGC AAC AAC GTG TTC CAG
ACC CAG GCC GGC TGT CTG ATC GGC GCC GAG
CAT GTG GAC ACC AGC TAC GAG TGT GAT ATC
CCC ATC GGC GCC GGC ATC TGC GCC AGC TAC
CAT ACC GTG AGC CTG CTG CGC AGC ACC AGC
CAG AAG AGC ATC GTG GCC TAC ACC ATG AGC
CTG GGC GCC

[0145] In certain embodiments described herein, a codon-optimized coding region encoding SEQ ID NO:6 is optimized according to codon usage in humans (*Homo sapiens*). Alternatively, a codon-optimized coding region encoding SEQ ID NO:6 may be optimized according to codon usage in any plant, animal, or microbial species. Codon-optimized coding regions encoding SEQ ID NO:6, optimized according to codon usage in humans are designed as follows. The amino acid composition of SEQ ID NO:6 is shown in Table 11.

TABLE 11

AMINO ACID		Number in SEQ ID NO:6
A	Ala	43
R	Arg	16
C	Cys	10
G	Gly	30
H	His	5
I	Ile	36
L	Leu	46
K	Lys	25
M	Met	10
F	Phe	28
P	Pro	19
S	Ser	35
T	Thr	38
W	Trp	4
Y	Tyr	17
V	Val	33
N	Asn	35
D	Asp	26
Q	Gln	34
E	Glu	23

[0146] Using the amino acid composition shown in Table 11, a human codon-optimized coding region which encodes SEQ ID NO:6 can be designed by any of the methods discussed herein. For "uniform" optimization, each amino acid is assigned the most frequent codon used in the human genome for that amino acid. According to this method, codons are assigned to the coding region encoding SEQ ID NO:6 as follows: the 28 phenylalanine codons are TTC, the

46 leucine codons are CTG, the 36 isoleucine codons are ATC, the 10 methionine codons are ATG, the 33 valine codons are GTG, the 35 serine codons are AGC, the 19 proline codons are CCC, the 38 threonine codons are ACC, the 43 alanine codons are GCC, the 17 tyrosine codons are TAC, the 5 histidine codons are CAC, the 34 glutamine codons are CAG, the 35 asparagine codons are AAC, the 25 lysine codons are AAG, the 26 aspartic acid codons are GAC, the 23 glutamic acid codons are GAG, the 10 cysteine codons are TGC, the 4 tryptophan codon is TGG, the 16 arginine codons are CGG, AGA, or AGG (the frequencies of usage of these three codons in the human genome are not significantly different), and the 30 glycine codons are GGC. The codon-optimized coding region designed by this method is presented herein as SEQ ID NO:29.

GAC AGC AGC ATC GCC TAC AGC AAC AAC ACC
ATC GCC ATC CCC ACC AAC TTC AGC ATC AGC
ATC ACC ACC GAG GTG ATG CCC GTG AGC ATG
GCC AAG ACC AGC GTG GAC TGC AAC ATG TAC
ATC TGC GGC GAC AGC ACC GAG TGC GCC AAC
CTG CTG CTG CAG TAC GGC AGC TTC TGC ACC
CAG CTG AAC CGG GCC CTG AGC GGC ATC GCC
GCC GAG CAG GAC CGG AAC ACC CGG GAG GTG
TTC GCC CAG GTG AAG CAG ATG TAC AAG ACC
CCC ACC CTG AAG TAC TTC GGC GGC TTC AAC
TTC AGC CAG ATC CTG CCC GAC CCC CTG AAG
CCC ACC AAG CGG AGC TTC ATC GAG GAC CTG
CTG TTC AAC AAG GTG ACC CTG GCC GAC GCC
GGC TTC ATG AAG CAG TAC GGC GAG TGC CTG
GGC GAC ATC AAC GCC CGG GAC CTG ATC TGC
GCC CAG AAG TTC AAC GGC CTG ACC GTG CTG
CCC CCC CTG CTG ACC GAC GAC ATG ATC GCC
GCC TAC ACC GCC GCC CTG GTG AGC GGC ACC
GCC ACC GCC GGC TGG ACC TTC GGC GCC GGC
GCC GCC CTG CAG ATC CCC TTC GCC ATG CAG
ATG GCC TAC CGG TTC AAC GGC ATC GGC GTG
ACC CAG AAC GTG CTG TAC GAG AAC CAG AAG
CAG ATC GCC AAC CAG TTC AAC AAG GCC ATC
AGC CAG ATC CAG GAG AGC CTG ACC ACC ACC
AGC ACC GCC CTG GGC AAG CTG CAG GAC GTG
GTG AAC CAG AAC GCC CAG GCC CTG AAC ACC
CTG GTG AAG CAG CTG AGC AGC AAC TTC GGC
GCC ATC AGC AGC GTG CTG AAC GAC ATC CTG
AGC CGG CTG GAC AAG GTG GAG GCC GAG GTG
CAG ATC GAC CGG CTG ATC ACC GGC CGG CTG
CAG AGC CTG CAG ACC TAC GTG ACC CAG CAG
CTG ATC CGG GCC GCC GAG ATC CGG GCC AGC
GCC AAC CTG GCC GCC ACC AAG ATG AGC GAG
TGC GTG CTG GGC CAG AGC AAG CGG GTG GAC
TTC TGC GGC AAG GGC TAC CAC CTG ATG AGC
TTC CCC CAG GCC GCC CCC CAC GGC GTG GTG

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TTC CTG CAC GTG ACC TAC GTG CCC AGC CAG
GAG CGG AAC TTC ACC ACC GCC CCC GCC ATC
TGC CAC GAG GGC AAG GCC TAC TTC CCC CGG
GAG GGC GTG TTC GTG TTC AAC GGC ACC AGC
TGG TTC ATC ACC CAG CGG AAC TTC TTC AGC
CCC CAG ATC ATC ACC ACC GAC AAC ACC TTC
GTG AGC GGC AAC TGC GAC GTG GTG ATC GGC
ATC ATC AAC AAC ACC GTG TAC GAC CCC CTG
CAG CCC GAG CTG GAC AGC TTC AAG GAG GAG
CTG GAC AAG TAC TTC AAG AAC CAC ACC AGC
CCC GAC GTG GAC CTG GGC GAC ATC AGC GGC
ATC AAC GCC AGC GTG GTG AAC ATC CAG AAG
GAG ATC GAC CGG CTG AAC GAG GTG GCC AAG
AAC CTG AAC GAG AGC CTG ATC GAC CTG CAG
GAG CTG GGC AAG TAC GAG CAG TAC ATC AAG
TGG CCC TGG
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[0147] A codon-optimized coding region encoding SEQ ID NO:56 designed by this method is presented herein as SEQ ID NO:64.

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ATG GAC AGC AGC ATC GCC TAC AGC AAC AAC ACC
ATC GCC ATC CCC ACC AAC TTC AGC ATC AGC
ATC ACC ACC GAG GTG ATG CCC GTG AGC ATG
GCC AAG ACC AGC GTG GAC TGC AAC ATG TAC
ATC TGC GGC GAC AGC ACC GAG TGC GCC AAC
CTG CTG CTG CAG TAC GGC AGC TTC TGC ACC
CAG CTG AAC CGG GCC CTG AGC GGC ATC GCC
GCC GAG CAG GAC CGG AAC ACC CGG GAG GTG
TTC GCC CAG GTG AAG CAG ATG TAC AAG ACC
CCC ACC CTG AAG TAC TTC GGC GGC TTC AAC
TTC AGC CAG ATC CTG CCC GAC CCC CTG AAG
CCC ACC AAG CGG AGC TTC ATC GAG GAC CTG
CTG TTC AAC AAG GTG ACC CTG GCC GAC GCC
GGC TTC ATG AAG CAG TAC GGC GAG TGC CTG
GGC GAC ATC AAC GCC CGG GAC CTG ATC TGC
GCC CAG AAG TTC AAC GGC CTG ACC GTG CTG
CCC CCC CTG CTG ACC GAC GAC ATG ATC GCC
GCC TAC ACC GCC GCC CTG GTG AGC GGC ACC
GCC ACC GCC GGC TGG ACC TTC GGC GCC GGC
GCC GCC CTG CAG ATC CCC TTC GCC ATG CAG
ATG GCC TAC CGG TTC AAC GGC ATC GGC GTG
ACC CAG AAC GTG CTG TAC GAG AAC CAG AAG
CAG ATC GCC AAC CAG TTC AAC AAG GCC ATC
AGC CAG ATC CAG GAG AGC CTG ACC ACC ACC
AGC ACC GCC CTG GGC AAG CTG CAG GAC GTG
GTG AAC CAG AAC GCC CAG GCC CTG AAC ACC
CTG GTG AAG CAG CTG AGC AGC AAC TTC GGC
GCC ATC AGC AGC GTG CTG AAC GAC ATC CTG
AGC CGG CTG GAC AAG GTG GAG GCC GAG GTG
CAG ATC GAC CGG CTG ATC ACC GGC CGG CTG
CAG AGC CTG CAG ACC TAC GTG ACC CAG CAG
CTG ATC CGG GCC GCC GAG ATC CGG GCC AGC
GCC AAC CTG GCC GCC ACC AAG ATG AGC GAG
TGC GTG CTG GGC CAG AGC AAG CGG GTG GAC
TTC TGC GGC AAG GGC TAC CAC CTG ATG AGC
TTC CCC CAG GCC GCC CCC CAC GGC GTG GTG
TTC CTG CAC GTG ACC TAC GTG CCC AGC CAG
GAG CGG AAC TTC ACC ACC GCC CCC GCC ATC
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        TGC CAC GAG GGC AAG GCC TAC TTC CCC CGG
        GAG GGC GTG TTC GTG TTC AAC GGC ACC AGC
        TGG TTC ATC ACC CAG CGG AAC TTC TTC AGC
        CCC CAG ATC ATC ACC ACC GAC AAC ACC TTC
        GTG AGC GGC AAC TGC GAC GTG GTG ATC GGC
        ATC ATC AAC AAC ACC GTG TAC GAC CCC CTG
        CAG CCC GAG CTG GAC AGC TTC AAG GAG GAG
        CTG GAC AAG TAC TTC AAG AAC CAC ACC AGC
        CCC GAC GTG GAC CTG GGC GAC ATC AGC GGC
        ATC AAC GCC AGC GTG GTG AAC ATC CAG AAG
        GAG ATC GAC CGG CTG AAC GAG GTG GCC AAG
        AAC CTG AAC GAG AGC CTG ATC GAC CTG CAG
        GAG CTG GGC AAG TAC GAG CAG TAC ATC AAG
TGG CCC TGG
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[0148] Alternatively, a human codon-optimized coding region which encodes SEQ ID NO:6 can be designed by the "full optimization" method, where each amino acid is assigned codons based on the frequency of usage in the human genome. These frequencies are shown in Table 4 above. Using this latter method, codons are assigned to the coding region encoding SEQ ID NO:6 as follows: about 13 of the 28 phenylalanine codons are TTT, and about 15 of the phenylalanine codons are TTC; about 3 of the 46 leucine codons are TTA, about 6 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 9 of the leucine codons are CTC, about 4 of the leucine codons are CTA, and about 18 of the leucine codons are CTG; about 13 of the 36 isoleucine codons are ATT, about 17 of the isoleucine codons are ATC, and about 6 of the isoleucine codons are ATA; the 10 methionine codons are ATG; about 6 of the 33 valine codons are GTT, about 15 of the valine codons are GTG, about 4 of the valine codons are GTA, and about 8 of the valine codons are GTC; about 6 of the 35 serine codons are TCT, about 8 of the serine codons are TCC, about 5 of the serine codons are TCA, about 2 of the serine codons are TCG, about 6 of the serine codons are AGT, and about 8 of the serine codons are AGC; about 5 of the 19 proline codons are CCT, about 6 of the proline codons are CCC, about 6 of the proline codons are CCA, and about 2 of the proline codons are CCG; about 9 of the 38 threonine codons are ACT, about 14 of the threonine codons are ACC, about 11 of the threonine codons are ACA, and about 4 of the threonine codons are ACG; about 11 of the 43 alanine codons are GCT, about 17 of the alanine codons are GCC, about 10 of

the alanine codons are GCA, and about 5 of the alanine codons are GCG; about 7 of the 17 tyrosine codons are TAT and about 10 of the tyrosine codons are TAC; about 2 of the 5 histidine codons are CAT and about 3 of the histidine codons are CAC; about 9 of the 34 glutamine codons are CAA and about 25 of the glutamine codons are CAG; about 16 of the 35 asparagine codons are AAT and about 19 of the asparagine codons are AAC; about 11 of the 25 lysine codons are AAA and about 14 of the lysine codons are AAG; about 12 of the 26 aspartic acid codons are GAT and about 14 of the aspartic acid codons are GAC; about 10 of the 23 glutamic acid codons are GAA and about 13 of the glutamic acid codons are GAG; about 5 of the 10 cysteine codons are TGT and about 5 of the cysteine codons are TGC; the 4 tryptophan codons are TGG; about 1 of the 16 arginine codons is CGT, about 3 of the arginine codons are CGC, about 2 of the arginine codons are CGA, about 3 of the arginine codons are CGG, about 4 of the arginine codons are AGA, and about 3 of the arginine codons are AGG; and about 5 of the 30 glycine codons are GGT, about 10 of the glycine codons are GGC, about 8 of the glycine codons are GGA, and about 7 of the glycine codons are GGG.

[0149] As described above, the term "about" means that the number of amino acids encoded by a certain codon may be one more or one less than the number given. It would be understood by those of ordinary skill in the art that the total number of any amino acid in the polypeptide sequence must remain constant, therefore, if there is one "more" of one codon encoding a give amino acid, there would have to be one "less" of another codon encoding that same amino acid.

[0150] A representative "fully optimized" codon-optimized coding region encoding SEQ ID NO:6, optimized according to codon usage in humans is presented herein as SEQ ID NO:28.

GAC AGT TCA ATC GCC TAT TCG AAC AAC ACT
ATA GCA ATC CCA ACA AAT TTT TCA ATT TCT
ATA ACA ACA GAG GTG ATG CCA GTG TCC ATG
GCA AAG ACT AGC GTA GAC TGC AAT ATG TAC
ATC TGC GGA GAT TCT ACA GAA TGT GCA AAC

TTG CTG CTA CAG TAT GGA TCG TTC TGT ACC
CAG CTC AAC CGG GCG CTG AGC GGC ATT GCT
GCC GAA CAG GAT CGC AAT ACG AGA GAG GTG
TTT GCT CAA GTG AAA CAA ATG TAT AAG ACC
CCA ACA TTG AAA TAC TTC GGT GGA TTC AAT
TTC AGT CAG ATT CTG CCA GAC CCA CTC AAA
CCC ACC AAG AGG AGC TTT ATT GAA GAT CTT
CTG TTC AAC AAA GTT ACC TTG GCC GAC GCT
GGG TTT ATG AAG CAA TAC GGT GAG TGC CTG
GGC GAC ATT AAC GCA CGA GAC CTG ATC TGC
GCC CAG AAG TTT AAC GGG CTC ACG GTT TTA
CCG CCA CTG CTG ACT GAT GAT ATG ATT GCC
GCT TAC ACT GCG GCC CTT GTG AGT GGT ACC
GCA ACT GCT GGC TGG ACG TTT GGC GCT GGG
GCG GCC TTA CAG ATC CCT TTT GCC ATG CAG
ATG GCC TAC AGG TTC AAT GGA ATT GGT GTC
ACT CAG AAT GTC CTG TAC GAG AAC CAG AAA
CAG ATC GCC AAC CAG TTC AAT AAA GCT ATT
TCA CAG ATT CAG GAA TCA CTT ACC ACA ACT
TCC ACG GCA CTC GGT AAA CTG CAG GAC GTG
GTG AAT CAG AAC GCT CAG GCA CTA AAT ACA
CTC GTC AAG CAA CTG AGT TCC AAT TTC GGG
GCC ATA TCT AGC GTA TTG AAC GAC ATC CTC
AGT CGG CTC GAC AAA GTG GAG GCC GAA GTC
CAA ATA GAC CGT CTT ATC ACA GGC AGA CTA
CAG TCA TTG CAG ACC TAC GTT ACC CAG CAG
TTG ATC CGC GCC GCT GAG ATA CGA GCC TCC
GCC AAT CTG GCC GCT ACC AAA ATG TCT GAG
TGT GTG CTC GGA CAA AGT AAG CGG GTG GAT
TTT TGC GGC AAG GGC TAT CAC CTC ATG TCC
TTC CCT CAA GCA GCA CCC CAC GGA GTC GTT
TTT CTG CAT GTG ACA TAC GTG CCT AGC CAG
GAG AGA AAC TTT ACC ACT GCG CCT GCC ATT
TGT CAT GAA GGC AAA GCT TAT TTT CCC CGC
GAG GGG GTG TTC GTT TTC AAC GGA ACT AGC
TGG TTT ATC ACA CAA AGG AAT TTC TTC TCC
CCC CAG ATC ATC ACC ACC GAC AAC ACC TTT
GTC TCT GGA AAC TGT GAC GTC GTT ATA GGC
ATC ATC AAT AAT ACA GTA TAC GAT CCC CTG
CAG CCC GAA CTT GAC TCT TTC AAG GAG GAA
CTA GAT AAG TAC TTC AAG AAT CAC ACC AGC
CCG GAT GTA GAT TTA GGG GAT ATT AGC GGG
ATT AAC GCA TCC GTG GTC AAC ATC CAA AAA
GAG ATT GAC AGA CTG AAC GAA GTG GCG AAG
AAC CTG AAT GAG TCC CTG ATC GAT CTT CAG
GAG CTG GGC AAG TAT GAA CAG TAT ATC AAG
TGG CCT TGG

[0151] A representative "fully optimized" codon-optimized coding region encoding SEQ ID NO:56, optimized according to codon usage in humans is presented herein as SEQ ID NO:65.

ATG GAC AGT TCA ATC GCC TAT TCG AAC AAC ACT
ATA GCA ATC CCA ACA AAT TTT TCA ATT TCT
ATA ACA ACA GAG GTG ATG CCA GTG TCC ATG
GCA AAG ACT AGC GTA GAC TGC AAT ATG TAC
ATC TGC GGA GAT TCT ACA GAA TGT GCA AAC
TTG CTG CTA CAG TAT GGA TCG TTC TGT ACC
CAG CTC AAC CGG GCG CTG AGC GGC ATT GCT
GCC GAA CAG GAT CGC AAT ACG AGA GAG GTG
TTT GCT CAA GTG AAA CAA ATG TAT AAG ACC
CCA ACA TTG AAA TAC TTC GGT GGA TTC AAT
TTC AGT CAG ATT CTG CCA GAC CCA CTC AAA
CCC ACC AAG AGG AGC TTT ATT GAA GAT CTT
CTG TTC AAC AAA GTT ACC TTG GCC GAC GCT
GGG TTT ATG AAG CAA TAC GGT GAG TGC CTG
GGC GAC ATT AAC GCA CGA GAC CTG ATC TGC
GCC CAG AAG TTT AAC GGG CTC ACG GTT TTA
CCG CCA CTG CTG ACT GAT GAT ATG ATT GCC
GCT TAC ACT GCG GCC CTT GTG AGT GGT ACC
GCA ACT GCT GGC TGG ACG TTT GGC GCT GGG
GCG GCC TTA CAG ATC CCT TTT GCC ATG CAG
ATG GCC TAC AGG TTC AAT GGA ATT GGT GTC
ACT CAG AAT GTC CTG TAC GAG AAC CAG AAA
CAG ATC GCC AAC CAG TTC AAT AAA GCT ATT
TCA CAG ATT CAG GAA TCA CTT ACC ACA ACT
TCC ACG GCA CTC GGT AAA CTG CAG GAC GTG
GTG AAT CAG AAC GCT CAG GCA CTA AAT ACA
CTC GTC AAG CAA CTG AGT TCC AAT TTC GGG
GCC ATA TCT AGC GTA TTG AAC GAC ATC CTC
AGT CGG CTC GAC AAA GTG GAG GCC GAA GTC
CAA ATA GAC CGT CTT ATC ACA GGC AGA CTA
CAG TCA TTG CAG ACC TAC GTT ACC CAG CAG
TTG ATC CGC GCC GCT GAG ATA CGA GCC TCC
GCC AAT CTG GCC GCT ACC AAA ATG TCT GAG
TGT GTG CTC GGA CAA AGT AAG CGG GTG GAT
TTT TGC GGC AAG GGC TAT CAC CTC ATG TCC
TTC CCT CAA GCA GCA CCC CAC GGA GTC GTT
TTT CTG CAT GTG ACA TAC GTG CCT AGC CAG
GAG AGA AAC TTT ACC ACT GCG CCT GCC ATT
TGT CAT GAA GGC AAA GCT TAT TTT CCC CGC
GAG GGG GTG TTC GTT TTC AAC GGA ACT AGC
TGG TTT ATC ACA CAA AGG AAT TTC TTC TCC
CCC CAG ATC ATC ACC ACC GAC AAC ACC TTT
GTC TCT GGA AAC TGT GAC GTC GTT ATA GGC
ATC ATC AAT AAT ACA GTA TAC GAT CCC CTG
CAG CCC GAA CTT GAC TCT TTC AAG GAG GAA
CTA GAT AAG TAC TTC AAG AAT CAC ACC AGC
CCG GAT GTA GAT TTA GGG GAT ATT AGC GGG
ATT AAC GCA TCC GTG GTC AAC ATC CAA AAA
GAG ATT GAC AGA CTG AAC GAA GTG GCG AAG
AAC CTG AAT GAG TCC CTG ATC GAT CTT CAG
GAG CTG GGC AAG TAT GAA CAG TAT ATC AAG
TGG CCT TGG

[0152] Another representative codon-optimized coding region encoding SEQ ID NO:6 is presented herein as SEQ ID NO:46.

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GAT AGC AGC ATA GCC TAC TCA AAC AAC ACG
ATC GCC ATC CCC ACA AAC TTT TCC ATT TCC
ATA ACT ACC GAG GTG ATG CCC GTG AGC ATG
GCC AAG ACA TCG GTA GAT TGC AAC ATG TAC
ATC TGT GGC GAT TCT ACA GAG TGT GCC AAC
CTG CTG CTG CAG TAC GGC TCT TTC TGC ACG
CAG CTG AAC AGG GCC CTG TCT GGC ATC GCC
GCC GAG CAG GAT CGG AAC ACA CGG GAG GTT
TTC GCC CAG GTA AAG CAG ATG TAT AAG ACG
CCC ACT CTG AAG TAC TTC GGC GGC TTC AAC
TTC TCT CAG ATA CTG CCC GAC CCC CTG AAG
CCC ACT AAG AGG TCT TTT ATC GAG GAT CTG
CTG TTC AAC AAG GTT ACC CTG GCC GAT GCC
GGC TTT ATG AAG CAG TAT GGC GAG TGC CTG
GGC GAC ATC AAC GCC AGA GAT CTG ATA TGC
GCC CAG AAG TTC AAC GGC CTG ACT GTG CTG
CCC CCC CTG CTG ACT GAC GAC ATG ATC GCC
GCC TAT ACC GCC GCC CTG GTG AGT GGC ACA
GCC ACT GCC GGC TGG ACA TTC GGC GCC GGC
GCC GCC CTG CAG ATC CCC TTC GCC ATG CAG
ATG GCC TAC AGA TTT AAC GGC ATT GGC GTC
ACT CAG AAC GTC CTG TAT GAG AAC CAG AAG
CAG ATC GCC AAC CAG TTT AAC AAG GCC ATA
AGC CAG ATC CAG GAG TCA CTG ACA ACG ACA
AGT ACC GCC CTG GGC AAG CTG CAG GAT GTA
GTG AAC CAG AAC GCC CAG GCC CTG AAC ACT
CTG GTT AAG CAG CTG TCT AGC AAC TTC GGC
GCC ATC AGT AGT GTT CTG AAC GAT ATT CTG
TCT AGG CTG GAC AAG GTC GAG GCC GAG GTG
CAG ATT GAT CGC CTG ATT ACC GGC AGA CTG
CAG AGT CTG CAG ACT TAT GTA ACT CAG CAG
CTG ATC AGA GCC GCC GAG ATT CGA GCC TCC
GCC AAC CTG GCC GCC ACA AAG ATG TCT GAG
TGC GTC CTG GGC CAG AGT AAG AGG GTT GAC
TTC TGC GGC AAG GGC TAT CAT CTG ATG TCT
TTT CCC CAG GCC GCC CCC CAC GGC GTC GTG
TTC CTG CAC GTA ACT TAC GTG CCC AGT CAG
GAG AGA AAC TTT ACC ACT GCC CCC GCC ATC
TGC CAC GAG GGC AAG GCC TAC TTC CCC AGA
GAG GGC GTG TTT GTG TTC AAC GGC ACA TCT
TGG TTC ATC ACC CAG AGG AAC TTT TTC AGC
CCC CAG ATC ATA ACA ACT GAC AAC ACT TTC
GTT TCG GGC AAC TGC GAC GTA GTG ATC GGC
ATA ATA AAC AAC ACC GTG TAC GAT CCC CTG
CAG CCC GAG CTG GAC AGC TTT AAG GAG GAG
CTG GAC AAG TAC TTT AAG AAC CAT ACC TCA
CCC GAT GTG GAC CTG GGC GAC ATT TCT GGC
ATA AAC GCC TCC GTC GTC AAC ATC CAG AAG
GAG ATA GAT AGA CTG AAC GAG GTT GCC AAG
AAC CTG AAC GAG TCC CTG ATC GAT CTG CAG
GAG CTG GGC AAG TAC GAG CAG TAT ATA AAG
TGG CCC TGG
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[0153] Another representative codon-optimized coding region encoding SEQ ID NO:56 is presented herein as SEQ ID NO:66.

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ATG GAT AGC AGC ATA GCC TAC TCA AAC AAC ACG
ATC GCC ATC CCC ACA AAC TTT TCC ATT TCC
ATA ACT ACC GAG GTG ATG CCC GTG AGC ATG
GCC AAG ACA TCG GTA GAT TGC AAC ATG TAC
ATC TGT GGC GAT TCT ACA GAG TGT GCC AAC
CTG CTG CTG CAG TAC GGC TCT TTC TGC ACG
CAG CTG AAC AGG GCC CTG TCT GGC ATC GCC
GCC GAG CAG GAT CGG AAC ACA CGG GAG GTT
TTC GCC CAG GTA AAG CAG ATG TAT AAG ACG
CCC ACT CTG AAG TAC TTC GGC GGC TTC AAC
TTC TCT CAG ATA CTG CCC GAC CCC CTG AAG
CCC ACT AAG AGG TCT TTT ATC GAG GAT CTG
CTG TTC AAC AAG GTT ACC CTG GCC GAT GCC
GGC TTT ATG AAG CAG TAT GGC GAG TGC CTG
GGC GAC ATC AAC GCC AGA GAT CTG ATA TGC
GCC CAG AAG TTC AAC GGC CTG ACT GTG CTG
CCC CCC CTG CTG ACT GAC GAC ATG ATC GCC
GCC TAT ACC GCC GCC CTG GTG AGT GGC ACA
GCC ACT GCC GGC TGG ACA TTC GGC GCC GGC
GCC GCC CTG CAG ATC CCC TTC GCC ATG CAG
ATG GCC TAC AGA TTT AAC GGC ATT GGC GTC
ACT CAG AAC GTC CTG TAT GAG AAC CAG AAG
CAG ATC GCC AAC CAG TTT AAC AAG GCC ATA
AGC CAG ATC CAG GAG TCA CTG ACA ACG ACA
AGT ACC GCC CTG GGC AAG CTG CAG GAT GTA
GTG AAC CAG AAC GCC CAG GCC CTG AAC ACT
CTG GTT AAG CAG CTG TCT AGC AAC TTC GGC
GCC ATC AGT AGT GTT CTG AAC GAT ATT CTG
TCT AGG CTG GAC AAG GTC GAG GCC GAG GTG
CAG ATT GAT CGC CTG ATT ACC GGC AGA CTG
CAG AGT CTG CAG ACT TAT GTA ACT CAG CAG
CTG ATC AGA GCC GCC GAG ATT CGA GCC TCC
GCC AAC CTG GCC GCC ACA AAG ATG TCT GAG
TGC GTC CTG GGC CAG AGT AAG AGG GTT GAC
TTC TGC GGC AAG GGC TAT CAT CTG ATG TCT
TTT CCC CAG GCC GCC CCC CAC GGC GTC GTG
TTC CTG CAC GTA ACT TAC GTG CCC AGT CAG
GAG AGA AAC TTT ACC ACT GCC CCC GCC ATC
TGC CAC GAG GGC AAG GCC TAC TTC CCC AGA
GAG GGC GTG TTT GTG TTC AAC GGC ACA TCT
TGG TTC ATC ACC CAG AGG AAC TTT TTC AGC
CCC CAG ATC ATA ACA ACT GAC AAC ACT TTC
GTT TCG GGC AAC TGC GAC GTA GTG ATC GGC
ATA ATA AAC AAC ACC GTG TAC GAT CCC CTG
CAG CCC GAG CTG GAC AGC TTT AAG GAG GAG
CTG GAC AAG TAC TTT AAG AAC CAT ACC TCA
CCC GAT GTG GAC CTG GGC GAC ATT TCT GGC
ATA AAC GCC TCC GTC GTC AAC ATC CAG AAG
GAG ATA GAT AGA CTG AAC GAG GTT GCC AAG
AAC CTG AAC GAG TCC CTG ATC GAT CTG CAG
GAG CTG GGC AAG TAC GAG CAG TAT ATA AAG
TGG CCC TGG
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[0154] In certain embodiments, a codon-optimized coding region encoding the full-length SARS-CoV spike protein (SEQ ID NO:23) is optimized according

to any plant, animal, or microbial species, including humans. A codon-optimized coding region encoding SEQ ID NO:23 was first established using the "uniform" optimization protocol described above. However, certain additional adjustments to the sequence were carried out in order to elminate, for example, newly opened reading frames being created on the opposite strand, splice acceptors, stretches of identical bases, or unwanted restriction enzyme sites. Making such adjustments is well within the capabilities of a person of ordinary skill in the art.

[0155] A codon-optimized coding region encoding SEQ ID NO:23 is conveniently synthesized as smaller fragments, which are then spliced together using restriction enzyme sites engineered into the sequence fragments. Examples of fragments of codon-optimized coding regions encoding SEQ ID NO:23 are as follows.

[0156] SEQ ID NO:57 has the following sequence:

GTCGACATGGTTATCTTTCTGCTGTTCCTCACCCTCACCAGCGGCAGCGATCTGGATAGGTGCACCACCT
TCGACGACGTGCAGGCCCCCAACTACACCCAGCACACCAGCAGCATGAGGGGCGTGTACTACCCCGACGA
GATTTTCAGAAGCGACACCCTGTACCTCACCCAGGACCTGTTCCTGCCCTTCTACAGCAACGTGACCGGC
TTCCACACCATCAACCACACCTTCGGCAACCCCGTGATCCCTTTCAAGGACGGCATCTACTTCGCCGCCA
CCGAGAAGAGCAATGTGGTGCGGGGCTGGGTGTTCGGCAGCACCATGAACAACAAGAGCCAGAGCGTGAT
CATCATCAACAACAGCACCAACGTGGTGATCCGGGCCTGCAATTTCGAGCTGTGCGACAACCCTTTCTTC
GCCGTGTCCAAACCTATGGGCACCCAGACCCACACCATGATCTTCGACAACGCCTTCAACTGCACCTTCG
AGTACATCAGCGACGCCTTCAGCCTGGATGTGAGCGAGAAGAGCGGCAACTTCAAGCACCTGCGGGAGTT
CGTGTTCAAGAACAAGGACGGCTTCCTGTACGTGTACAAGGGCTACCAGCCCATCGACGTGGTGAGAGAC
CTGCCCAGCGGCTTCAACACCCTGAAGCCCATCTTCAAGCTGCCCCTGGGCATCAACATCACCAACTTCC
GGGCCATCCTCACCGCCTTTAGCCCTGCCCAGGATATCTGGGGCACCAGCGCCGCTGCCTACTTCGTGGG
CTACCTGAAGCCTACCACCTTCATGCTGAAGTACGACGAGAACGGCACCATCACCGATGCCGTGGACTGC
AGCCAGAACCCCCTGGCCGAGCTGAAGTGCAGCGTGAAGAGCTTCGAGATCGACAAGGGCATCTACCAGA
CCAGCAACTTCAGAGTGGTGCCTAGCGGCGATGTGGTGAGGTTCCCCAATATCACCAACCTGTGCCCCTT
CGGCGAGGTGTTCAACGCCACCAAGTTCCCTAGCGTGTACGCCTGGGAGCGGAAGAAGATCAGCAACTGC
GTGGCCGATTACAGCGTGCTGTACAACTCCACCTTCTTCAGCACCTTCAAGTGCTACGGCGTGAGCGCCA
CCAAGCTGAACGACCTGTGCTTCAGCAACGTGTACGCCGACTCATTCGTGGTGAAGGGCGACGACGTGAG
ACAGATCGCCCCTGGCCAGACCGGCGTGATCGCCGACTACAACTACAAGCTT

[0157] Nucleotides 7 to 1242 of SEQ ID NO:57 encode amino acids 1 to 412 of SEQ ID NO:23, with the exception that amino acid 2 (Phenylalanine, (F)) of SEQ ID NO:23 is replaced with valine (V). The translation product of nucleotides 7 to 1242 of SEQ ID NO:57 is presented herein as SEQ ID NO:58.

MVIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDL
FLPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVII
NNSTNVVIRACNFELCDNPFFAVSKPMGTQTHTMIFDNAFNCTFEYISDAFSLDVSEK
SGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAI

LTAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVK
SFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVA
DYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIAD
YNYKL

[0158] Nucleotides 1 to 6 of SEQ ID NO:57, GTCGAC, is a recognition site for the restriction enzyme Sal I. Nucleotides 1237 to 1242 of SEQ ID NO:57, AAGCTT, is a recognition site for the restriction enzyme Hind III.

[0159] SEQ ID NO:59 has the following sequence:

AAGCTTCCCGACGACTTCATGGGCTGCGTGCTGGCCTGGAACACCA
GAAACATCGACGCCACCTCCACCGGCAACTACAATTACAAGTACCG
CTACCTGAGGCACGGCAAGCTGAGACCCTTCGAGCGGGACATCTCC
AACGTGCCCTTCAGCCCCGACGGCAAGCCCTGCACCCCCCCTGCCC
TGAACTGCTACTGGCCCCTGAACGACTACGGCTTCTACACCACCAC
CGGCATCGGCTATCAGCCCTACAGAGTGGTGGTGCTGAGCTTCGAG
CTGCTGAACGCCCCTGCCACCGTGTGCGGCCCCAAGCTGAGCACCG
ACCTCATCAAGAACCAGTGCGTGAACTTCAACTTCAACGGCCTCAC
CGGCACCGGCGTGCTCACCCCCAGCAGCAAGAGATTCCAGCCCTTC
CAGCAGTTCGGCAGGGACGTGAGCGATTTCACCGACAGCGTGAGG
GATCCTAAGACCAGCGAGATCCTGGACATCAGCCCTTGCAGCTTCG
GCGGCGTGTCCGTGATCACCCCCGGCACCAACGCCAGCAGCGAGG
TGGCCGTGCTGTACCAGGACGTGAACTGCACCGACGTGAGCACCGC
CATCCACGCCGACCAGCTCACCCCCGCCTGGAGAATCTACAGCACC
GGCAACAACGTGTTCCAGACCCAGGCCGGCTGCCTCATCGGCGCCG
AGCACGTGGACACCAGCTACGAGTGCGACATCCCCATCGGAGCCG
GCATCTGCGCCAGCTACCACACCGTGAGCCTGCTGAGAAGCACCAG
CCAGAAGAGCATCGTGGCCTACACCATGAGCCTGGGCGCCGACAG
CAGCATCGCCTACAGCAACAACACCATCGCCATCCCCACCAACTTC
AGCATCTCCATCACCACCGAGGTGATGCCCGTGAGCATGGCCAAGA
CCAGCGTGGATTGCAACATGTACATCTGCGGCGACAGCACCGAGTG
CGCCAACCTGCTGCTGCAGTACGGCAGCTTCTGCACCCAGCTGAAC
AGAGCCCTGAGCGGCATTGCCGCCGAGCAGGACAGAAACACCAGG
GAGGTGTTCGCCCAGGTGAAGCAGATGTATAAGACCCCCACCCTGA
AGTACTTCGGCGGGTTCAACTTCAGCCAGATCCTGCCCGATCCTCT
GAAGCCCACCAAGCGGAGCTTCATCGAGGACCTGCTGTTCAACAA
GGTGACCCTGGCCGACGCCGGCTTTATGAAGCAGTACGGCGAGTGC
CTGGGCGATATCAACGCCAGGGACCTCATCTGCGCCCAGAAGTTCA
ACGGCTTGACCGTGCTGCCCCCTCTGCTCACCGATGATATGATCGC
CGCCTATACAGCCGCCCTGGTGTCAGGCACCGCCACCGCCGGCTGG
ACCTTTGGCGCCGGAGCCGCCCTGCAGATCCCCTTCGCCATGCAGA
TGGCCTACCGGT

[0160] Nucleotides 1 to 1431 of SEQ ID NO:59 encode amino acids 411 to 887 of SEQ ID NO:23. Nucleotides 1 to 6 of SEQ ID NO:59, AAGCTT, is a recognition site for the restriction enzyme Hind III. Nucleotides 1237 to 1242 of SEQ ID NO:59, ACCGGT, is a recognition site for the restriction enzymes Age I and PinA I.

[0161] SEQ ID NO:60 has the following sequence:

ACCGGTTCAATGGCATCGGCGTGACCCAGAACGTGCTGTACGAGAACCAGAAGC
AGATCGCCAACCAGTTCAATAAGGCCATCTCCCAGATCCAGGAGAGCCTCACCAC
CACAAGCACCGCCCTGGGCAAGCTGCAGGACGTGGTGAACCAGAACGCCCAGGC
CCTGAATACCCTGGTGAAGCAGCTGAGCAGCAACTTCGGCGCCATCAGCAGCGTG
CTGAACGACATCCTGAGCAGGCTGGATAAGGTGGAGGCCGAGGTGCAGATCGAC
AGACTCATCACCGGCAGACTGCAGAGCCTGCAGACCTACGTGACCCAGCAGCTCA
TCAGAGCCGCCGAGATCAGAGCCAGCGCCAATCTGGCCGCCACCAAGATGAGCG
AGTGCGTGCTGGGCCAGAGCAAGAGAGTGGACTTCTGCGGCAAGGGCTATCACCT
CATGAGCTTCCCTCAGGCCGCTCCCCACGGCGTGGTGTTCCTGCACGTGACCTACG
TGCCTAGCCAGGAGAGGAATTTCACCACCGCCCCAGCCATCTGCCACGAGGGCAA
GGCCTACTTCCCCAGAGAGGGCGTGTTCGTGTTTAACGGCACCAGCTGGTTCATC
ACCCAGCGGAACTTCTTCAGCCCCCAGATCATCACCACAGACAACACCTTCGTGT
CCGGCAATTGCGACGTGGTCATCGGCATCATCAATAACACCGTGTACGACCCCCT
GCAGCCCGAGCTGGATAGCTTCAAGGAGGAGCTGGACAAGTACTTCAAGAACCA
CACCTCCCCCGACGTGGACCTGGGCGACATCAGCGGCATCAATGCCAGCGTGGTG
AACATCCAGAAGGAGATCGACCGGCTGAACGAGGTGGCCAAGAACCTGAACGAG
AGCCTCATCGACCTGCAGGAGCTGGGAAAGTACGAGCAGTACATCAAGTGGCCCT
GGTACGTGTGGCTGGGCTTCATCGCCGGCCTCATCGCCATCGTGATGGTGACCATC
CTGCTGTGCTGCATGACCAGCTGCTGCTCCTGCCTGAAGGGCGCCTGCAGCTGTG
GCAGCTGCTGCAAGTTCGACGAGGACGACTCAGAGCCCGTGCTGAAGGGCGTGA
AGCTGCACTACACCTGAAGATCT

[0162] Nucleotides 3 to 1109 of SEQ ID NO:60 encode amino acids 887 to 1255 of SEQ ID NO:23. Nucleotides 1 to 6 of SEQ ID NO:60, ACCGGT, is a recognition site for the restriction enzymes Age I and PinA I. Nucleotides 1113 to 1118 of SEQ ID NO:59, AGATCT, is a recognition site for the restriction enzyme Bgl II.

[0163] SEQ ID NOs 57, 59, and 60 are then spliced together using the restriction enzyme sites described above to produce a codon-optimized coding region encoding SEQ ID NO:23 in its entirety, with the exception that amino acid 2 (Phenylalanine, (F)) of SEQ ID NO:23 is replaced with valine (V). The spliced sequence is presented herein as SEQ ID NO:61.

GTCGACATGGTTATCTTTCTGCTGTTCCTCACCCTCACCAGCGGCAG
CGATCTGGATAGGTGCACCACCTTCGACGACGTGCAGGCCCCCAAC

TACACCCAGCACACCAGCAGCATGAGGGGCGTGTACTACCCCGAC
GAGATTTTCAGAAGCGACACCCTGTACCTCACCCAGGACCTGTTCC
TGCCCTTCTACAGCAACGTGACCGGCTTCCACACCATCAACCACAC
CTTCGGCAACCCCGTGATCCCTTTCAAGGACGGCATCTACTTCGCC
GCCACCGAGAAGAGCAATGTGGTGCGGGGCTGGGTGTTCGGCAGC
ACCATGAACAACAAGAGCCAGAGCGTGATCATCATCAACAACAGC
ACCAACGTGGTGATCCGGGCCTGCAATTTCGAGCTGTGCGACAACC
CTTTCTTCGCCGTGTCCAAACCTATGGGCACCCAGACCCACACCAT
GATCTTCGACAACGCCTTCAACTGCACCTTCGAGTACATCAGCGAC
GCCTTCAGCCTGGATGTGAGCGAGAAGAGCGGCAACTTCAAGCAC
CTGCGGGAGTTCGTGTTCAAGAACAAGGACGGCTTCCTGTACGTGT
ACAAGGGCTACCAGCCCATCGACGTGGTGAGAGACCTGCCCAGCG
GCTTCAACACCCTGAAGCCCATCTTCAAGCTGCCCCTGGGCATCAA
CATCACCAACTTCCGGGCCATCCTCACCGCCTTTAGCCCTGCCCAG
GATATCTGGGGCACCAGCGCCGCTGCCTACTTCGTGGGCTACCTGA
AGCCTACCACCTTCATGCTGAAGTACGACGAGAACGGCACCATCAC
CGATGCCGTGGACTGCAGCCAGAACCCCCTGGCCGAGCTGAAGTG
CAGCGTGAAGAGCTTCGAGATCGACAAGGGCATCTACCAGACCAG
CAACTTCAGAGTGGTGCCTAGCGGCGATGTGGTGAGGTTCCCCAAT
ATCACCAACCTGTGCCCCTTCGGCGAGGTGTTCAACGCCACCAAGT
TCCCTAGCGTGTACGCCTGGGAGCGGAAGAAGATCAGCAACTGCG
TGGCCGATTACAGCGTGCTGTACAACTCCACCTTCTTCAGCACCTTC
AAGTGCTACGGCGTGAGCGCCACCAAGCTGAACGACCTGTGCTTCA
GCAACGTGTACGCCGACTCATTCGTGGTGAAGGGCGACGACGTGA
GACAGATCGCCCCTGGCCAGACCGGCGTGATCGCCGACTACAACTA
CAAGCTTCCCGACGACTTCATGGGCTGCGTGCTGGCCTGGAACACC
AGAAACATCGACGCCACCTCCACCGGCAACTACAATTACAAGTACC
GCTACCTGAGGCACGGCAAGCTGAGACCCTTCGAGCGGGACATCTC
CAACGTGCCCTTCAGCCCCGACGGCAAGCCCTGCACCCCCCCTGCC
CTGAACTGCTACTGGCCCCTGAACGACTACGGCTTCTACACCACCA
CCGGCATCGGCTATCAGCCCTACAGAGTGGTGGTGCTGAGCTTCGA
GCTGCTGAACGCCCCTGCCACCGTGTGCGGCCCCAAGCTGAGCACC
GACCTCATCAAGAACCAGTGCGTGAACTTCAACTTCAACGGCCTCA
CCGGCACCGGCGTGCTCACCCCCAGCAGCAAGAGATTCCAGCCCTT
CCAGCAGTTCGGCAGGGACGTGAGCGATTTCACCGACAGCGTGAG
GGATCCTAAGACCAGCGAGATCCTGGACATCAGCCCTTGCAGCTTC
GGCGGCGTGTCCGTGATCACCCCCGGCACCAACGCCAGCAGCGAG
GTGGCCGTGCTGTACCAGGACGTGAACTGCACCGACGTGAGCACC
GCCATCCACGCCGACCAGCTCACCCCCGCCTGGAGAATCTACAGCA
CCGGCAACAACGTGTTCCAGACCCAGGCCGGCTGCCTCATCGGCGC
CGAGCACGTGGACACCAGCTACGAGTGCGACATCCCCATCGGAGC
CGGCATCTGCGCCAGCTACCACACCGTGAGCCTGCTGAGAAGCACC
AGCCAGAAGAGCATCGTGGCCTACACCATGAGCCTGGGCGCCGAC
AGCAGCATCGCCTACAGCAACAACACCATCGCCATCCCCACCAACT
TCAGCATCTCCATCACCACCGAGGTGATGCCCGTGAGCATGGCCAA
GACCAGCGTGGATTGCAACATGTACATCTGCGGCGACAGCACCGA

GTGCGCCAACCTGCTGCTGCAGTACGGCAGCTTCTGCACCCAGCTG
AACAGAGCCCTGAGCGGCATTGCCGCCGAGCAGGACAGAAACACC
AGGGAGGTGTTCGCCCAGGTGAAGCAGATGTATAAGACCCCCACC
CTGAAGTACTTCGGCGGGTTCAACTTCAGCCAGATCCTGCCCGATC
CTCTGAAGCCCACCAAGCGGAGCTTCATCGAGGACCTGCTGTTCAA
CAAGGTGACCCTGGCCGACGCCGGCTTTATGAAGCAGTACGGCGA
GTGCCTGGGCGATATCAACGCCAGGGACCTCATCTGCGCCCAGAAG
TTCAACGGCTTGACCGTGCTGCCCCCTCTGCTCACCGATGATATGAT
CGCCGCCTATACAGCCGCCCTGGTGTCAGGCACCGCCACCGCCGGC
TGGACCTTTGGCGCCGGAGCCGCCCTGCAGATCCCCTTCGCCATGC
AGATGGCCTACCGGTTCAATGGCATCGGCGTGACCCAGAACGTGCT
GTACGAGAACCAGAAGCAGATCGCCAACCAGTTCAATAAGGCCAT
CTCCCAGATCCAGGAGAGCCTCACCACCACAAGCACCGCCCTGGGC
AAGCTGCAGGACGTGGTGAACCAGAACGCCCAGGCCCTGAATACC
CTGGTGAAGCAGCTGAGCAGCAACTTCGGCGCCATCAGCAGCGTG
CTGAACGACATCCTGAGCAGGCTGGATAAGGTGGAGGCCGAGGTG
CAGATCGACAGACTCATCACCGGCAGACTGCAGAGCCTGCAGACC
TACGTGACCCAGCAGCTCATCAGAGCCGCCGAGATCAGAGCCAGC
GCCAATCTGGCCGCCACCAAGATGAGCGAGTGCGTGCTGGGCCAG
AGCAAGAGAGTGGACTTCTGCGGCAAGGGCTATCACCTCATGAGCT
TCCCTCAGGCCGCTCCCCACGGCGTGGTGTTCCTGCACGTGACCTA
CGTGCCTAGCCAGGAGAGGAATTTCACCACCGCCCCAGCCATCTGC
CACGAGGGCAAGGCCTACTTCCCCAGAGAGGGCGTGTTCGTGTTTA
ACGGCACCAGCTGGTTCATCACCCAGCGGAACTTCTTCAGCCCCCA
GATCATCACCACAGACAACACCTTCGTGTCCGGCAATTGCGACGTG
GTCATCGGCATCATCAATAACACCGTGTACGACCCCCTGCAGCCCG
AGCTGGATAGCTTCAAGGAGGAGCTGGACAAGTACTTCAAGAACC
ACACCTCCCCCGACGTGGACCTGGGCGACATCAGCGGCATCAATGC
CAGCGTGGTGAACATCCAGAAGGAGATCGACCGGCTGAACGAGGT
GGCCAAGAACCTGAACGAGAGCCTCATCGACCTGCAGGAGCTGGG
AAAGTACGAGCAGTACATCAAGTGGCCCTGGTACGTGTGGCTGGG
CTTCATCGCCGGCCTCATCGCCATCGTGATGGTGACCATCCTGCTGT
GCTGCATGACCAGCTGCTGCTCCTGCCTGAAGGGCGCCTGCAGCTG
TGGCAGCTGCTGCAAGTTCGACGAGGACGACTCAGAGCCCGTGCTG
AAGGGCGTGAAGCTGCACTACACCTGAAGATCT

[0164] The translation product of nucleotides 7 to 3771 of SEQ ID NO:61 is presented herein as SEQ ID NO:62

.

MVIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDL
FLPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIII
NNSTNVVIRACNFELCDNPFFAVSKPMGTQTHTMIFDNAFNCTFEYISDAFSLDVSEK
SGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAI
LTAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVK
SFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVA
DYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIAD

YNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDG
KPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKN
QCVNFNFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCSFGGV
SVITPGTNASSEVAVLYQDVNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGA
EHVDTSYECDIPIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTN
FSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIAAEQD
RNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGF
MKQYGECLGDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFG
AGAALQIPFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGK
LQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQ
TYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVV
FLHVTYVPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNTF
VSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQ
KEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWLGFIAGLIAIVMVTILLCCMT
SCCSCLKGACSCGSCCKFDEDDSEPVLKGVKLHYT

[0165] In certain embodiments described herein, a codon-optimized coding region encoding SEQ ID NO:8 is optimized according to codon usage in humans (*Homo sapiens*). Alternatively, a codon-optimized coding region encoding SEQ ID NO:8 may be optimized according to codon usage in any plant, animal, or microbial species.Codon-optimized coding regions encoding SEQ ID NO:8, optimized according to codon usage in humans are designed as follows. The amino acid composition of SEQ ID NO:8 is shown in Table 12.

TABLE 12

AMINO ACID		Number in SEQ ID NO:8
A	Ala	84
R	Arg	41
C	Cys	33
G	Gly	77
H	His	14
I	Ile	73
L	Leu	92
K	Lys	57
M	Met	19
F	Phe	79
P	Pro	57
S	Ser	93
T	Thr	94
W	Trp	10
Y	Tyr	52
V	Val	89

N	Asn	81
D	Asp	71
Q	Gln	55
E	Glu	40

[0166] Using the amino acid composition shown in Table 12, a human codon-optimized coding region which encodes SEQ ID NO:8 can be designed by any of the methods discussed herein. For "uniform" optimization, each amino acid is assigned the most frequent codon used in the human genome for that amino acid. According to this method, codons are assigned to the coding region encoding SEQ ID NO:8 as follows: the 79 phenylalanine codons are TTC, the 92 leucine codons are CTG, the 73 isoleucine codons are ATC, the 19 methionine codons are ATG, the 89 valine codons are GTG, the 93 serine codons are AGC, the 57 proline codons are CCC, the 94 threonine codons are ACC, the 84 alanine codons are GCC, the 52 tyrosine codons are TAC, the 14 histidine codons are CAC, the 55 glutamine codons are CAG, the 81 asparagine codons are AAC, the 57 lysine codons are AAG, the 71 aspartic acid codons are GAC, the 40 glutamic acid codons are GAG, the 33 cysteine codons are TGC, the 10 tryptophan codon is TGG, the 41 arginine codons are CGG, AGA, or AGG (the frequencies of usage of these three codons in the human genome are not significantly different), and the 77 glycine codons are GGC. The codon-optimized coding region designed by this method is presented herein as SEQ ID NO:31.

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ATG GAC GCC ATG AAG CGG GGC CTG TGC TGC
GTG CTG CTG CTG TGC GGC GCC GTG TTC GTG
AGC CCC AGC GCC CGG GGC AGC GGC AGC GAC
CTG GAC CGG TGC ACC ACC TTC GAC GAC GTG
CAG GCC CCC AAC TAC ACC CAG CAC ACC AGC
AGC ATG CGG GGC GTG TAC TAC CCC GAC GAG
ATC TTC CGG AGC GAC ACC CTG TAC CTG ACC
CAG GAC CTG TTC CTG CCC TTC TAC AGC AAC
GTG ACC GGC TTC CAC ACC ATC AAC CAC ACC
TTC GGC AAC CCC GTG ATC CCC TTC AAG GAC
GGC ATC TAC TTC GCC GCC ACC GAG AAG AGC
AAC GTG GTG CGG GGC TGG GTG TTC GGC AGC
ACC ATG AAC AAC AAG AGC CAG AGC GTG ATC
ATC ATC AAC AAC AGC ACC AAC GTG GTG ATC
CGG GCC TGC AAC TTC GAG CTG TGC GAC AAC
CCC TTC TTC GCC GTG AGC AAG CCC ATG GGC
ACC CAG ACC CAC ACC ATG ATC TTC GAC AAC
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GCC TTC AAC TGC ACC TTC GAG TAC ATC AGC
GAC GCC TTC AGC CTG GAC GTG AGC GAG AAG
AGC GGC AAC TTC AAG CAC CTG CGG GAG TTC
GTG TTC AAG AAC AAG GAC GGC TTC CTG TAC
GTG TAC AAG GGC TAC CAG CCC ATC GAC GTG
GTG CGG GAC CTG CCC AGC GGC TTC AAC ACC
CTG AAG CCC ATC TTC AAG CTG CCC CTG GGC
ATC AAC ATC ACC AAC TTC CGG GCC ATC CTG
ACC GCC TTC AGC CCC GCC CAG GAC ATC TGG
GGC ACC AGC GCC GCC GCC TAC TTC GTG GGC
TAC CTG AAG CCC ACC ACC TTC ATG CTG AAG
TAC GAC GAG AAC GGC ACC ATC ACC GAC GCC
GTG GAC TGC AGC CAG AAC CCC CTG GCC GAG
CTG AAG TGC AGC GTG AAG AGC TTC GAG ATC
GAC AAG GGC ATC TAC CAG ACC AGC AAC TTC
CGG GTG GTG CCC AGC GGC GAC GTG GTG CGG
TTC CCC AAC ATC ACC AAC CTG TGC CCC TTC
GGC GAG GTG TTC AAC GCC ACC AAG TTC CCC
AGC GTG TAC GCC TGG GAG CGG AAG AAG ATC
AGC AAC TGC GTG GCC GAC TAC AGC GTG CTG
TAC AAC AGC ACC TTC TTC AGC ACC TTC AAG
TGC TAC GGC GTG AGC GCC ACC AAG CTG AAC
GAC CTG TGC TTC AGC AAC GTG TAC GCC GAC
AGC TTC GTG GTG AAG GGC GAC GAC GTG CGG
CAG ATC GCC CCC GGC CAG ACC GGC GTG ATC
GCC GAC TAC AAC TAC AAG CTG CCC GAC GAC
TTC ATG GGC TGC GTG CTG GCC TGG AAC ACC
CGG AAC ATC GAC GCC ACC AGC ACC GGC AAC
TAC AAC TAC AAG TAC CGG TAC CTG CGG CAC
GGC AAG CTG CGG CCC TTC GAG CGG GAC ATC
AGC AAC GTG CCC TTC AGC CCC GAC GGC AAG
CCC TGC ACC CCC CCC GCC CTG AAC TGC TAC
TGG CCC CTG AAC GAC TAC GGC TTC TAC ACC
ACC ACC GGC ATC GGC TAC CAG CCC TAC CGG
GTG GTG GTG CTG AGC TTC GAG CTG CTG AAC
GCC CCC GCC ACC GTG TGC GGC CCC AAG CTG
AGC ACC GAC CTG ATC AAG AAC CAG TGC GTG
AAC TTC AAC TTC AAC GGC CTG ACC GGC ACC
GGC GTG CTG ACC CCC AGC AGC AAG CGG TTC
CAG CCC TTC CAG CAG TTC GGC CGG GAC GTG
AGC GAC TTC ACC GAC AGC GTG CGG GAC CCC
AAG ACC AGC GAG ATC CTG GAC ATC AGC CCC
TGC AGC TTC GGC GGC GTG AGC GTG ATC ACC
CCC GGC ACC AAC GCC AGC AGC GAG GTG GCC
GTG CTG TAC CAG GAC GTG AAC TGC ACC GAC
GTG AGC ACC GCC ATC CAC GCC GAC CAG CTG
ACC CCC GCC TGG CGG ATC TAC AGC ACC GGC
AAC AAC GTG TTC CAG ACC CAG GCC GGC TGC
CTG ATC GGC GCC GAG CAC GTG GAC ACC AGC
TAC GAG TGC GAC ATC CCC ATC GGC GCC GGC
ATC TGC GCC AGC TAC CAC ACC GTG AGC CTG
CTG CGG AGC ACC AGC CAG AAG AGC ATC GTG
GCC TAC ACC ATG AGC CTG GGC GCC GAC AGC
AGC ATC GCC TAC AGC AAC AAC ACC ATC GCC
ATC CCC ACC AAC TTC AGC ATC AGC ATC ACC
ACC GAG GTG ATG CCC GTG AGC ATG GCC AAG
ACC AGC GTG GAC TGC AAC ATG TAC ATC TGC
GGC GAC AGC ACC GAG TGC GCC AAC CTG CTG
CTG CAG TAC GGC AGC TTC TGC ACC CAG CTG
AAC CGG GCC CTG AGC GGC ATC GCC GCC GAG
CAG GAC CGG AAC ACC CGG GAG GTG TTC GCC
CAG GTG AAG CAG ATG TAC AAG ACC CCC ACC
CTG AAG TAC TTC GGC GGC TTC AAC TTC AGC

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CAG ATC CTG CCC GAC CCC CTG AAG CCC ACC
AAG CGG AGC TTC ATC GAG GAC CTG CTG TTC
AAC AAG GTG ACC CTG GCC GAC GCC GGC TTC
ATG AAG CAG TAC GGC GAG TGC CTG GGC GAC
ATC AAC GCC CGG GAC CTG ATC TGC GCC CAG
AAG TTC AAC GGC CTG ACC GTG CTG CCC CCC
CTG CTG ACC GAC GAC ATG ATC GCC GCC TAC
ACC GCC GCC CTG GTG AGC GGC ACC GCC ACC
GCC GGC TGG ACC TTC GGC GCC GGC GCC GCC
CTG CAG ATC CCC TTC GCC ATG CAG ATG GCC
TAC CGG TTC AAC GGC ATC GGC GTG ACC CAG
AAC GTG CTG TAC GAG AAC CAG AAG CAG ATC
GCC AAC CAG TTC AAC AAG GCC ATC AGC CAG
ATC CAG GAG AGC CTG ACC ACC ACC AGC ACC
GCC CTG GGC AAG CTG CAG GAC GTG GTG AAC
CAG AAC GCC CAG GCC CTG AAC ACC CTG GTG
AAG CAG CTG AGC AGC AAC TTC GGC GCC ATC
AGC AGC GTG CTG AAC GAC ATC CTG AGC CGG
CTG GAC AAG GTG GAG GCC GAG GTG CAG ATC
GAC CGG CTG ATC ACC GGC CGG CTG CAG AGC
CTG CAG ACC TAC GTG ACC CAG CAG CTG ATC
CGG GCC GCC GAG ATC CGG GCC AGC GCC AAC
CTG GCC GCC ACC AAG ATG AGC GAG TGC GTG
CTG GGC CAG AGC AAG CGG GTG GAC TTC TGC
GGC AAG GGC TAC CAC CTG ATG AGC TTC CCC
CAG GCC GCC CCC CAC GGC GTG GTG TTC CTG
CAC GTG ACC TAC GTG CCC AGC CAG GAG CGG
AAC TTC ACC ACC GCC CCC GCC ATC TGC CAC
GAG GGC AAG GCC TAC TTC CCC CGG GAG GGC
GTG TTC GTG TTC AAC GGC ACC AGC TGG TTC
ATC ACC CAG CGG AAC TTC TTC AGC CCC CAG
ATC ATC ACC ACC GAC AAC ACC TTC GTG AGC
GGC AAC TGC GAC GTG GTG ATC GGC ATC ATC
AAC AAC ACC GTG TAC GAC CCC CTG CAG CCC
GAG CTG GAC AGC TTC AAG GAG GAG CTG GAC
AAG TAC TTC AAG AAC CAC ACC AGC CCC GAC
GTG GAC CTG GGC GAC ATC AGC GGC ATC AAC
GCC AGC GTG GTG AAC ATC CAG AAG GAG ATC
GAC CGG CTG AAC GAG GTG GCC AAG AAC CTG
AAC GAG AGC CTG ATC GAC CTG CAG GAG CTG
GGC AAG TAC GAG CAG TAC ATC AAG TGG CCC
TGG
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[0167] Alternatively, a human codon-optimized coding region which encodes SEQ ID NO:8 can be designed by the "full optimization" method, where each amino acid is assigned codons based on the frequency of usage in the human genome. These frequencies are shown in Table 4 above. Using this latter method, codons are assigned to the coding region encoding SEQ ID NO:8 as follows: about 36 of the 79 phenylalanine codons are TTT, and about 43 of the phenylalanine codons are TTC; about 7 of the 92 leucine codons are TTA, about 12 of the leucine codons are TTG, about 12 of the leucine codons are CTT, about 18 of the leucine codons are CTC, about 7 of the leucine codons

are CTA, and about 36 of the leucine codons are CTG; about 26 of the 73 isoleucine codons are ATT, about 35 of the isoleucine codons are ATC, and about 12 of the isoleucine codons are ATA; the 19 methionine codons are ATG; about 16 of the 89 valine codons are GTT, about 41 of the valine codons are GTG, about 11 of the valine codons are GTA, and about 21 of the valine codons are GTC; about 17 of the 93 serine codons are TCT, about 20 of the serine codons are TCC, about 14 of the serine codons are TCA, about 5 of the serine codons are TCG, about 15 of the serine codons are AGT, and about 22 of the serine codons are AGC; about 16 of the 57 proline codons are CCT, about 19 of the proline codons are CCC, about 16 of the proline codons are CCA, and about 6 of the proline codons are CCG; about 23 of the 94 threonine codons are ACT, about 34 of the threonine codons are ACC, about 26 of the threonine codons are ACA, and about 11 of the threonine codons are ACG; about 22 of the 84 alanine codons are GCT, about 34 of the alanine codons are GCC, about 19 of the alanine codons are GCA, and about 9 of the alanine codons are GCG; about 23 of the 52 tyrosine codons are TAT and about 29 of the tyrosine codons are TAC; about 6 of the 14 histidine codons are CAT and about 8 of the histidine codons are CAC; about 14 of the 55 glutamine codons are CAA and about 41 of the glutamine codons are CAG; about 37 of the 81 asparagine codons are AAT and about 44 of the asparagine codons are AAC; about 24 of the 57 lysine codons are AAA and about 33 of the lysine codons are AAG; about 33 of the 71 aspartic acid codons are GAT and about 38 of the aspartic acid codons are GAC; about 17 of the 40 glutamic acid codons are GAA and about 23 of the glutamic acid codons are GAG; about 15 of the 33 cysteine codons are TGT and about 18 of the cysteine codons are TGC; the 10 tryptophan codons are TGG; about 3 of the 41 arginine codons are CGT, about 8 of the arginine codons are CGC, about 5 of the arginine codons are CGA, about 8 of the arginine codons are CGG, about 9 of the arginine codons are AGA, and about 8 of the arginine codons are AGG; and about 13 of the 77 glycine codons are GGT, about 26 of the glycine codons are GGC, about 19 of the glycine codons are GGA, and about 19 of the glycine codons are GGG.

[0168] As described above, the term "about" means that the number of amino acids encoded by a certain codon may be one more or one less than the number given. It would be understood by those of ordinary skill in the art that the total number of any amino acid in the polypeptide sequence must remain constant, therefore, if there is one "more" of one codon encoding a give amino acid, there would have to be one "less" of another codon encoding that same amino acid.

[0169] A representative "fully optimized" codon-optimized coding region encoding SEQ ID NO:8, optimized according to codon usage in humans is presented herein as SEQ ID NO:30.

ATG GAT GCA ATG AAG CGG GGC CTG TGC TGC
GTG CTC CTG CTC TGC GGG GCG GTG TTT GTG
AGC CCC AGT GCC AGA GGT AGC GGC AGC GAT
TTG GAT AGG TGC ACC ACA TTT GAT GAC GTG
CAG GCT CCC AAT TAC ACC CAG CAC ACC AGT
TCT ATG AGA GGA GTA TAC TAC CCT GAC GAG
ATC TTC CGC AGT GAT ACC CTA TAT TTA ACA
CAA GAT TTA TTC TTA CCC TTC TAC TCC AAC
GTC ACA GGG TTT CAC ACC ATC AAC CAC ACC
TTC GGC AAC CCC GTG ATC CCG TTT AAA GAT
GGC ATT TAT TTC GCA GCC ACA GAG AAG TCG
AAT GTA GTG CGG GGT TGG GTG TTT GGA TCA
ACA ATG AAT AAT AAA TCT CAG TCC GTG ATC
ATT ATT AAC AAC TCT ACG AAT GTG GTT ATA
CGA GCC TGT AAT TTC GAG TTA TGC GAT AAT
CCA TTT TTC GCG GTC AGT AAA CCA ATG GGC
ACT CAG ACC CAT ACG ATG ATT TTC GAT AAC
GCA TTC AAT TGT ACG TTT GAA TAC ATT TCT
GAT GCT TTT TCA CTC GAC GTT TCA GAA AAG
TCT GGG AAC TTC AAG CAT TTA AGA GAG TTC
GTC TTT AAA AAT AAA GAC GGG TTC CTG TAC
GTG TAT AAA GGA TAC CAG CCT ATC GAC GTG
GTG CGG GAC CTG CCA AGC GGT TTT AAT ACC
CTG AAG CCC ATC TTT AAG CTG CCC CTG GGA
ATC AAT ATT ACA AAC TTC AGG GCT ATC CTC
ACC GCT TTT AGC CCA GCT CAG GAC ATA TGG
GGA ACC TCC GCC GCC GCC TAC TTC GTC GGA
TAT TTG AAA CCA ACC ACA TTC ATG CTG AAG
TAT GAC GAA AAT GGG ACG ATT ACC GAC GCC
GTA GAC TGT AGT CAG AAC CCT TTG GCG GAG
TTG AAG TGC TCA GTC AAG AGC TTT GAG ATC
GAC AAG GGA ATT TAT CAA ACT AGC AAC TTC
AGG GTG GTG CCC TCC GGA GAT GTA GTT CGC
TTC CCC AAC ATC ACC AAC CTG TGC CCG TTC
GGT GAG GTG TTT AAT GCA ACT AAA TTC CCC
TCA GTG TAT GCC TGG GAA AGA AAG AAA ATT

AGC AAC TGT GTT GCC GAT TAC AGC GTC CTT
TAT AAC TCA ACA TTC TTC TCT ACC TTT AAG
TGC TAT GGT GTG TCC GCC ACT AAG TTG AAC
GAC CTC TGC TTT AGT AAC GTG TAC GCT GAT
TCC TTC GTG GTG AAA GGG GAT GAC GTG CGT
CAG ATT GCA CCG GGC CAG ACC GGA GTA ATC
GCC GAT TAC AAT TAC AAG TTG CCT GAC GAC
TTC ATG GGC TGC GTT CTA GCA TGG AAT ACC
CGC AAC ATA GAT GCC ACC TCA ACG GGG AAC
TAC AAC TAC AAG TAC AGA TAT CTG AGA CAC
GGT AAG CTG CGG CCT TTT GAG CGG GAT ATC
TCC AAT GTG CCT TTT AGC CCC GAT GGC AAA
CCA TGC ACC CCA CCT GCC CTG AAT TGT TAT
TGG CCT TTG AAC GAT TAT GGA TTC TAC ACT
ACC ACT GGG ATC GGT TAT CAA CCC TAC CGG
GTC GTC GTC CTG AGT TTT GAA CTC TTG AAC
GCG CCT GCA ACA GTC TGC GGA CCC AAG CTG
TCG ACA GAC CTT ATC AAG AAT CAG TGT GTG
AAC TTT AAC TTC AAT GGG CTC ACC GGT ACC
GGT GTT CTG ACT CCA TCT AGT AAG CGA TTT
CAA CCA TTC CAA CAG TTC GGC CGT GAC GTT
TCC GAT TTT ACG GAT TCG GTG CGT GAT CCA
AAA ACA TCA GAG ATC CTT GAC ATA TCG CCG
TGT TCT TTT GGA GGC GTG TCT GTG ATT ACA
CCA GGC ACT AAT GCT AGT AGC GAA GTC GCT
GTA CTA TAC CAG GAC GTG AAC TGC ACC GAC
GTG AGC ACG GCA ATC CAC GCT GAC CAG CTG
ACC CCC GCC TGG CGC ATC TAC AGT ACA GGC
AAT AAC GTC TTT CAG ACC CAG GCC GGC TGT
CTG ATT GGG GCT GAG CAC GTC GAC ACT TCC
TAT GAA TGT GAT ATT CCC ATC GGC GCT GGA
ATT TGT GCT AGC TAT CAC ACA GTC TCC CTT
TTA AGA TCA ACC AGC CAG AAA TCT ATT GTG
GCT TAC ACA ATG TCT CTC GGC GCA GAC TCA
TCA ATT GCC TAT AGC AAC AAT ACC ATT GCA
ATC CCT ACC AAT TTT AGT ATA TCC ATA ACC
ACC GAG GTG ATG CCC GTG TCT ATG GCG AAA
ACT TCC GTC GAT TGC AAC ATG TAT ATC TGC
GGG GAC TCC ACA GAA TGC GCC AAC CTG CTT
CTG CAG TAT GGA AGC TTC TGT ACT CAA CTC
AAC CGC GCA TTG TCT GGG ATT GCC GCC GAG
CAG GAT AGG AAT ACT AGA GAG GTG TTC GCT
CAG GTT AAA CAA ATG TAC AAG ACA CCG ACA
CTT AAG TAC TTC GGA GGT TTT AAC TTT TCC
CAG ATA CTC CCT GAC CCT CTA AAG CCT ACT
AAA CGC AGT TTC ATC GAG GAT CTC CTG TTT
AAT AAG GTG ACA CTC GCC GAT GCT GGC TTC
ATG AAA CAA TAC GGA GAA TGC CTG GGA GAC
ATT AAC GCC AGA GAC CTG ATC TGT GCC CAG
AAG TTC AAC GGT CTG ACA GTA CTT CCT CCC
CTT CTG ACG GAC GAC ATG ATT GCT GCA TAC
ACA GCC GCC CTA GTT AGC GGC ACA GCC ACA
GCT GGG TGG ACC TTT GGC GCT GGC GCA GCG
TTG CAG ATT CCA TTC GCG ATG CAG ATG GCT
TAC CGA TTT AAC GGG ATC GGC GTG ACT CAG

AAT GTT TTG TAT GAG AAC CAG AAA CAG ATC
GCT AAT CAG TTT AAC AAG GCA ATC AGC CAG
ATA CAA GAA TCT CTG ACT ACC ACA AGC ACC
GCT CTG GGA AAA CTG CAG GAC GTG GTG AAT
CAG AAT GCA CAG GCC CTC AAC ACG CTC GTG
AAG CAG CTT AGT TCC AAT TTC GGG GCC ATC
TCC TCC GTT TTA AAT GAT ATC CTG AGT CGC
CTG GAC AAG GTC GAG GCC GAA GTT CAG ATC
GAC CGC CTG ATC ACA GGG AGG CTA CAA TCA
TTG CAG ACT TAC GTG ACT CAG CAG CTC ATA
AGG GCT GCA GAG ATT AGG GCC TCT GCA AAC
CTT GCC GCG ACC AAG ATG TCC GAG TGT GTT
CTC GGT CAG TCC AAA CGG GTT GAC TTT TGT
GGC AAA GGC TAC CAT CTG ATG AGC TTC CCC
CAG GCC GCA CCC CAT GGC GTA GTC TTT CTG
CAC GTA ACT TAT GTG CCA TCC CAA GAA AGG
AAC TTC ACT ACG GCG CCA GCC ATA TGC CAT
GAA GGT AAA GCA TAT TTC CCT CGA GAA GGG
GTA TTT GTT TTC AAC GGG ACT AGC TGG TTT
ATT ACG CAG CGG AAT TTC TTC TCA CCA CAA
ATC ATC ACT ACT GAT AAC ACA TTC GTC AGC
GGC AAT TGT GAC GTC GTC ATT GGA ATT ATA
AAC AAC ACT GTG TAC GAT CCT CTG CAG CCG
GAA CTG GAT TCT TTT AAG GAG GAG CTC GAC
AAG TAC TTC AAA AAC CAT ACC TCG CCC GAC
GTG GAC CTA GGC GAT ATC TCT GGG ATT AAT
GCC TCA GTA GTC AAC ATC CAG AAG GAG ATA
GAC CGA CTT AAT GAG GTT GCC AAG AAT CTG
AAT GAG AGT CTC ATC GAT CTG CAA GAA CTT
GGC AAG TAT GAA CAA TAT ATC AAA TGG CCA
TGG

[0170] In certain embodiments described herein, a codon-optimized coding region encoding SEQ ID NO:10 is optimized according to codon usage in humans (*Homo sapiens*). Alternatively, a codon-optimized coding region encoding SEQ ID NO:10 may be optimized according to codon usage in any plant, animal, or microbial species.Codon-optimized coding regions encoding SEQ ID NO:10, optimized according to codon usage in humans are designed as follows. The amino acid composition of SEQ ID NO:10 is shown in Table 13.

TABLE 13

AMINO ACID		Number in SEQ ID NO:10
A	Ala	41
R	Arg	25
C	Cys	23

G	Gly	47
H	His	9
I	Ile	37
L	Leu	46
K	Lys	32
M	Met	9
F	Phe	51
P	Pro	38
S	Ser	58
T	Thr	56
W	Trp	6
Y	Tyr	35
V	Val	56
N	Asn	46
D	Asp	45
Q	Gln	21
E	Glu	17

[0171] Using the amino acid composition shown in Table 13, a human codon-optimized coding region which encodes SEQ ID NO:10 can be designed by any of the methods discussed herein. For "uniform" optimization, each amino acid is assigned the most frequent codon used in the human genome for that amino acid. According to this method, codons are assigned to the coding region encoding SEQ ID NO:10 as follows: the 51 phenylalanine codons are TTC, the 46 leucine codons are CTG, the 37 isoleucine codons are ATC, the 9 methionine codons are ATG, the 56 valine codons are GTG, the 58 serine codons are AGC, the 38 proline codons are CCC, the 56 threonine codons are ACC, the 41 alanine codons are GCC, the 35 tyrosine codons are TAC, the 9 histidine codons are CAC, the 21 glutamine codons are CAG, the 46 asparagine codons are AAC, the 32 lysine codons are AAG, the 45 aspartic acid codons are GAC, the 17 glutamic acid codons are GAG, the 23 cysteine codons are TGC, the 6 tryptophan codons are TGG, the 25 arginine codons are CGG, AGA, or AGG (the frequencies of usage of these three codons in the human genome are not significantly different), and the 47 glycine codons are GGC. The codon-optimized coding region designed by this method is presented herein as SEQ ID NO:33.

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ATG GAC GCC ATG AAG CGG GGC CTG TGC TGC
GTG CTG CTG CTG TGC GGC GCC GTG TTC GTG
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AGC CCC AGC GCC CGG GGC AGC GGC AGC GAC
CTG GAC CGG TGC ACC ACC TTC GAC GAC GTG
CAG GCC CCC AAC TAC ACC CAG CAC ACC AGC
AGC ATG CGG GGC GTG TAC TAC CCC GAC GAG
ATC TTC CGG AGC GAC ACC CTG TAC CTG ACC
CAG GAC CTG TTC CTG CCC TTC TAC AGC AAC
GTG ACC GGC TTC CAC ACC ATC AAC CAC ACC
TTC GGC AAC CCC GTG ATC CCC TTC AAG GAC
GGC ATC TAC TTC GCC GCC ACC GAG AAG AGC
AAC GTG GTG CGG GGC TGG GTG TTC GGC AGC
ACC ATG AAC AAC AAG AGC CAG AGC GTG ATC
ATC ATC AAC AAC AGC ACC AAC GTG GTG ATC
CGG GCC TGC AAC TTC GAG CTG TGC GAC AAC
CCC TTC TTC GCC GTG AGC AAG CCC ATG GGC
ACC CAG ACC CAC ACC ATG ATC TTC GAC AAC
GCC TTC AAC TGC ACC TTC GAG TAC ATC AGC
GAC GCC TTC AGC CTG GAC GTG AGC GAG AAG
AGC GGC AAC TTC AAG CAC CTG CGG GAG TTC
GTG TTC AAG AAC AAG GAC GGC TTC CTG TAC
GTG TAC AAG GGC TAC CAG CCC ATC GAC GTG
GTG CGG GAC CTG CCC AGC GGC TTC AAC ACC
CTG AAG CCC ATC TTC AAG CTG CCC CTG GGC
ATC AAC ATC ACC AAC TTC CGG GCC ATC CTG
ACC GCC TTC AGC CCC GCC CAG GAC ATC TGG
GGC ACC AGC GCC GCC GCC TAC TTC GTG GGC
TAC CTG AAG CCC ACC ACC TTC ATG CTG AAG
TAC GAC GAG AAC GGC ACC ATC ACC GAC GCC
GTG GAC TGC AGC CAG AAC CCC CTG GCC GAG
CTG AAG TGC AGC GTG AAG AGC TTC GAG ATC
GAC AAG GGC ATC TAC CAG ACC AGC AAC TTC
CGG GTG GTG CCC AGC GGC GAC GTG GTG CGG
TTC CCC AAC ATC ACC AAC CTG TGC CCC TTC
GGC GAG GTG TTC AAC GCC ACC AAG TTC CCC
AGC GTG TAC GCC TGG GAG CGG AAG AAG ATC
AGC AAC TGC GTG GCC GAC TAC AGC GTG CTG
TAC AAC AGC ACC TTC TTC AGC ACC TTC AAG
TGC TAC GGC GTG AGC GCC ACC AAG CTG AAC
GAC CTG TGC TTC AGC AAC GTG TAC GCC GAC
AGC TTC GTG GTG AAG GGC GAC GAC GTG CGG
CAG ATC GCC CCC GGC CAG ACC GGC GTG ATC
GCC GAC TAC AAC TAC AAG CTG CCC GAC GAC
TTC ATG GGC TGC GTG CTG GCC TGG AAC ACC
CGG AAC ATC GAC GCC ACC AGC ACC GGC AAC
TAC AAC TAC AAG TAC CGG TAC CTG CGG CAC
GGC AAG CTG CGG CCC TTC GAG CGG GAC ATC
AGC AAC GTG CCC TTC AGC CCC GAC GGC AAG
CCC TGC ACC CCC CCC GCC CTG AAC TGC TAC
TGG CCC CTG AAC GAC TAC GGC TTC TAC ACC
ACC ACC GGC ATC GGC TAC CAG CCC TAC CGG
GTG GTG GTG CTG AGC TTC GAG CTG CTG AAC
GCC CCC GCC ACC GTG TGC GGC CCC AAG CTG
AGC ACC GAC CTG ATC AAG AAC CAG TGC GTG
AAC TTC AAC TTC AAC GGC CTG ACC GGC ACC
GGC GTG CTG ACC CCC AGC AGC AAG CGG TTC
CAG CCC TTC CAG CAG TTC GGC CGG GAC GTG
AGC GAC TTC ACC GAC AGC GTG CGG GAC CCC
AAG ACC AGC GAG ATC CTG GAC ATC AGC CCC
TGC AGC TTC GGC GGC GTG AGC GTG ATC ACC
CCC GGC ACC AAC GCC AGC AGC GAG GTG GCC
GTG CTG TAC CAG GAC GTG AAC TGC ACC GAC
GTG AGC ACC GCC ATC CAC GCC GAC CAG CTG
ACC CCC GCC TGG CGG ATC TAC AGC ACC GGC
AAC AAC GTG TTC CAG ACC CAG GCC GGC TGC

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CTG ATC GGC GCC GAG CAC GTG GAC ACC AGC
TAC GAG TGC GAC ATC CCC ATC GGC GCC GGC
ATC TGC GCC AGC TAC CAC ACC GTG AGC CTG
CTG CGG AGC ACC AGC CAG AAG AGC ATC GTG
GCC TAC ACC ATG AGC CTG GGC
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[0172] Alternatively, a human codon-optimized coding region which encodes SEQ ID NO:10 can be designed by the "full optimization" method, where each amino acid is assigned codons based on the frequency of usage in the human genome. These frequencies are shown in Table 4 above. Using this latter method, codons are assigned to the coding region encoding SEQ ID NO:10 as follows: about 23 of the 51 phenylalanine codons are TTT, and about 28 of the phenylalanine codons are TTC; about 3 of the 46 leucine codons are TTA, about 6 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 9 of the leucine codons are CTC, about 4 of the leucine codons are CTA, and about 18 of the leucine codons are CTG; about 13 of the 37 isoleucine codons are ATT, about 18 of the isoleucine codons are ATC, and about 6 of the isoleucine codons are ATA; the 9 methionine codons are ATG; about 10 of the 56 valine codons are GTT, about 26 of the valine codons are GTG, about 7 of the valine codons are GTA, and about 13 of the valine codons are GTC; about 11 of the 58 serine codons are TCT, about 13 of the serine codons are TCC, about 9 of the serine codons are TCA, about 3 of the serine codons are TCG, about 8 of the serine codons are AGT, and about 14 of the serine codons are AGC; about 11 of the 38 proline codons are CCT, about 13 of the proline codons are CCC, about 10 of the proline codons are CCA, and about 4 of the proline codons are CCG; about 14 of the 56 threonine codons are ACT, about 20 of the threonine codons are ACC, about 16 of the threonine codons are ACA, and about 6 of the threonine codons are ACG; about 11 of the 41 alanine codons are GCT, about 16 of the alanine codons are GCC, about 10 of the alanine codons are GCA, and about 4 of the alanine codons are GCG; about 15 of the 35 tyrosine codons are TAT and about 20 of the tyrosine codons are TAC; about 4 of the 9 histidine codons are CAT and about 5 of the histidine codons are CAC; about 5 of the 21 glutamine codons

are CAA and about 16 of the glutamine codons are CAG; about 21 of the 46 asparagine codons are AAT and about 25 of the asparagine codons are AAC; about 14 of the 32 lysine codons are AAA and about 18 of the lysine codons are AAG; about 21 of the 45 aspartic acid codons are GAT and about 24 of the aspartic acid codons are GAC; about 7 of the 17 glutamic acid codons are GAA and about 10 of the glutamic acid codons are GAG; about 10 of the 23 cysteine codons are TGT and about 13 of the cysteine codons are TGC; the 6 tryptophan codons are TGG; about 2 of the 25 arginine codons are CGT, about 5 of the arginine codons are CGC, about 3 of the arginine codons are CGA, about 5 of the arginine codons are CGG, about 5 of the arginine codons are AGA, and about 5 of the arginine codons are AGG; and about 8 of the 47 glycine codons are GGT, about 16 of the glycine codons are GGC, about 11 of the glycine codons are GGA, and about 12 of the glycine codons are GGG.

[0173] As described above, the term "about" means that the number of amino acids encoded by a certain codon may be one more or one less than the number given. It would be understood by those of ordinary skill in the art that the total number of any amino acid in the polypeptide sequence must remain constant, therefore, if there is one "more" of one codon encoding a give amino acid, there would have to be one "less" of another codon encoding that same amino acid.

[0174] A representative "fully optimized" codon-optimized coding region encoding SEQ ID NO: 10, optimized according to codon usage in humans is presented herein as SEQ ID NO:32.

ATG GAC GCC ATG AAG CGA GGA CTG TGC TGC
GTT TTG TTG CTG TGC GGC GCA GTT TTT GTC
AGT CCA TCC GCC CGG GGG TCG GGA TCT GAC
CTA GAT AGA TGC ACG ACC TTC GAT GAC GTG
CAG GCA CCA AAT TAC ACC CAA CAT ACT TCA
TCC ATG CGC GGC GTT TAC TAT CCC GAC GAA
ATC TTC CGG AGT GAC ACC CTG TAT CTG ACT
CAG GAC CTG TTT CTG CCC TTC TAC AGC AAT
GTG ACA GGC TTT CAC ACC ATT AAC CAT ACC
TTC GGG AAT CCA GTA ATC CCT TTT AAG GAT
GGG ATT TAC TTT GCT GCT ACT GAG AAA AGT
AAT GTT GTC AGG GGG TGG GTT TTT GGC TCA

ACA ATG AAC AAT AAG TCT CAG AGT GTC ATC
ATC ATT AAC AAT TCT ACC AAT GTA GTC ATC
AGA GCA TGC AAC TTC GAG CTC TGT GAT AAC
CCT TTC TTT GCT GTG TCT AAG CCC ATG GGC
ACT CAA ACA CAT ACC ATG ATC TTC GAC AAT
GCG TTC AAT TGT ACC TTT GAG TAT ATA TCA
GAC GCC TTC AGC CTA GAC GTC TCG GAA AAG
TCC GGA AAC TTT AAA CAC CTG CGG GAA TTC
GTG TTT AAG AAC AAA GAT GGA TTT TTG TAC
GTA TAC AAG GGT TAT CAG CCT ATC GAT GTC
GTG CGT GAT CTG CCC TCC GGC TTC AAC ACC
CTG AAG CCT ATA TTC AAA CTA CCC CTA GGG
ATC AAC ATC ACC AAT TTT AGG GCA ATA CTT
ACG GCA TTT TCC CCA GCC CAG GAC ATC TGG
GGA ACT TCC GCC GCT GCC TAC TTT GTG GGC
TAT CTC AAG CCT ACT ACT TTC ATG CTT AAG
TAT GAT GAG AAT GGC ACA ATC ACG GAT GCA
GTG GAT TGC TCG CAG AAT CCA CTT GCT GAG
CTG AAA TGC TCC GTA AAG AGC TTC GAA ATT
GAT AAA GGA ATC TAT CAG ACC AGC AAC TTC
CGG GTC GTG CCC TCT GGC GAC GTT GTC CGG
TTC CCC AAC ATC ACC AAC CTC TGC CCA TTC
GGC GAG GTG TTC AAC GCT ACA AAA TTC CCA
AGT GTC TAC GCC TGG GAG AGG AAA AAG ATC
TCT AAT TGT GTG GCA GAT TAT TCC GTG TTA
TAC AAC AGC ACA TTC TTC TCA ACG TTC AAG
TGT TAT GGC GTG AGC GCC ACC AAG CTT AAC
GAC CTC TGC TTC TCC AAT GTA TAC GCT GAC
TCT TTT GTG GTT AAG GGA GAC GAT GTG CGA
CAG ATC GCC CCG GGG CAA ACC GGA GTG ATT
GCG GAC TAC AAC TAT AAA CTG CCC GAC GAT
TTC ATG GGT TGT GTG CTT GCT TGG AAT ACG
AGG AAC ATT GAC GCA ACG AGC ACC GGG AAC
TAT AAT TAC AAA TAT CGT TAC CTG CGC CAT
GGG AAA CTC AGA CCT TTT GAA CGA GAT ATT
AGC AAC GTC CCT TTC TCA CCG GAT GGG AAG
CCC TGT ACC CCA CCT GCC CTG AAC TGC TAT
TGG CCT CTC AAC GAC TAC GGC TTC TAC ACT
ACC ACA GGG ATC GGG TAC CAG CCC TAT CGC
GTG GTG GTT CTC TCC TTT GAA CTC CTT AAT
GCT CCC GCG ACT GTG TGT GGG CCG AAG TTG
AGT ACT GAC TTA ATA AAA AAT CAA TGC GTA
AAC TTT AAC TTT AAT GGC TTG ACA GGT ACA
GGT GTG CTC ACA CCG AGT AGC AAA AGG TTC
CAG CCA TTT CAG CAA TTT GGC AGA GAT GTG
TCT GAC TTT ACA GAC AGC GTG CGC GAT CCT
AAG ACT TCT GAG ATT TTA GAC ATC TCA CCT
TGT TCC TTT GGA GGA GTG AGC GTG ATA ACT
CCC GGT ACC AAC GCC TCA TCC GAA GTG GCT
GTC CTG TAT CAG GAC GTT AAT TGC ACC GAT
GTC TCT ACA GCC ATT CAC GCC GAT CAG CTG
ACA CCA GCT TGG CGC ATC TAC AGT ACC GGT
AAC AAT GTT TTC CAG ACT CAG GCC GGT TGT
CTG ATT GGC GCC GAG CAC GTC GAC ACA TCT
TAC GAG TGC GAT ATT CCC ATA GGT GCC GGC

ATT TGT GCG AGC TAC CAC ACT GTA TCA CTG
CTG AGA AGC ACA AGC CAG AAA TCA ATT GTG
GCA TAC ACA ATG TCC TTG GGA GCA

[0175] In certain embodiments described herein, a codon-optimized coding region encoding SEQ ID NO:12 is optimized according to codon usage in humans (*Homo sapiens*). Alternatively, a codon-optimized coding region encoding SEQ ID NO:12 may be optimized according to codon usage in any plant, animal, or microbial species.Codon-optimized coding regions encoding SEQ ID NO:12, optimized according to codon usage in humans are designed as follows. The amino acid composition of SEQ ID NO:12 is shown in Table 14.

TABLE 14

AMINO ACID		Number in SEQ ID NO:12
A	Ala	46
R	Arg	18
C	Cys	13
G	Gly	34
H	His	5
I	Ile	36
L	Leu	50
K	Lys	26
M	Met	12
F	Phe	29
P	Pro	20
S	Ser	38
T	Thr	38
W	Trp	4
Y	Tyr	17
V	Val	36
N	Asn	35
D	Asp	27
Q	Gln	34
E	Glu	23

[0176] Using the amino acid composition shown in Table 14, a human codon-optimized coding region which encodes SEQ ID NO:12 can be designed by any of the methods discussed herein. For "uniform" optimization, each amino acid is assigned the most frequent codon used in the human genome for that

amino acid. According to this method, codons are assigned to the coding region encoding SEQ ID NO:12 as follows: the 29 phenylalanine codons are TTC, the 50 leucine codons are CTG, the 36 isoleucine codons are ATC, the 12 methionine codons are ATG, the 36 valine codons are GTG, the 38 serine codons are AGC, the 20 proline codons are CCC, the 38 threonine codons are ACC, the 46 alanine codons are GCC, the 17 tyrosine codons are TAC, the 5 histidine codons are CAC, the 34 glutamine codons are CAG, the 35 asparagine codons are AAC, the 26 lysine codons are AAG, the 35 aspartic acid codons are GAC, the 23 glutamic acid codons are GAG, the 13 cysteine codons are TGC, the 4 tryptophan codon is TGG, the 18 arginine codons are CGG, AGA, or AGG (the frequencies of usage of these three codons in the human genome are not significantly different), and the 34 glycine codons are GGC. The codon-optimized coding region designed by this method is presented herein as SEQ ID NO:35.

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ATG GAC GCC ATG AAG CGG GGC CTG TGC TGC
GTG CTG CTG CTG TGC GGC GCC GTG TTC GTG
AGC CCC AGC GCC CGG GGC AGC GGC GAC AGC
AGC ATC GCC TAC AGC AAC AAC ACC ATC GCC
ATC CCC ACC AAC TTC AGC ATC AGC ATC ACC
ACC GAG GTG ATG CCC GTG AGC ATG GCC AAG
ACC AGC GTG GAC TGC AAC ATG TAC ATC TGC
GGC GAC AGC ACC GAG TGC GCC AAC CTG CTG
CTG CAG TAC GGC AGC TTC TGC ACC CAG CTG
AAC CGG GCC CTG AGC GGC ATC GCC GCC GAG
CAG GAC CGG AAC ACC CGG GAG GTG TTC GCC
CAG GTG AAG CAG ATG TAC AAG ACC CCC ACC
CTG AAG TAC TTC GGC GGC TTC AAC TTC AGC
CAG ATC CTG CCC GAC CCC CTG AAG CCC ACC
AAG CGG AGC TTC ATC GAG GAC CTG CTG TTC
AAC AAG GTG ACC CTG GCC GAC GCC GGC TTC
ATG AAG CAG TAC GGC GAG TGC CTG GGC GAC
ATC AAC GCC CGG GAC CTG ATC TGC GCC CAG
AAG TTC AAC GGC CTG ACC GTG CTG CCC CCC
CTG CTG ACC GAC GAC ATG ATC GCC GCC TAC
ACC GCC GCC CTG GTG AGC GGC ACC GCC ACC
GCC GGC TGG ACC TTC GGC GCC GGC GCC GCC
CTG CAG ATC CCC TTC GCC ATG CAG ATG GCC
TAC CGG TTC AAC GGC ATC GGC GTG ACC CAG
AAC GTG CTG TAC GAG AAC CAG AAG CAG ATC
GCC AAC CAG TTC AAC AAG GCC ATC AGC CAG
ATC CAG GAG AGC CTG ACC ACC ACC AGC ACC
GCC CTG GGC AAG CTG CAG GAC GTG GTG AAC
CAG AAC GCC CAG GCC CTG AAC ACC CTG GTG
AAG CAG CTG AGC AGC AAC TTC GGC GCC ATC
AGC AGC GTG CTG AAC GAC ATC CTG AGC CGG
CTG GAC AAG GTG GAG GCC GAG GTG CAG ATC
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GAC CGG CTG ATC ACC GGC CGG CTG CAG AGC
CTG CAG ACC TAC GTG ACC CAG CAG CTG ATC
CGG GCC GCC GAG ATC CGG GCC AGC GCC AAC
CTG GCC GCC ACC AAG ATG AGC GAG TGC GTG
CTG GGC CAG AGC AAG CGG GTG GAC TTC TGC
GGC AAG GGC TAC CAC CTG ATG AGC TTC CCC
CAG GCC GCC CCC CAC GGC GTG GTG TTC CTG
CAC GTG ACC TAC GTG CCC AGC CAG GAG CGG
AAC TTC ACC ACC GCC CCC GCC ATC TGC CAC
GAG GGC AAG GCC TAC TTC CCC CGG GAG GGC
GTG TTC GTG TTC AAC GGC ACC AGC TGG TTC
ATC ACC CAG CGG AAC TTC TTC AGC CCC CAG
ATC ATC ACC ACC GAC AAC ACC TTC GTG AGC
GGC AAC TGC GAC GTG GTG ATC GGC ATC ATC
AAC AAC ACC GTG TAC GAC CCC CTG CAG CCC
GAG CTG GAC AGC TTC AAG GAG GAG CTG GAC
AAG TAC TTC AAG AAC CAC ACC AGC CCC GAC
GTG GAC CTG GGC GAC ATC AGC GGC ATC AAC
GCC AGC GTG GTG AAC ATC CAG AAG GAG ATC
GAC CGG CTG AAC GAG GTG GCC AAG AAC CTG
AAC GAG AGC CTG ATC GAC CTG CAG GAG CTG
GGC AAG TAC GAG CAG TAC ATC AAG TGG CCC
TGG
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[0177] Alternatively, a human codon-optimized coding region which encodes SEQ ID NO:12 can be designed by the "full optimization" method, where each amino acid is assigned codons based on the frequency of usage in the human genome. These frequencies are shown in Table 4 above. Using this latter method, codons are assigned to the coding region encoding SEQ ID NO:12 as follows: about 13 of the 29 phenylalanine codons are TTT, and about 16 of the phenylalanine codons are TTC; about 4 of the 50 leucine codons are TTA, about 6 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 10 of the leucine codons are CTC, about 4 of the leucine codons are CTA, and about 20 of the leucine codons are CTG; about13 of the 36 isoleucine codons are ATT, about 17 of the isoleucine codons are ATC, and about 6 of the isoleucine codons are ATA; the 12 methionine codons are ATG; about 6 of the 36 valine codons are GTT, about 9 of the valine codons are GTG, about 4 of the valine codons are GTA, and about 17 of the valine codons are GTG; about 7 of the 38 serine codons are TCT, about 8 of the serine codons are TCC, about 6 of the serine codons are TCA, about 2 of the serine codons are TCG, about 6 of the serine codons are AGT, and about 9 of the serine codons are AGC; about 6 of the 20 proline codons are CCT, about 7

of the proline codons are CCC, about 5 of the proline codons are CCA, and about 2 of the proline codons are CCG; about 9 of the 38 threonine codons are ACT, about 14 of the threonine codons are ACC, about 11 of the threonine codons are ACA, and about 4 of the threonine codons are ACG; about 12 of the 46 alanine codons are GCT, about 19 of the alanine codons are GCC, about 10 of the alanine codons are GCA, and about 5 of the alanine codons are GCG; about 7 of the 17 tyrosine codons are TAT and about 10 of the tyrosine codons are TAC; about 2 of the 5 histidine codons are CAT and about 3 of the histidine codons are CAC; about 9 of the 34 glutamine codons are CAA and about 25 of the glutamine codons are CAG; about 16 of the 35 asparagine codons are AAT and about 19 of the asparagine codons are AAC; about 11 of the 26 lysine codons are AAA and about 15 of the lysine codons are AAG; about 12 of the 27 aspartic acid codons are GAT and about 15 of the aspartic acid codons are GAC; about 16 of the 23 glutamic acid codons are GAA and about 13 of the glutamic acid codons are GAG; about 6 of the 13 cysteine codons are TGT and about 7 of the cysteine codons are TGC; the 4 tryptophan codons are TGG; about 1 of the 18 arginine codons are CGT, about 3 of the arginine codons are CGC, about 2 of the arginine codons are CGA, about 4 of the arginine codons are CGG, about 4 of the arginine codons are AGA, and about 4 of the arginine codons are AGG; and about 6 of the 34 glycine codons are GGT, about 12 of the glycine codons are GGC, about 8 of the glycine codons are GGA, and about 8 of the glycine codons are GGG.

[0178] As described above, the term "about" means that the number of amino acids encoded by a certain codon may be one more or one less than the number given. It would be understood by those of ordinary skill in the art that the total number of any amino acid in the polypeptide sequence must remain constant, therefore, if there is one "more" of one codon encoding a give amino acid, there would have to be one "less" of another codon encoding that same amino acid.

[0179] A representative "fully optimized" codon-optimized coding region encoding SEQ ID NO:12, optimized according to codon usage in humans is presented herein as SEQ ID NO:34.

ATG GAT GCA ATG AAA AGA GGC CTG TGT TGT
GTT CTG CTG CTG TGT GGG GCG GTA TTT GTG
AGT CCC TCT GCC AGG GGA AGC GGC GAC AGC
AGT ATA GCC TAC TCA AAC AAT ACC ATC GCC
ATT CCT ACA AAT TTT TCC ATC TCA ATC ACG
ACG GAA GTC ATG CCA GTT AGC ATG GCC AAA
ACC TCT GTC GAC TGC AAC ATG TAC ATC TGC
GGA GAC TCT ACT GAG TGC GCA AAC CTG CTC
TTG CAG TAT GGC TCG TTT TGC ACC CAG TTG
AAT CGG GCC CTC AGT GGC ATT GCC GCA GAA
CAA GAT CGG AAT ACC AGG GAG GTC TTC GCG
CAA GTC AAG CAG ATG TAC AAA ACC CCT ACA
CTC AAA TAC TTC GGG GGG TTC AAC TTT AGC
CAA ATC CTG CCA GAC CCC CTC AAG CCT ACT
AAG CGC AGT TTT ATC GAA GAC TTA CTC TTT
AAT AAG GTG ACA TTA GCT GAT GCC GGA TTC
ATG AAG CAG TAC GGA GAG TGC CTG GGG GAT
ATC AAC GCG CGG GAC CTA ATC TGT GCC CAG
AAG TTC AAC GGT CTG ACA GTG CTT CCG CCT
CTC CTG ACC GAT GAT ATG ATC GCA GCT TAC
ACC GCC GCA CTG GTT AGT GGT ACG GCC ACA
GCA GGC TGG ACC TTC GGT GCC GGT GCT GCC
CTG CAA ATC CCA TTC GCG ATG CAG ATG GCA
TAC AGA TTT AAC GGC ATT GGA GTC ACC CAG
AAT GTC CTA TAC GAG AAC CAG AAG CAA ATC
GCT AAC CAG TTC AAC AAA GCC ATA TCC CAG
ATT CAG GAG TCC CTT ACT ACA ACC AGT ACT
GCT TTA GGT AAA CTG CAA GAT GTA GTG AAC
CAG AAC GCT CAG GCC TTA AAT ACC CTT GTT
AAA CAG CTA TCC TCA AAC TTT GGG GCT ATC
TCC TCC GTG CTC AAC GAT ATC CTG AGC CGC
CTC GAT AAG GTG GAA GCG GAG GTC CAG ATC
GAT AGA CTT ATT ACA GGC AGG CTT CAG TCT
CTC CAG ACC TAT GTC ACA CAA CAG CTC ATT
CGT GCT GCA GAG ATC CGC GCT TCC GCC AAC
TTG GCT GCA ACA AAG ATG TCT GAA TGT GTG
CTG GGA CAG AGC AAG AGA GTG GAC TTT TGT
GGG AAA GGC TAT CAC TTG ATG AGC TTC CCC
CAG GCC GCC CCC CAT GGA GTG GTA TTC CTA
CAC GTG ACG TAC GTT CCA TCT CAA GAA CGA
AAT TTC ACC ACC GCA CCT GCC ATT TGC CAC
GAA GGG AAG GCT TAT TTC CCT CGA GAG GGC
GTG TTC GTT TTT AAC GGG ACT TCA TGG TTT
ATA ACT CAA AGG AAT TTC TTC TCG CCC CAG
ATA ATT ACA ACA GAC AAC ACT TTT GTG AGC
GGC AAT TGC GAC GTG GTC ATA GGT ATT ATT
AAT AAT ACT GTG TAT GAC CCG CTG CAG CCC
GAA CTG GAC AGC TTT AAA GAG GAG CTG GAC

AAA TAC TTC AAG AAT CAT ACT TCA CCC GAC
GTG GAT CTG GGC GAC ATA TCC GGA ATC AAT
GCC TCT GTG GTA AAC ATT CAG AAG GAG ATC
GAT CGG CTG AAC GAA GTG GCT AAG AAT CTG
AAT GAA TCA TTG ATT GAC CTT CAG GAG TTG
GGC AAG TAT GAG CAG TAT ATT AAA TGG CCA
TGG

[0180] Another representative codon-optimized coding region encoding SEQ ID NO:12 is presented herein as SEQ ID NO:47.

ATG GAT GCC ATG AAG CGA GGC CTG TGT TGC
GTA CTG CTG CTG TGC GGC GCC GTG TTT GTG
AGC CCC AGC GCC CGG GGC AGT GGC GAC AGC
AGC ATC GCC TAT TCG AAC AAC ACT ATT GCC
ATA CCC ACA AAC TTC TCT ATA TCT ATA ACT
ACG GAG GTG ATG CCC GTG TCT ATG GCC AAG
ACT AGT GTA GAC TGC AAC ATG TAC ATC TGC
GGC GAC TCT ACT GAG TGC GCC AAC CTG CTG
CTG CAG TAT GGC TCT TTC TGC ACC CAG CTG
AAC AGA GCC CTG AGT GGC ATC GCC GCC GAG
CAG GAC CGG AAC ACA AGA GAG GTT TTC GCC
CAG GTA AAG CAG ATG TAC AAG ACC CCC ACT
CTG AAG TAT TTT GGC GGC TTC AAC TTC TCT
CAG ATC CTG CCC GAT CCC CTG AAG CCC ACC
AAG AGG TCT TTC ATC GAG GAC CTG CTG TTC
AAC AAG GTC ACT CTG GCC GAT GCC GGC TTC
ATG AAG CAG TAC GGC GAG TGC CTG GGC GAC
ATT AAC GCC CGC GAC CTG ATC TGT GCC CAG
AAG TTT AAC GGC CTG ACG GTC CTG CCC CCC
CTG CTG ACA GAT GAT ATG ATC GCC GCC TAC
ACT GCC GCC CTG GTC TCT GGC ACC GCC ACC
GCC GGC TGG ACT TTC GGC GCC GGC GCC GCC
CTG CAG ATC CCC TTC GCC ATG CAG ATG GCC
TAT AGA TTT AAC GGC ATA GGC GTA ACT CAG
AAC GTC CTG TAC GAG AAC CAG AAG CAG ATC
GCC AAC CAG TTT AAC AAG GCC ATC TCC CAG
ATT CAG GAG AGC CTG ACA ACC ACT AGC ACT
GCC CTG GGC AAG CTG CAG GAC GTG GTG AAC
CAG AAC GCC CAG GCC CTG AAC ACA CTG GTT
AAG CAG CTG AGT TCT AAC TTT GGC GCC ATA
TCC TCG GTG CTG AAC GAC ATA CTG TCA AGG
CTG GAC AAG GTC GAG GCC GAG GTT CAG ATA
GAT AGA CTG ATC ACA GGC AGA CTG CAG AGC
CTG CAG ACC TAC GTT ACA CAG CAG CTG ATC
AGA GCC GCC GAG ATC AGA GCC TCA GCC AAC
CTG GCC GCC ACG AAG ATG TCT GAG TGC GTC
CTG GGC CAG TCT AAG AGA GTC GAT TTC TGC
GGC AAG GGC TAC CAC CTG ATG AGT TTC CCC
CAG GCC GCC CCC CAT GGC GTT GTA TTC CTG
CAT GTG ACA TAT GTT CCC TCC CAG GAG AGG
AAC TTT ACC ACG GCC CCC GCC ATC TGC CAC
GAG GGC AAG GCC TAC TTC CCC AGA GAG GGC
GTG TTC GTT TTT AAC GGC ACT AGC TGG TTT
ATT ACC CAG AGG AAC TTC TTC TCC CCC CAG
ATT ATA ACA ACA GAT AAC ACT TTC GTG TCC
GGC AAC TGC GAT GTT GTG ATA GGC ATC ATT
AAC AAC ACA GTG TAC GAT CCC CTG CAG CCC

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GAG CTG GAT AGT TTT AAG GAG GAG CTG GAC
AAG TAT TTT AAG AAC CAC ACT TCC CCC GAT
GTA GAC CTG GGC GAT ATC AGT GGC ATA AAC
GCC AGT GTC GTG AAC ATA CAG AAG GAG ATC
GAT AGG CTG AAC GAG GTG GCC AAG AAC CTG
AAC GAG TCA CTG ATC GAT CTG CAG GAG CTG
GGC AAG TAC GAG CAG TAT ATT AAG TGG CCC
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[0181] A representative codon-optimized coding region encoding SEQ ID NO:12 according to the "standardized optimization" method is presented herein as SEQ ID NO: 69.

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ATG GAT GCC ATG AAG CGC GGC CTG TGC TGT
GTG CTG CTG CTG TGT GGC GCC GTG TTC GTG
AGC CCC AGC GCC CGC GGC AGC GGC GAT AGC
AGC ATC GCC TAC AGC AAC AAC ACC ATC GCC
ATC CCC ACC AAC TTC AGC ATC AGC ATC ACC
ACC GAG GTG ATG CCC GTG AGC ATG GCC AAG
ACC AGC GTG GAT TGC AAC ATG TAC ATC TGC
GGC GAC AGC ACC GAG TGC GCC AAC CTG CTG
CTG CAG TAC GGC AGC TTC TGC ACC CAG CTG
AAC CGC GCC CTG AGC GGC ATC GCC GCC GAG
CAG GAC CGC AAC ACC CGC GAG GTG TTC GCC
CAG GTG AAG CAG ATG TAC AAG ACC CCC ACC
CTG AAG TAC TTC GGC GGC TTC AAC TTC AGC
CAG ATC CTG CCC GAC CCC CTG AAG CCC ACC
AAG CGC AGC TTC ATC GAG GAT CTG CTG TTC
AAC AAG GTG ACC CTG GCC GAC GCC GGC TTC
ATG AAG CAG TAC GGC GAG TGC CTG GGC GAC
ATC AAC GCC CGC GAC CTG ATC TGC GCC CAG
AAG TTC AAC GGC CTG ACC GTG CTG CCC CCC
CTG CTG ACC GAT GAC ATG ATC GCC GCC TAC
ACC GCC GCC CTG GTG AGC GGC ACC GCC ACC
GCC GGC TGG ACC TTC GGC GCC GGC GCC GCC
CTG CAG ATC CCC TTC GCC ATG CAG ATG GCC
TAC CGC TTC AAC GGC ATC GGC GTG ACC CAG
AAC GTG CTG TAC GAG AAC CAG AAG CAG ATC
GCC AAC CAG TTC AAC AAG GCC ATC AGC CAG
ATC CAG GAG AGC CTG ACC ACC ACC AGC ACC
GCC CTG GGC AAG CTG CAG GAT GTG GTG AAC
CAG AAC GCC CAG GCC CTG AAC ACC CTG GTG
AAG CAG CTG AGC AGC AAC TTC GGC GCC ATC
AGC AGC GTG CTG AAC GAT ATC CTG AGC CGC
CTG GAT AAG GTG GAG GCC GAG GTG CAG ATC
GAC CGC CTG ATC ACC GGC CGC CTG CAG AGC
CTG CAG ACC TAC GTG ACC CAG CAG CTG ATC
CGC GCC GCC GAG ATC CGC GCC AGC GCC AAC
CTG GCC GCC ACC AAG ATG AGC GAG TGC GTG
CTG GGC CAG AGC AAG CGC GTG GAT TTC TGC
GGC AAG GGC TAC CAC CTG ATG AGC TTC CCC
CAG GCC GCC CCC CAC GGC GTG GTG TTC CTG
CAT GTG ACC TAC GTG CCC AGC CAG GAG CGC
AAC TTC ACC ACC GCC CCC GCC ATC TGC CAC
GAG GGC AAG GCC TAC TTC CCC CGC GAG GGC
GTG TTC GTG TTC AAC GGC ACC AGC TGG TTC
ATC ACC CAG CGC AAC TTC TTC AGC CCC CAG
ATC ATC ACC ACC GAC AAC ACC TTC GTG AGC
GGC AAC TGC GAC GTG GTG ATC GGC ATC ATC
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AAC AAC ACC GTG TAC GAT CCC CTG CAG CCC
GAG CTG GAT AGC TTC AAG GAG GAG CTG GAC
AAG TAC TTC AAG AAC CAT ACC AGC CCC GAT
GTG GAT CTG GGC GAC ATC AGC GGC ATC AAC
GCC AGC GTG GTG AAC ATC CAG AAG GAG ATC
GAT CGC CTG AAC GAG GTG GCC AAG AAC CTG
AAC GAG AGC CTG ATC GAT CTG CAG GAG CTG
GGC AAG TAC GAG CAG TAC ATC AAG TGG CCC
TGG
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[0182] In certain embodiments described herein, a codon-optimized coding region encoding SEQ ID NO:14 is optimized according to codon usage in humans (*Homo sapiens*). Alternatively, a codon-optimized coding region encoding SEQ ID NO:14 may be optimized according to codon usage in any plant, animal, or microbial species. Codon-optimized coding regions encoding SEQ ID NO:14, optimized according to codon usage in humans are designed as follows. The amino acid composition of SEQ ID NO:14 is shown in Table 15.

TABLE 15

AMINO ACID		Number in SEQ ID NO:14
A	Ala	34
R	Arg	31
C	Cys	0
G	Gly	45
H	His	5
I	Ile	11
L	Leu	26
K	Lys	29
M	Met	7
F	Phe	13
P	Pro	31
S	Ser	35
T	Thr	33
W	Trp	5
Y	Tyr	11
V	Val	11
N	Asn	25
D	Asp	22
Q	Gln	34
E	Glu	14

[0183] Using the amino acid composition shown in Table 15, a human codon-optimized coding region which encodes SEQ ID NO:14 can be designed by any of the methods discussed herein. For "uniform" optimization, each amino acid is assigned the most frequent codon used in the human genome for that amino acid. According to this method, codons are assigned to the coding region encoding SEQ ID NO:14 as follows: the 13 phenylalanine codons are TTC, the 26 leucine codons are CTG, the 11 isoleucine codons are ATC, the 7 methionine codons are ATG, the 11 valine codons are GTG, the 35 serine codons are AGC, the 31 proline codons are CCC, the 33 threonine codons are ACC, the 34 alanine codons are GCC, the 11 tyrosine codons are TAC, the 5 histidine codons are CAC, the 34 glutamine codons are CAG, the 25 asparagine codons are AAC, the 29 lysine codons are AAG, the 22 aspartic acid codons are GAC, the 14 glutamic acid codons are GAG, the 5 tryptophan codons are TGG, the 31 arginine codons are CGG, AGA, or AGG (the frequencies of usage of these three codons in the human genome are not significantly different), and the 45 glycine codons are GGC. The codon-optimized N coding region designed by this method is presented herein as SEQ ID NO:37.

ATGAGCGACAACGGCCCCCAGAGCAACCAGAGAAGCGCCCCCAGAATCAC
CTTCGGCGGCCCCACCGACAGCACCGACAACAACCAGAACGGCGGCAGAA
ACGGCGCCAGACCCAAGCAGAGAAGACCCCAGGGCCTGCCCAACAACACC
GCCAGCTGGTTCACCGCCCTGACCCAGCACGGCAAGGAGGAGCTGAGATT
CCCCAGAGGCCAGGGCGTGCCCATCAACACCAACAGCGGCCCCGACGACC
AGATCGGCTACTACAGAAGAGCCACCAGAAGAGTGAGAGGCGGCGACGGC
AAGATGAAGGAGCTGAGCCCCAGATGGTACTTCTACTACCTGGGCACCGG
CCCCGAGGCCAGCCTGCCCTACGGCGCCAACAAGGAGGGCATCGTGTGGG
TGGCCACCGAGGGCGCCCTGAACACCCCCAAGGACCACATCGGCACCAGA
AACCCCAACAACAACGCCGCCACCGTGCTGCAGCTGCCCCAGGGCACCAC
CCTGCCCAAGGGCTTCTACGCCGAGGGCAGCAGAGGCGGCAGCCAGGCCA
GCAGCAGAAGCAGCAGCAGAAGCAGAGGCAACAGCAGAAACAGCACCCCC
GGCAGCAGCAGAGGCAACAGCCCCGCCAGAATGGCCAGCGGCGGCGGCGA
GACCGCCCTGGCCCTGCTGCTGCTGGACAGACTGAACCAGCTGGAGAGCA
AGGTGAGCGGCAAGGGCCAGCAGCAGCAGGGCCAGACCGTGACCAAGAAG
AGCGCCGCCGAGGCCAGCAAGAAGCCCAGACAGAAGAGAACCGCCACCAA
GCAGTACAACGTGACCCAGGCCTTCGGCAGAAGAGGCCCCGAGCAGACCC
AGGGCAACTTCGGCGACCAGGACCTGATCAGACAGGGCACCGACTACAAG
CACTGGCCCCAGATCGCCCAGTTCGCCCCCAGCGCCAGCGCCTTCTTCGG
CATGAGCAGAATCGGCATGGAGGTGACCCCCAGCGGCACCTGGCTGACCT
ACCACGGCGCCATCAAGCTGGACGACAAGGACCCCCAGTTCAAGGACAAC

GTGATCCTGCTGAACAAGCACATCGACGCCTACAAGACCTTCCCCCCCAC
CGAGCCCAAGAAGGACAAGAAGAAGAAGACCGACGAGGCCCAGCCCCTGC
CCCAGAGACAGAAGAAGCAGCCCACCGTGACCCTGCTGCCCGCCGCCGAC
ATGGACGACTTCAGCAGACAGCTGCAGAACAGCATGAGCGGCGCCAGCGC
CGACAGCACCCAGGCC

[0184] Alternatively, a human codon-optimized coding region which encodes SEQ ID NO:14 can be designed by the "full optimization" method, where each amino acid is assigned codons based on the frequency of usage in the human genome. These frequencies are shown in Table 4 above. Using this latter method, codons are assigned to the coding region encoding SEQ ID NO:14 as follows: about 4 of the 13 phenylalanine codons are TTT, and about 9 of the phenylalanine codons are TTC; about 1 of the 26 leucine codons are TTA, about 6 of the leucine codons are TTG, about 7 of the leucine codons are CTT, about 3 of the leucine codons are CTC, about 5 of the leucine codons are CTA, and about 4 of the leucine codons are CTG; about 7 of the 11 isoleucine codons are ATT, about 3 of the isoleucine codons are ATC, and about 1 of the isoleucine codons are ATA; the 7 methionine codons are ATG; about 4 of the 11 valine codons are GTT, about 4 of the valine codons are GTC, about 1 of the valine codons is GTA, and about 2 of the valine codons are GTG; about 10 of the 35 serine codons are TCT, about 3 of the serine codons are TCC, about 9 of the serine codons are TCA, about 1 of the serine codons is TCG, about 7 of the serine codons are AGT, and about 5 of the serine codons are AGC; about 10 of the 31 proline codons are CCT, about 9 of the proline codons are CCC, about 10 of the proline codons are CCA, and about 2 of the proline codons are CCG; about 17 of the 33 threonine codons are ACT, about 5 of the threonine codons are ACC, about 11 of the threonine codons are ACA, and about 0 of the threonine codons is ACG; about 14 of the 34 alanine codons are GCT, about 8 of the alanine codons are GCC, about 9 of the alanine codons are GCA, and about 3 of the alanine codons are GCG; about 2 of the 11 tyrosine codons are TAT and about 9 of the tyrosine codons are TAC; about 3 of the 5 histidine codons are CAT and about 2 of the histidine codons are CAC; about 24 of the 34 glutamine codons are CAA and about 10 of the

glutamine codons are CAG; about 16 of the 25 asparagine codons are AAT and about 9 of the asparagine codons are AAC; about 20 of the 29 lysine codons are AAA and about 9 of the lysine codons are AAG; about 10 of the 22 aspartic acid codons are GAT and about 12 of the aspartic acid codons are GAC; about 7 of the 14 glutamic acid codons are GAA and about 7 of the glutamic acid codons are GAG; the 5 tryptophan codons are TGG; about 5 of the 31 arginine codons are CGT, about 8 of the arginine codons are CGC, about 6 of the arginine codons are CGA, about 0 of the arginine codons are CGG, about 10 of the arginine codons are AGA, and about 2 of the arginine codons are AGG; and about 10 of the 45 glycine codons are GGT, about 16 of the glycine codons are GGC, about 16 of the glycine codons are GGA, and about 3 of the glycine codons are GGG.

[0185] As described above, the term "about" means that the number of amino acids encoded by a certain codon may be one more or one less than the number given. It would be understood by those of ordinary skill in the art that the total number of any amino acid in the polypeptide sequence must remain constant, therefore, if there is one "more" of one codon encoding a give amino acid, there would have to be one "less" of another codon encoding that same amino acid.

[0186] A representative "fully optimized" codon-optimized coding region encoding SEQ ID NO:14, optimized according to codon usage in humans is presented herein as SEQ ID NO:36.

ATG TCC GAT AAT GGT CCC CAG TCT AAC CAG
AGG TCG GCG CCA AGA ATC ACA TTC GGG GGC
CCA ACA GAC AGT ACC GAT AAC AAC CAG AAC
GGC GGA AGA AAC GGG GCC AGG CCC AAG CAG
CGG AGA CCT CAG GGA TTA CCA AAT AAT ACC
GCA AGC TGG TTC ACA GCC CTG ACC CAG CAT
GGA AAA GAG GAA CTG AGA TTC CCT AGA GGA
CAA GGG GTG CCT ATT AAT ACT AAT AGC GGG
CCT GAC GAT CAA ATT GGC TAT TAT CGA CGT
GCG ACT CGC CGT GTT AGA GGG GGG GAC GGG
AAG ATG AAG GAG CTT AGC CCA CGC TGG TAC
TTT TAC TAT CTG GGA ACC GGA CCT GAA GCT
AGT CTG CCC TAC GGC GCT AAC AAG GAG GGA
ATA GTA TGG GTC GCC ACG GAA GGT GCG TTG

AAT ACT CCG AAA GAT CAC ATC GGC ACC AGA
AAT CCT AAC AAT AAC GCC GCA ACC GTG CTA
CAA TTA CCC CAG GGA ACT ACT CTG CCG AAG
GGG TTC TAT GCG GAG GGA AGC CGC GGC GGC
TCA CAA GCC AGT TCA CGC TCC AGC TCC CGG
TCG AGG GGT AAT TCC CGA AAC AGC ACC CCG
GGA TCA TCT AGG GGA AAC TCT CCC GCC CGG
ATG GCC TCA GGC GGC GGC GAA ACA GCT CTG
GCT CTG CTA TTG CTG GAC CGG CTC AAC CAG
CTC GAG TCC AAA GTC TCT GGT AAA GGT CAG
CAG CAG CAG GGT CAA ACA GTG ACC AAA AAA
AGT GCA GCC GAG GCC AGC AAG AAA CCA CGC
CAG AAA CGT ACG GCC ACA AAG CAA TAC AAT
GTG ACC CAA GCC TTT GGA AGG CGG GGG CCC
GAA CAG ACA CAG GGC AAT TTC GGC GAT CAA
GAT TTG ATA CGA CAG GGC ACT GAC TAC AAA
CAC TGG CCG CAG ATC GCT CAG TTT GCA CCT
AGC GCC TCC GCT TTC TTT GGC ATG AGT CGG
ATT GGC ATG GAG GTG ACA CCA TCA GGT ACT
TGG TTA ACG TAC CAC GGG GCA ATC AAA CTT
GAT GAT AAA GAT CCC CAG TTT AAG GAC AAC
GTT ATC CTC CTG AAT AAG CAT ATT GAC GCC
TAT AAG ACC TTC CCC CCA ACC GAA CCA AAG
AAG GAC AAG AAG AAG AAG ACA GAC GAG GCA
CAG CCT CTC CCC CAG AGG CAG AAA AAG CAG
CCT ACT GTC ACC CTT CTG CCC GCT GCA GAC
ATG GAT GAC TTT TCC CGC CAA CTC CAG AAC
TCT ATG AGT GGG GCT TCC GCT GAC TCT ACG
CAG GCC TGA

[0187] Another representative codon-optimized coding region encoding SEQ ID NO:14 is presented herein as SEQ ID NO:63. SEQ ID NO:14 is encoded by nucleotides 7 to 1275 of SEQ ID NO:63.

GTCGACATGAGCGACAACGGCCCCCAGAGCAACCAGAGAAGCGCCCCCAGAATC
ACCTTTGGCGGCCCTACCGACAGCACCGACAACAACCAGAACGGCGGCAGAAAC
GGCGCCAGACCCAAGCAGAGGAGACCCCAGGGCCTGCCCAACAACACCGCCAGC
TGGTTCACCGCCCTCACCCAGCACGGCAAGGAGGAGCTGAGATTCCCCAGAGGCC
AGGGCGTGCCCATCAATACCAACAGCGGCCCAGACGATCAGATCGGCTACTACCG
GAGGGCCACCAGAAGAGTGAGAGGCGGCGACGGCAAGATGAAGGAGCTGAGCC
CCCGGTGGTACTTCTACTACCTGGGCACCGGCCCTGAGGCCAGCCTGCCCTACGG
CGCCAACAAGGAGGGCATCGTGTGGGTGGCCACCGAGGGCGCCCTGAATACCCC
CAAGGACCACATCGGCACCAGGAACCCCAACAACAATGCCGCCACCGTGCTGCA
GCTGCCCCAGGGCACCACCCTGCCCAAGGGCTTCTACGCCGAGGGCAGCAGAGG
CGGCAGCCAGGCCAGCAGCAGAAGCAGCAGCAGGAGCAGGGGCAACAGCAGAA
ATAGCACCCCCGGCAGCAGCAGAGGAAATTCACCCGCCAGAATGGCCAGCGGCG
GAGGCGAGACCGCCCTGGCCCTGCTGCTCCTGGACAGGCTGAATCAGCTGGAGAG
CAAGGTGAGCGGCAAGGGCCAGCAACAGCAGGGACAGACCGTGACCAAGAAGTC
TGCCGCCGAGGCCAGCAAGAAGCCCAGGCAGAAGAGAACCGCCACCAAGCAGTA
CAATGTGACCCAGGCCTTCGGCAGAAGAGGCCCCGAGCAGACCCAGGGCAATTT
CGGCGACCAGGACCTCATCAGACAGGGCACCGACTACAAGCACTGGCCTCAGAT
CGCCCAGTTCGCCCCCAGCGCCAGCGCCTTCTTCGGCATGAGCCGGATCGGCATG

GAGGTGACCCCCAGCGGCACCTGGCTCACCTACCACGGCGCCATCAAGCTGGACG
ACAAGGACCCCCAGTTCAAGGACAACGTGATCCTGCTGAACAAGCACATCGACG
CCTACAAGACCTTCCCACCCACCGAGCCCAAGAAGGACAAGAAGAAGAAAACCG
ACGAGGCCCAGCCCCTGCCCCAGAGACAGAAGAAGCAGCCCACCGTGACCCTGC
TGCCTGCCGCCGACATGGACGACTTCAGCCGCCAGCTGCAGAATAGCATGAGCGG
CGCCTCTGCCGATTCAACCCAGGCCTGAAGATCT

[0188] In certain embodiments described herein, a codon-optimized coding region encoding SEQ ID NO:16 is optimized according to codon usage in humans (*Homo sapiens*). Alternatively, a codon-optimized coding region encoding SEQ ID NO:16 may be optimized according to codon usage in any plant, animal, or microbial species. Codon-optimized coding regions encoding SEQ ID NO:16, optimized according to codon usage in humans are designed as follows. The amino acid composition of SEQ ID NO:16 is shown in Table16.

TABLE 16

AMINO ACID		Number in SEQ ID NO:16
A	Ala	33
R	Arg	31
C	Cys	0
G	Gly	45
H	His	5
I	Ile	11
L	Leu	26
K	Lys	22
M	Met	7
F	Phe	12
P	Pro	28
S	Ser	35
T	Thr	30
W	Trp	5
Y	Tyr	11
V	Val	11
N	Asn	25
D	Asp	20
Q	Gln	33
E	Glu	12

[0189] Using the amino acid composition shown in Table 16, a human codon-optimized coding region which encodes SEQ ID NO:16 can be designed by any of the methods discussed herein. For "uniform" optimization, each amino

acid is assigned the most frequent codon used in the human genome for that amino acid. According to this method, codons are assigned to the coding region encoding SEQ ID NO:16 as follows: the 12 phenylalanine codons are TTC, the 26 leucine codons are CTG, the 11 isoleucine codons are ATC, the 7 methionine codons are ATG, the 11 valine codons are GTG, the 35 serine codons are AGC, the 28 proline codons are CCC, the 30 threonine codons are ACC, the 33 alanine codons are GCC, the 11 tyrosine codons are TAC, the 5 histidine codons are CAC, the 33 glutamine codons are CAG, the 25 asparagine codons are AAC, the 22 lysine codons are AAG, the 20 aspartic acid codons are GAC, the 12 glutamic acid codons are GAG, the 5 tryptophan codons are TGG, the 31 arginine codons are CGG, AGA, or AGG (the frequencies of usage of these three codons in the human genome are not significantly different), and the 45 glycine codons are GGC. The codon-optimized N (minus NLS) coding region designed by this method is presented herein as SEQ ID NO:39.

ATGAGCGACAACGGCCCCCAGAGCAACCAGAGAAGCGCCCCCAGAATCAC
CTTCGGCGGCCCCACCGACAGCACCGACAACAACCAGAACGGCGGCAGAA
ACGGCGCCAGACCCAAGCAGAGAAGACCCCAGGGCCTGCCCAACAACACC
GCCAGCTGGTTCACCGCCCTGACCCAGCACGGCAAGGAGGAGCTGAGATT
CCCCAGAGGCCAGGGCGTGCCCATCAACACCAACAGCGGCCCCGACGACC
AGATCGGCTACTACAGAAGAGCCACCAGAAGAGTGAGAGGCGGCGACGGC
AAGATGAAGGAGCTGAGCCCCAGATGGTACTTCTACTACCTGGGCACCGG
CCCCGAGGCCAGCCTGCCCTACGGCGCCAACAAGGAGGGCATCGTGTGGG
TGGCCACCGAGGGCGCCCTGAACACCCCCAAGGACCACATCGGCACCAGA
AACCCCAACAACAACGCCGCCACCGTGCTGCAGCTGCCCCAGGGCACCAC
CCTGCCCAAGGGCTTCTACGCCGAGGGCAGCAGAGGCGGCAGCCAGGCCA
GCAGCAGAAGCAGCAGCAGAAGCAGAGGCAACAGCAGAAACAGCACCCCC
GGCAGCAGCAGAGGCAACAGCCCCGCCAGAATGGCCAGCGGCGGCGGCGA
GACCGCCCTGGCCCTGCTGCTGCTGGACAGACTGAACCAGCTGGAGAGCA
AGGTGAGCGGCAAGGGCCAGCAGCAGCAGGGCCAGACCGTGACCAAGAAG
AGCGCCGCCGAGGCCAGCAAGAAGCCCAGACAGAAGAGAACCGCCACCAA
GCAGTACAACGTGACCCAGGCCTTCGGCAGAAGAGGCCCCGAGCAGACCC
AGGGCAACTTCGGCGACCAGGACCTGATCAGACAGGGCACCGACTACAAG
CACTGGCCCCAGATCGCCCAGTTCGCCCCCAGCGCCAGCGCCTTCTTCGG
CATGAGCAGAATCGGCATGGAGGTGACCCCCAGCGGCACCTGGCTGACCT
ACCACGGCGCCATCAAGCTGGACGACAAGGACCCCCAGTTCAAGGACAAC
GTGATCCTGCTGAACAAGCACATCGACGCCTACCCCCTGCCCCAGAGACA
GAAGAAGCAGCCCACCGTGACCCTGCTGCCCGCCGCCGACATGGACGACT
TCAGCAGACAGCTGCAGAACAGCATGAGCGGCGCCAGCGCCGACAGCACC
CAGGCC

[0190] Alternatively, a human codon-optimized coding region which encodes SEQ ID NO:16 can be designed by the "full optimization" method, where each amino acid is assigned codons based on the frequency of usage in the human genome. These frequencies are shown in Table 4 above. Using this latter method, codons are assigned to the coding region encoding SEQ ID NO:16 as follows: about 5 of the 12 phenylalanine codons are TTT, and about 7 of the phenylalanine codons are TTC; about 3 of the 26 leucine codons are TTA, about 3 of the leucine codons are TTG, about 3 of the leucine codons are CTT, about 5 of the leucine codons are CTC, about 2 of the leucine codons are CTA, and about 10 of the leucine codons are CTG; about 4 of the 11 isoleucine codons are ATT, about 5 of the isoleucine codons are ATC, and about 2 of the isoleucine codons are ATA; the 7 methionine codons are ATG; about 2 of the 11 valine codons are GTT, about 3 of the valine codons are GTC, about 1 of the valine codons is GTA, and about 5 of the valine codons are GTG; about 6 of the 35 serine codons are TCT, about 8 of the serine codons are TCC, about 5 of the serine codons are TCA, about 2 of the serine codons are TCG, about 6 of the serine codons are AGT, and about 8 of the serine codons are AGC; about 8 of the 28 proline codons are CCT, about 9 of the proline codons are CCC, about 8 of the proline codons are CCA, and about 3 of the proline codons are CCG; about 7 of the 30 threonine codons are ACT, about 11 of the threonine codons are ACC, about 9 of the threonine codons are ACA, and about 3 of the threonine codons are ACG; about 9 of the 33 alanine codons are GCT, about 13 of the alanine codons are GCC, about 7 of the alanine codons are GCA, and about 4 of the alanine codons are GCG; about 5 of the 11 tyrosine codons are TAT and about 6 of the tyrosine codons are TAC; about 2 of the 5 histidine codons are CAT and about 3 of the histidine codons are CAC; about 9 of the 33 glutamine codons are CAA and about 24 of the glutamine codons are CAG; about 12 of the 25 asparagine codons are AAT and about 13 of the asparagine codons are AAC; about 9 of the 22 lysine codons are AAA and about 13 of the lysine codons are AAG; about 9 of the 20 aspartic acid codons are GAT and about 11 of the aspartic acid codons are

GAC; about 5 of the 12 glutamic acid codons are GAA and about 7 of the glutamic acid codons are GAG; the 5 tryptophan codons are TGG; about 3 of the 31 arginine codons are CGT, about 6 of the arginine codons are CGC, about 3 of the arginine codons are CGA, about 6 of the arginine codons are CGG, about 7 of the arginine codons are AGA, and about 6 of the arginine codons are AGG; and about 7 of the 45 glycine codons are GGT, about 15 of the glycine codons are GGC, about 12 of the glycine codons are GGA, and about 11 of the glycine codons are GGG.

[0191] As described above, the term "about" means that the number of amino acids encoded by a certain codon may be one more or one less than the number given. It would be understood by those of ordinary skill in the art that the total number of any amino acid in the polypeptide sequence must remain constant, therefore, if there is one "more" of one codon encoding a give amino acid, there would have to be one "less" of another codon encoding that same amino acid.

[0192] A representative "fully optimized" codon-optimized coding region encoding SEQ ID NO:16, optimized according to codon usage in humans is presented herein as SEQ ID NO:38.

ATG AGT GAT AAT GGC CCC CAG TCT AAC CAG
AGG AGC GCA CCG CGG ATC ACG TTC GGT GGC
CCA ACC GAC TCA ACA GAC AAT AAT CAG AAC
GGA GGA CGC AAT GGT GCA CGT CCT AAG CAG
AGA CGC CCC CAA GGG CTG CCT AAT AAT ACA
GCA AGT TGG TTT ACC GCA CTC ACA CAA CAT
GGA AAG GAA GAG TTG CGG TTC CCC CGC GGC
CAG GGC GTG CCC ATC AAC ACA AAT AGC GGA
CCC GAC GAT CAG ATC GGA TAT TAC CGA AGA
GCT ACA AGG AGA GTT CGC GGC GGG GAT GGC
AAG ATG AAG GAG CTA TCA CCA CGA TGG TAC
TTC TAT TAC CTC GGG ACA GGC CCA GAG GCC
TCG CTA CCA TAC GGG GCC AAC AAG GAG GGT
ATT GTC TGG GTC GCT ACC GAA GGG GCC CTG
AAT ACA CCT AAA GAC CAC ATA GGT ACC AGA
AAT CCC AAC AAT AAC GCC GCG ACC GTG TTA
CAG CTT CCT CAG GGA ACG ACC CTT CCA AAA
GGG TTT TAC GCC GAA GGA TCT CGG GGA GGG
TCA CAG GCT AGC TCC CGT AGC TCC TCA AGG
TCC AGG GGG AAT TCT AGA AAC AGT ACA CCC
GGC TCT AGC CGT GGT AAC TCC CCA GCT CGC

ATG GCA TCC GGC GGA GGG GAA ACC GCT CTG
GCT CTG CTC CTG TTA GAT CGG TTG AAC CAA
CTG GAA TCG AAG GTA TCC GGA AAG GGA CAG
CAG CAG CAA GGC CAG ACT GTG ACT AAG AAG
TCC GCG GCC GAG GCC AGT AAG AAA CCC CGC
CAG AAA CGA ACT GCC ACC AAA CAG TAT AAT
GTG ACA CAG GCC TTC GGC AGA CGG GGT CCA
GAG CAG ACC CAA GGC AAC TTC GGG GAT CAG
GAC CTG ATT CGG CAG GGT ACC GAC TAT AAG
CAC TGG CCG CAA ATT GCT CAG TTT GCT CCC
AGT GCG AGT GCC TTC TTC GGC ATG TCT AGG
ATC GGG ATG GAG GTT ACT CCT AGC GGC ACT
TGG CTT ACT TAT CAC GGA GCC ATC AAA CTC
GAT GAT AAG GAC CCA CAG TTT AAG GAT AAC
GTG ATT CTG CTG AAC AAA CAT ATA GAC GCG
TAC CCT CTC CCG CAA AGG CAG AAA AAA CAG
CCT ACC GTC ACG TTA CTG CCT GCC GCA GAC
ATG GAC GAC TTT TCT AGA CAG TTG CAA AAC
AGC ATG TCA GGC GCA TCC GCC GAT AGC ACT
CAA GCT TGA

[0193] In certain embodiments described herein, a codon-optimized coding region encoding SEQ ID NO:19 is optimized according to codon usage in humans (*Homo sapiens*). Alternatively, a codon-optimized coding region encoding SEQ ID NO:19 may be optimized according to codon usage in any plant, animal, or microbial species. Codon-optimized coding regions encoding SEQ ID NO:19, optimized according to codon usage in humans are designed as follows. The amino acid composition of SEQ ID NO:19 is shown in Table 17.

TABLE 17

AMINO ACID		Number in SEQ ID NO:19
A	Ala	19
R	Arg	15
C	Cys	3
G	Gly	15
H	His	3
I	Ile	18
L	Leu	31
K	Lys	6
M	Met	7
F	Phe	11
P	Pro	6
S	Ser	11

T	Thr	13
W	Trp	7
Y	Tyr	9
V	Val	16
N	Asn	13
D	Asp	6
Q	Gln	5
E	Glu	7

[0194] Using the amino acid composition shown in Table 17, a human codon-optimized coding region which encodes SEQ ID NO:19 can be designed by any of the methods discussed herein. For "uniform" optimization, each amino acid is assigned the most frequent codon used in the human genome for that amino acid. According to this method, codons are assigned to the coding region encoding SEQ ID NO:19 as follows: the 11 phenylalanine codons are TTC, the 31 leucine codons are CTG, the 18 isoleucine codons are ATC, the 7 methionine codons are ATG, the 16 valine codons are GTG, the 11 serine codons are AGC, the 6 proline codons are CCC, the 13 threonine codons are ACC, the 19 alanine codons are GCC, the 19 tyrosine codons are TAC, the 3 histidine codons are CAC, the 5 glutamine codons are CAG, the 13 asparagine codons are AAC, the 6 lysine codons are AAG, the 6 aspartic acid codons are GAC, the 7 glutamic acid codons are GAG, the 3 cysteine codons are TGC, the 7 tryptophan codons are TGG, the 15 arginine codons are CGG, AGA, or AGG (the frequencies of usage of these three codons in the human genome are not significantly different), and the 43 glycine codons are GGC. The codon-optimized M coding region designed by this method is presented herein as SEQ ID NO:41.

ATGGCCGACAACGGCACCATCACCGTGGAGGAGCTGAAGCAGCTGCTGGA
GCAGTGGAACCTGGTGATCGGCTTCCTGTTCCTGGCCTGGATCATGCTGC
TGCAGTTCGCCTACAGCAACAGAAACAGATTCCTGTACATCATCAAGCTG
GTGTTCCTGTGGCTGCTGTGGCCCGTGACCCTGGCCTGCTTCGTGCTGGC
CGCCGTGTACAGAATCAACTGGGTGACCGGCGGCATCGCCATCGCCATGG
CCTGCATCGTGGGCCTGATGTGGCTGAGCTACTTCGTGGCCAGCTTCAGA
CTGTTCGCCAGAACCAGAAGCATGTGGAGCTTCAACCCCGAGACCAACAT
CCTGCTGAACGTGCCCCTGAGAGGCACCATCGTGACCAGACCCCTGATGG
AGAGCGAGCTGGTGATCGGCGCCGTGATCATCAGAGGCCACCTGAGAATG
GCCGGCCACCCCCTGGGCAGATGCGACATCAAGGACCTGCCCAAGGAGAT
CACCGTGGCCACCAGCAGAACCCTGAGCTACTACAAGCTGGGCGCCAGCC

AGAGAGTGGGCACCGACAGCGGCTTCGCCGCCTACAACAGATACAGAATC
GGCAACTACAAGCTGAACACCGACCACGCCGGCAGCAACGACAACATCGC
CCTGCTGGTGCAG

[0195] Alternatively, a human codon-optimized coding region which encodes SEQ ID NO:19 can be designed by the "full optimization" method, where each amino acid is assigned codons based on the frequency of usage in the human genome. These frequencies are shown in Table 4 above. Using this latter method, codons are assigned to the coding region encoding SEQ ID NO:19 as follows: about 5 of the 11 phenylalanine codons are TTT, and about 6 of the phenylalanine codons are TTC; about 3 of the 31 leucine codons are TTA, about 4 of the leucine codons are TTG, about 4 of the leucine codons are CTT, about 6 of the leucine codons are CTC, about 2 of the leucine codons are CTA, and about 12 of the leucine codons are CTG; about 6 of the 18 isoleucine codons are ATT, about 9 of the isoleucine codons are ATC, and about 3 of the isoleucine codons are ATA; the 7 methionine codons are ATG; about 3 of the 16 valine codons are GTT, about 4 of the valine codons are GTC, about 2 of the valine codons are GTA, and about 7 of the valine codons are GTG; about 2 of the 11 serine codons are TCT, about 2 of the serine codons are TCC, about 2 of the serine codons are TCA, about 1 of the serine codons is TCG, about 1 of the serine codons is AGT, and about 3 of the serine codons are AGC; about 2 of the 6 proline codons are CCT, about 2 of the proline codons are CCC, about 1 of the proline codons is CCA, and about 1 of the proline codons is CCG; about 3 of the 13 threonine codons are ACT, about 5 of the threonine codons are ACC, about 4 of the threonine codons are ACA, and about 1 of the threonine codons is ACG; about 5 of the 19 alanine codons are GCT, about 8 of the alanine codons are GCC, about 4 of the alanine codons are GCA, and about 2 of the alanine codons are GCG; about 4 of the 9 tyrosine codons are TAT and about 5 of the tyrosine codons are TAC; about 1 of the 3 histidine codons is CAT and about 2 of the histidine codons are CAC; about 1 of the 5 glutamine codons is CAA and about 4 of the glutamine codons are CAG; about 6 of the 13 asparagine codons are AAT and about 7 of

the asparagine codons are AAC; about 3 of the 6 lysine codons are AAA and about 3 of the lysine codons are AAG; about 3 of the 6 aspartic acid codons are GAT and about 3 of the aspartic acid codons are GAC; about 3 of the 7 glutamic acid codons are GAA and about 4 of the glutamic acid codons are GAG; about 1 of the 3 cysteine codons is TGT and about 2 of the cysteine codons are TGC; the 7 tryptophan codons are TGG; about 1 of the 15 arginine codons is CGT, about 3 of the arginine codons are CGC, about 2 of the arginine codons are CGA, about 3 of the arginine codons are CGG, about 3 of the arginine codons are AGA, and about 3 of the arginine codons are AGG; and about 2 of the 15 glycine codons are GGT, about 5 of the glycine codons are GGC, about 4 of the glycine codons are GGA, and about 4 of the glycine codons are GGG.

[0196] As described above, the term "about" means that the number of amino acids encoded by a certain codon may be one more or one less than the number given. It would be understood by those of ordinary skill in the art that the total number of any amino acid in the polypeptide sequence must remain constant, therefore, if there is one "more" of one codon encoding a give amino acid, there would have to be one "less" of another codon encoding that same amino acid.

[0197] A representative "fully optimized" codon-optimized coding region encoding SEQ ID NO:19, optimized according to codon usage in humans is presented herein as SEQ ID NO:40.

ATG GCT GAC AAC GGC ACC ATA ACC GTC GAG
GAG CTT AAA CAG TTA TTA GAA CAA TGG AAC
TTG GTG ATA GGA TTC CTC TTT CTG GCA TGG
ATC ATG TTG CTT CAG TTC GCC TAT TCT AAC
CGC AAT AGG TTT TTG TAC ATT ATC AAG CTG
GTC TTC CTT TGG CTG CTC TGG CCC GTA ACA
CTA GCC TGT TTT GTT TTG GCG GCC GTG TAT
CGG ATC AAT TGG GTG ACA GGT GGC ATT GCT
ATT GCG ATG GCT TGC ATC GTG GGG CTG ATG
TGG CTG TCG TAT TTC GTT GCC TCA TTC CGG
CTG TTT GCC CGA ACA AGG AGT ATG TGG TCT
TTT AAC CCC GAG ACC AAT ATT CTG CTC AAT
GTG CCT TTA CGC GGC ACT ATC GTG ACC CGG
CCT CTA ATG GAA TCC GAG CTG GTA ATT GGC

GCA GTC ATC ATA AGG GGG CAC CTC AGA ATG
GCC GGG CAC CCA CTT GGG AGA TGC GAC ATC
AAG GAT CTG CCG AAG GAA ATT ACT GTT GCA
ACT TCA CGA ACG CTG AGC TAT TAC AAA CTG
GGA GCT AGC CAG AGA GTG GGT ACC GAC TCC
GGC TTC GCT GCC TAC AAC CGC TAC CGT ATC
GGA AAT TAC AAA CTC AAC ACA GAT CAT GCA
GGA AGC AAT GAT AAC ATC GCC CTC CTG GTC
CAG TGA

[0198] In certain embodiments described herein, a codon-optimized coding region encoding SEQ ID NO:21 is optimized according to codon usage in humans (*Homo sapiens*). Alternatively, a codon-optimized coding region encoding SEQ ID NO:21 may be optimized according to codon usage in any plant, animal, or microbial species. Codon-optimized coding regions encoding SEQ ID NO:21, optimized according to codon usage in humans are designed as follows. The amino acid composition of SEQ ID NO:21 is shown in Table 18.

TABLE 18

AMINO ACID		Number in SEQ ID NO:21
A	Ala	4
R	Arg	2
C	Cys	3
G	Gly	2
H	His	0
I	Ile	3
L	Leu	14
K	Lys	2
M	Met	1
F	Phe	4
P	Pro	2
S	Ser	7
T	Thr	5
W	Trp	0
Y	Tyr	4
V	Val	14
N	Asn	5
D	Asp	1
Q	Gln	0
E	Glu	3

[0199] Using the amino acid composition shown in Table 18, a human codon-optimized coding region which encodes SEQ ID NO:21 can be designed by any of the methods discussed herein. For "uniform" optimization, each amino acid is assigned the most frequent codon used in the human genome for that amino acid. According to this method, codons are assigned to the coding region encoding SEQ ID NO:21 as follows: the 4 phenylalanine codons are TTC, the 14 leucine codons are CTG, the 18 isoleucine codons are 3, the 1 methionine codon is ATG, the 14 valine codons are GTG, the 7 serine codons are AGC, the 2 proline codons are CCC, the 5 threonine codons are ACC, the 4 alanine codons are GCC, the 4 tyrosine codons are TAC, the 5 asparagine codons are AAC, the 2 lysine codons are AAG, the 1 aspartic acid codon is GAC, the 3 glutamic acid codons are GAG, the 3 cysteine codons are TGC, the 1 tryptophan codon is TGG, the 2 arginine codons are CGG, AGA, or AGG (the frequencies of usage of these three codons in the human genome are not significantly different), and the 2 glycine codons are GGC. The codon-optimized E coding region designed by this method is presented herein as SEQ ID NO:43.

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ATG TAC AGC TTC GTG AGC GAG GAG ACC GGC
ACC CTG ATC GTG AAC AGC GTG CTG CTG TTC
CTG GCC TTC GTG GTG TTC CTG CTG GTG ACC
CTG GCC ATC CTG ACC GCC CTG CGG CTG TGC
GCC TAC TGC TGC AAC ATC GTG AAC GTG AGC
CTG GTG AAG CCC ACC GTG TAC GTG TAC AGC
CGG GTG AAG AAC CTG AAC AGC AGC GAG GGC
GTG CCC GAC CTG CTG GTG TGA
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[0200] Alternatively, a human codon-optimized coding region which encodes SEQ ID NO:21 can be designed by an optimization method, where each amino acid is assigned codons based on the frequency of usage in the human genome. These frequencies are shown in Table 4 above. Using this latter method, codons are assigned to the coding region encoding SEQ ID NO:21 as follows: about 1 of the 4 phenylalanine codons are TTT, and about 3 of the phenylalanine codons are TTC; about 2 of the 14 leucine codons are TTA,

about 2 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 0 of the leucine codons are CTC, about 2 of the leucine codons are CTA, and about 2 of the leucine codons are CTG; about 1 of the 3 isoleucine codons are ATT, about 1 of the isoleucine codons are ATC, and about 1 of the isoleucine codons are ATA; the 1 methionine codons are ATG; about 6 of the 14 valine codons are GTT, about 3 of the valine codons are GTC, about 3 of the valine codons are GTA, and about 2 of the valine codons are GTG; about 2 of the 7 serine codons are TCT, about 0 of the serine codons are TCC, about 1 of the serine codons are TCA, about 2 of the serine codons is TCG, about 1 of the serine codons is AGT, and about 1 of the serine codons are AGC; about 1 of the 2 proline codons are CCT, about 0 of the proline codons are CCC, about 1 of the proline codons is CCA, and about 0 of the proline codons is CCG; about 1 of the 5 threonine codons are ACT, about 0 of the threonine codons are ACC, about 2 of the threonine codons are ACA, and about 2 of the threonine codons is ACG; about 1 of the 4 alanine codons are GCT, about 1 of the alanine codons are GCC, about 0 of the alanine codons are GCA, and about 2 of the alanine codons are GCG; about 0 of the 4 tyrosine codons are TAT and about 4 of the tyrosine codons are TAC; about 3 of the 5 asparagine codons are AAT and about 2 of the asparagine codons are AAC; about 2 of the 2 lysine codons are AAA and about 0 of the lysine codons are AAG; about 1 of the 1 aspartic acid codons are GAT and about 0 of the aspartic acid codons are GAC; about 3 of the 3 glutamic acid codons are GAA and about 0 of the glutamic acid codons are GAG; about 1 of the 3 cysteine codons is TGT and about 2 of the cysteine codons are TGC; about 1 of the 2 arginine codons is CGT, about 0 of the arginine codons are CGC, about 1 of the arginine codons are CGA, about 0 of the arginine codons are CGG, about 0 of the arginine codons are AGA, and about 0 of the arginine codons are AGG; and about 1 of the 2 glycine codons are GGT, about 0 of the glycine codons are GGC, about 1 of the glycine codons are GGA, and about 0 of the glycine codons are GGG.

[0201] As described above, the term "about" means that the number of amino acids encoded by a certain codon may be one more or one less than the

number given. It would be understood by those of ordinary skill in the art that the total number of any amino acid in the polypeptide sequence must remain constant, therefore, if there is one "more" of one codon encoding a give amino acid, there would have to be one "less" of another codon encoding that same amino acid.

[0202] A representative fully codon-optimized coding region encoding SEQ ID NO:21, optimized according to codon usage in humans is presented herein as SEQ ID NO:42.

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ATG TAC AGC TTT GTG TCT GAA GAA ACA GGA
ACG TTG ATA GTT AAT AGT GTT TTG CTT TTC
TTA GCG TTC GTA GTC TTC CTT CTT GTC ACA
CTT GCC ATT TTA ACT GCG CTT CGT CTA TGC
GCT TAC TGT TGC AAT ATC GTA AAC GTG TCG
CTT GTT AAA CCA ACG GTT TAC GTA TAC TCG
CGA GTT AAA AAC CTG AAT TCT TCA GAA GGT
GTT CCT GAT CTG CTA GTC TAA
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[0203] Another representative codon-optimized coding region encoding SEQ ID NO:21 is presented herein as SEQ ID NO:48.

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ATG TAT AGT TTT GTG AGT GAG GAG ACG GGC
ACC CTG ATT GTC AAC TCA GTG CTG CTG TTC
CTG GCC TTT GTT GTC TTC CTG CTG GTA ACT
CTG GCC ATC CTG ACT GCC CTG AGA CTG TGC
GCC TAC TGC TGC AAC ATC GTG AAC GTC TCT
CTG GTA AAG CCC ACA GTT TAC GTG TAT TCT
AGG GTG AAG AAC CTG AAC TCC AGC GAG GGC
GTT CCC GAT CTG CTG GTA TGA
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[0204] Randomly assigning codons at an optimized frequency to encode a given polypeptide sequence using the "uniform optimization," "full optimization," "minimal optimization," or other optimization methods, can be done manually by calculating codon frequencies for each amino acid, and then assigning the codons to the polypeptide sequence randomly. Additionally, various algorithms and computer software programs are readily available to those of ordinary skill in the art. For example, the "EditSeq" function in the Lasergene Package, available from DNAstar, Inc., Madison, WI, the backtranslation function in the VectorNTI Suite, available from InforMax,

Inc., Bethesda, MD, and the "backtranslate" function in the GCG--Wisconsin Package, available from Accelrys, Inc., San Diego, CA. In addition, various resources are publicly available to codon-optimize coding region sequences. For example, the "backtranslation" function found at http://www.entelechon.com/ eng/backtranslation.html (visited July 9, 2002), and the "backtranseq" function available at http://bioinfo.pbi.nrc.ca:8090/EMBOSS/index.html (visited October 15, 2002). Constructing a rudimentary algorithm to assign codons based on a given frequency can also easily be accomplished with basic mathematical functions by one of ordinary skill in the art.

[0205] A number of options are available for synthesizing codon-optimized coding regions designed by any of the methods described above, using standard and routine molecular biological manipulations well known to those of ordinary skill in the art. In one approach, a series of complementary oligonucleotide pairs of 80-90 nucleotides each in length and spanning the length of the desired sequence are synthesized by standard methods. These oligonucleotide pairs are synthesized such that upon annealing, they form double stranded fragments of 80-90 base pairs, containing cohesive ends, *e.g.*, each oligonucleotide in the pair is synthesized to extend 3, 4, 5, 6, 7, 8, 9, 10, or more bases beyond the region that is complementary to the other oligonucleotide in the pair. The single-stranded ends of each pair of oligonucleotides is designed to anneal with the single-stranded end of another pair of oligonucleotides. The oligonucleotide pairs are allowed to anneal, and approximately five to six of these double-stranded fragments are then allowed to anneal together via the cohesive single stranded ends, and then they ligated together and cloned into a standard bacterial cloning vector, for example, a TOPO® vector available from Invitrogen Corporation, Carlsbad, CA. The construct is then sequenced by standard methods. Several of these constructs consisting of 5 to 6 fragments of 80 to 90 base pair fragments ligated together, *i.e.*, fragments of about 500 base pairs, are prepared, such that the entire desired sequence is represented in a series of plasmid constructs. The inserts

of these plasmids are then cut with appropriate restriction enzymes and ligated together to form the final construct. The final construct is then cloned into a standard bacterial cloning vector, and sequenced. Additional methods would be immediately apparent to the skilled artisan. In addition, gene synthesis is readily available commercially.

[0206] The codon-optimized coding regions can be versions encoding any gene products from any strain, derivative, or variant of SARS-CoV, or fragments, variants, or derivatives of such gene products. For example, nucleic acid fragments of codon-optimized coding regions encoding the S, N, E or M polypeptides, or fragments, variants or derivatives thereof. Codon-optimized coding regions encoding other SARS-CoV polypeptides or fragments, variants, or derivatives thereof (*e.g.,* those encoding certain predicted open reading frames in the SARS-CoV genome), are included within the present invention. Additional, non-codon-optimized polynucleotides encoding SARS-CoV polypeptides or other polypeptides may be included as well.

Compositions and Methods

[0207] In certain embodiments, the present invention is directed to compositions and methods of raising a detectable immune in a vertebrate by administering *in vivo*, into a tissue of a vertebrate, one or more polynucleotides comprising at least one wild-type coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof, and/or at least one codon-optimized coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof. In addition, the present invention is directed to compositions and methods of raising a detectable immune response in a vertebrate by administering to the vertebrate a composition comprising one or more polynucleotides as described herein, and at least one isolated SARS-CoV component, or isolated polypeptide. The SARS-CoV component may be inactivated virus, attenuated virus, a viral vector

expressing an isolated SARS-CoV polypeptide, or a SARS-CoV virus protein, fragment, variant or derivative thereof.

[0208] The polynucleotides comprising at least one coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof, and/or at least one codon-optimized coding region encoding a SARS-CoV polypeptide may be administered either prior to, at the same time (simultaneously), or subsequent to the administration of the SARS-CoV component, or isolated polypeptide.

[0209] The SARS-CoV component, or isolated polypeptide in combination with polynucleotides comprising at least one coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof, and/or at least one codon-optimized coding region encoding a SARS-CoV polypeptide compositions may be referred to as "combinatorial polynucleotide vaccine compositions" or "single formulation heterologous prime-boost vaccine compositions."

[0210] The isolated SARS-CoV polypeptides of the invention may be in any form, and are generated using techniques well known in the art. Examples include isolated SARS-CoV proteins produced recombinantly, isolated SARS-CoV proteins directly purified from their natural milieu, recombinant (non-SARS-CoV) virus vectors expressing an isolated SARS-CoV protein, or proteins delivered in the form of an inactivated SARS-CoV vaccine, such as conventional vaccines.

[0211] When utilized, an isolated SARS-CoV component, or polypeptide or fragment, variant or derivative thereof is administered in an immunologically effective amount. Canine coronavirus, known to infect swine, turkeys, mice, calves, dogs, cats, rodents, avians and humans, may be administered as a live viral vector vaccine at a dose rate per dog of 10^5-10^8 pfu, or as a typical subunit vaccine at 10 *u*g-1 mg of polypeptide, according to U.S. Patent No. 5,661,006, incorporated by reference herein in its entirety. Similarly, Bovine coronavirus is administered to animals in an antigen vaccine composition at dose of about 1 to about 100 micrograms of subunit antigen, according to U.S.

Patent No. 5,369,026, incorporated by reference herein in its entirety. The effective amount of SARS-CoV component or isolated polypeptide, and polynucleotides as described herein are determinable by one of ordinary skill in the art based upon several factors, including the antigen being expressed, the age and weight of the subject, and the precise condition requiring treatment and its severity, and route of administration.

[0212] In the instant invention, the combination of conventional antigen vaccine compositions with the polynucleotides comprising at least one coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof, and/or at least one codon-optimized coding region encoding a SARS-CoV polypeptide compositions provides for therapeutically beneficial effects at dose sparing concentrations. For example, immunological responses sufficient for a therapeutically beneficial effect in patients predetermined for an approved commercial product, such as for the typical animal coronavirus products described above, may be attained by using less of the product when supplemented or enhanced with the appropriate amount of polynucleotides comprising at least one coding region encoding a SARS-CoV or codon-optimized nucleic acid. Thus, dose sparing is contemplated by administration of conventional coronavirus vaccines administered in combination with the nucleic acids of the invention.

[0213] In particular, the dose of an antigen SARS-CoV vaccine may be reduced by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60% or at least 70% when administered in combination with the nucleic acid compositions of the invention.

[0214] Similarly, a desirable level of an immunological response afforded by a DNA-based pharmaceutical alone may be attained with less DNA by including an aliquot of antigen SARS-CoV vaccine. Further, using a combination of conventional and DNA-based pharmaceuticals may allow both materials to be used in lesser amounts, while still affording the desired level of immune response arising from administration of either component alone in higher amounts (*e.g.*, one may use less of either immunological product when

they are used in combination). This may be manifest not only by using lower amounts of materials being delivered at any time, but also to leads to reducing the number of administrations in a vaccination regime (*e.g.*, 2 versus 3 or 4 injections), and/or to reducing the kinetics of the immunological response (*e.g.*, desired response levels are attained in 3 weeks instead of 6 weeks after immunization).

[0215] In particular, the dose of DNA-based pharmaceuticals, may be reduced by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60% or at least 70% when administered in combination with antigen SARS-CoV vaccines.

[0216] Determining the precise amounts of DNA based pharmaceutical and SARS-CoV antigen is based on a number of factors as described above, and is readily determined by one of ordinary skill in the art.

[0217] In addition to dose sparing, the claimed combinatorial compositions provide for a broadening of the immune response and/or enhanced beneficial immune responses. Such broadened or enhanced immune responses are achieved by: adding DNA to enhance cellular responses to a conventional vaccine; adding a conventional vaccine to a DNA pharmaceutical to enhance humoral response; using a combination that induces additional epitopes (both humoral and/or cellular) to be recognized and/or responded to in a more desirable way (epitope broadening); employing a DNA-conventional vaccine combination designed for a particular desired spectrum of immunological responses; and/or obtaining a desirable spectrum by using higher amounts of either component. The broadened immune response is measurable by one of ordinary skill in the art by standard immunological assays specific for the desirable response spectrum.

[0218] Both broadening and dose sparing may be obtained simultaneously.

[0219] In addition, the present invention is directed to compositions and methods of raising a detectable immune response in a vertebrate by administering to the vertebrate a composition comprising one or more SARS-CoV polynucleotides as described herein. The compositions of the invention

may comprise at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10 polynucleotides, as described herein, encoding different SARS-CoV polypeptides or fragments, variants or derivatives thereof in the same composition.

[0220] The coding regions encoding SARS-CoV polypeptides or fragments, variants, or derivatives thereof may be codon optimized for a particular vertebrate. Codon optimization is carried out by the methods described herein; for example, in certain embodiments codon-optimized coding regions encoding polypeptides of SARS-CoV, or nucleic acid fragments of such coding regions encoding fragments, variants, or derivatives thereof are optimized according to the codon usage of the particular vertebrate. The polynucleotides of the invention are incorporated into the cells of the vertebrate *in vivo*, and an immunologically effective amount of a SARS-CoV polypeptide or a fragment, variant, or derivative thereof is produced *in vivo*. The coding regions encoding a SARS-CoV polypeptide or a fragment, variant, or derivative thereof may be codon optimized for mammals, *e.g.*, humans, apes, monkeys (*e.g.*, owl, squirrel, cebus, rhesus, African green, patas, cynomolgus, and cercopithecus), orangutans, baboons, gibbons, and chimpanzees, dogs, wolves, cats, lions, and tigers, horses, donkeys, zebras, cows, pigs, sheep, deer, giraffes, bears, rabbits, mice, ferrets, seals, whales; birds, *e.g.*, ducks, geese, terns, shearwaters, gulls, turkeys, chickens, quail, pheasants, geese, starlings and budgerigars; or other vertebrates.

[0221] In particular, the present invention relates to codon-optimized coding regions encoding polypeptides of SARS-CoV, or fragments, variants, or derivatives thereof, or nucleic acid fragments of such coding regions or fragments, variants, or derivatives thereof, which have been optimized according to human codon usage. For example, human codon-optimized coding regions encoding polypeptides of SARS-CoV, or fragments, variants, or derivatives thereof are prepared by substituting one or more codons preferred for use in human genes for the codons naturally used in the DNA sequence encoding the SARS-CoV polypeptide or a fragment, variant, or

derivative thereof. Also provided are polynucleotides, vectors, and other expression constructs comprising wild-type coding regions or codon-optimized coding regions encoding polypeptides of SARS-CoV, or nucleic acid fragments of such wild-type coding regions or codon-optimized coding regions including variants, or derivatives thereof. Also provided are pharmaceutical compositions comprising polynucleotides, vectors, and other expression constructs comprising wild-type coding regions or codon-optimized coding regions encoding polypeptides of SARS-CoV, or nucleic acid fragments of such coding regions encoding variants, or derivatives thereof; and various methods of using such polynucleotides, vectors and other expression constructs. Coding regions encoding SARS-CoV polypeptides may be uniformly optimized, fully optimized, or minimally optimized, or otherwise optimized, as described herein.

[0222] The present invention is further directed towards polynucleotides comprising coding regions or codon-optimized coding regions encoding polypeptides of SARS-CoV antigens, for example, (predicted ORF's), optionally in conjunction with other antigens. The invention is also directed to polynucleotides comprising nucleic acid fragments or codon-optimized nucleic acid fragments encoding fragments, variants and derivatives of these polypeptides.

[0223] In certain embodiments, the present invention provides an isolated polynucleotide comprising a nucleic acid fragment, where the nucleic acid fragment is a fragment of a coding region or a codon optimized coding region encoding a polypeptide at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a SARS-CoV polypeptide, *e.g.*, S, N, E or M, and where the nucleic acid fragment is a variant of a coding region or a codon optimized coding region encoding an SARS-CoV polypeptide, *e.g.*, S, N, E or M. The human codon-optimized coding region can be optimized for any vertebrate species and by any of the methods described herein.

[0224] As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag *et al.* (*Comp. App. Biosci.* 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is expressed as percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining, Penalty=30 Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

Isolated SARS-CoV Polypeptides

[0225] The present invention is further drawn to compositions which include at least one polynucleotide comprising one or more nucleic acid fragments, where each nucleic acid fragment is a fragment of a coding region or a codon-optimized coding region operably encoding an SARS-CoV polypeptide or fragment, variant, or derivative thereof; together with and one or more isolated SARS-CoV, components, polypeptides or fragments, variants or derivatives thereof, *i.e.*, "combinatorial polynucleotide vaccine compositions" or "single formulation heterologous prime-boost vaccine compositions." The isolated SARS-CoV polypeptides of the invention may be in any form, and are generated using techniques well known in the art. Examples include isolated SARS-CoV proteins produced recombinantly, isolated SARS-CoV proteins

directly purified from their natural milieu, and recombinant (non-SARS-CoV) virus vectors expressing an isolated SARS-CoV protein.

[0226] Similarly, the isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof to be delivered (either a recombinant protein, a purified subunit, or viral vector expressing an isolated SARS-CoV polypeptide) may be any isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof, including but not limited to the S, S1, S2, N, E or M proteins or fragments, variants or derivatives thereof. Fragments include, but are not limited to the soluble portion of the S protein and the S1 and S2 domains of the S protein. In certain embodiments, a derivative protein may be a fusion protein. It should be noted that any isolated SARS-CoV polypeptide or fragment, variant, or derivative thereof described herein may be combined in a composition with any polynucleotide comprising a nucleic acid fragment, where the nucleic acid fragment is a fragment of a coding region or a codon-optimized coding region operably encoding a SARS-CoV polypeptide or fragment, variant, or derivative thereof. The proteins may be different, the same, or may be combined in any combination of one or more isolated SARS-CoV proteins and one or more polynucleotides.

[0227] In certain embodiments, the isolated SARS-CoV polypeptides, or fragments, derivatives or variants thereof may be fused to or conjugated to a second isolated SARS-CoV polypeptide, or fragment, derivative or variant thereof, or may be fused to other heterologous proteins, including for example, hepatitis B proteins including, but not limited to the hepatitis B core antigen (HBcAg), or those derived from diphtheria or tetanus. The second isolated SARS-CoV polypeptide or other heterologous protein may act as a "carrier" that potentiates the immunogenicity of the SARS-CoV polypeptide or a fragment, variant, or derivative thereof to which it is attached. Hepatitis B virus proteins and fragments and variants thereof useful as carriers within the scope of the invention are disclosed in U.S. Patent No. 6,231,864 and U.S. Patent No. 5,143,726, incorporated by reference in their entireties.

Polynucleotides comprising coding regions encoding said fused or conjugated proteins are also within the scope of the invention.

Methods and Administration

[0228] The present invention also provides methods for delivering a SARS-CoV polypeptide or a fragment, variant, or derivative thereof to a human, which comprise administering to a human one or more of the polynucleotide compositions described herein such that upon administration of polynucleotide compositions such as those described herein, a SARS-CoV polypeptide or a fragment, variant, or derivative thereof is expressed in human cells, in an amount sufficient to generate an immune response to SARS-CoV; or administering the SARS-CoV polypeptide or a fragment, variant, or derivative thereof itself to the human in an amount sufficient to generate an immune response.

[0229] The present invention further provides methods for delivering a SARS-CoV polypeptide or a fragment, variant, or derivative thereof to a human, which comprise administering to a vertebrate one or more of the compositions described herein; such that upon administration of compositions such as those described herein, an immune response is generated in the vertebrate.

[0230] The term "vertebrate" is intended to encompass a singular "vertebrate" as well as plural "vertebrates" and comprises mammals and birds, as well as fish, reptiles, and amphibians.

[0231] The term "mammal" is intended to encompass a singular "mammal" and plural "mammals," and includes, but is not limited to humans; primates such as apes, monkeys (*e.g.*, owl, squirrel, cebus, rhesus, African green, patas, cynomolgus, and cercopithecus), orangutans, baboons, gibbons, and chimpanzees; canids such as dogs and wolves; felids such as cats, lions, and tigers; equines such as horses, donkeys, and zebras, food animals such as cows, pigs, and sheep; ungulates such as deer and giraffes; ursids such as

bears; and others such as rabbits, mice, ferrets, seals, whales. In particular, the mammal can be a human subject, a food animal or a companion animal.

[0232] The term "bird" is intended to encompass a singular "bird" and plural "birds," and includes, but is not limited to feral water birds such as ducks, geese, terns, shearwaters, and gulls; as well as domestic avian species such as turkeys, chickens, quail, pheasants, geese, and ducks. The term "bird" also encompasses passerine birds such as starlings and budgerigars.

[0233] The present invention further provides a method for generating, enhancing or modulating an immune response to SARS-CoV comprising administering to a vertebrate one or more of the compositions described herein. In this method, the compositions may include one or more isolated polynucleotides comprising at least one nucleic acid fragment where the nucleic acid fragment is a fragment of a coding region or a codon-optimized coding region encoding an SARS-CoV polypeptide, or a fragment, variant, or derivative thereof. In another embodiment, the compositions may include multiple (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) polynucleotides as described herein, such polynucleotides encoding different SARS CoV polypeptides in the same composition.

[0234] In another embodiment, the compositions may include both a polynucleotide as described above; and also an isolated SARS-CoV polypeptide, or a fragment, variant, or derivative thereof, wherein the protein is provided as a recombinant protein, in particular, a fusion protein, a purified subunit, viral vector expressing the protein, or inactivated virus. Thus, the latter compositions include both a polynucleotide encoding a SARS-CoV polypeptide or a fragment, variant, or derivative thereof and an isolated SARS-CoV polypeptide or a fragment, variant, or derivative thereof. The SARS-CoV polypeptide or a fragment, variant, or derivative thereof encoded by the polynucleotide of the compositions need not be the same as the isolated SARS-CoV polypeptide or a fragment, variant, or derivative thereof of the compositions. Compositions to be used according to this method may be univalent, bivalent, trivalent or multivalent.

[0235] The polynucleotides of the compositions may comprise a fragment of a coding region or a human (or other vertebrate) codon-optimized coding region encoding a protein of SARS-CoV, or a fragment, variant, or derivative thereof. The polynucleotides are incorporated into the cells of the vertebrate *in vivo*, and an antigenic amount of the SARS-CoV polypeptide, or fragment, variant, or derivative thereof, is produced *in vivo*. Upon administration of the composition according to this method, the SARS-CoV polypeptide or a fragment, variant, or derivative thereof is expressed in the vertebrate in an amount sufficient to elicit an immune response. Such an immune response might be used, for example, to generate antibodies to the SARS-CoV for use in diagnostic assays or as laboratory reagents, or as therapeutic or preventative vaccines as described herein.

[0236] The present invention further provides a method for generating, enhancing, or modulating a protective and/or therapeutic immune response to SARS-CoV in a vertebrate, comprising administering to a vertebrate in need of therapeutic and/or preventative immunity one or more of the compositions described herein. In this method, the compositions include one or more polynucleotides comprising at least one nucleic acid fragment, where the nucleic acid fragment is a fragment of a wild-type coding region or a codon-optimized coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof. In a further embodiment, the composition used in this method includes both an isolated polynucleotide comprising at least one nucleic acid fragment, where the nucleic acid fragment is a fragment of a wild-type coding region or a codon-optimized coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof; and at least one isolated SARS-CoV polypeptide, or a fragment, variant, or derivative thereof. Thus, the latter composition includes both an isolated polynucleotide encoding a SARS-CoV polypeptide or a fragment, variant, or derivative thereof and an isolated SARS-CoV polypeptide or a fragment, variant, or derivative thereof, for example, a recombinant protein, a purified subunit, or viral vector expressing the protein. Upon administration of the composition according to

this method, the SARS-CoV polypeptide or a fragment, variant, or derivative thereof is expressed in the vertebrate in a therapeutically or prophylactically effective amount.

[0237] In certain embodiments, the polynucleotide or polypeptide compositions of the present invention may be administered to a vertebrate where the vertebrate is used as an *in vivo* model to observe the effects of individual or multiple SARS-CoV polypeptides *in vivo*. This approach would not only eliminate the species specific barrier to studying SARS-CoV, but would allow for the study of the immunopathology of SARS-CoV polypeptides as well as SARS-CoV polypeptide specific effects with out using infectious SARS-CoV virus. An *in vivo* vertebrate model of SARS infection would be useful, for example, in developing treatments for one or more aspects of SARS infection by mimicking those aspects of infection without the potential hazards associated with handling the infectious virus

[0238] As used herein, an "immune response" refers to the ability of a vertebrate to elicit an immune reaction to a composition delivered to that vertebrate. Examples of immune responses include an antibody response or a cellular, *e.g.*, T-cell, response. One or more compositions of the present invention may be used to prevent SARS-CoV infection in vertebrates, *e.g.*, as a prophylactic or prevenative vaccine (also sometimes referred to in the art as a "protective" vaccine), to establish or enhance immunity to SARS-CoV in a healthy individual prior to exposure to SARS-CoV or contraction of Severe Acute Respiratory Syndrome (SARS), thus preventing the syndrome or reducing the severity of SARS symptoms. As used herein, "a detectable immune response" refers to an immunogenic response to the polynucleotides and polypeptides of the present invention, which can be measured or observed by standard protocols. These protocols include, but are not limited to, immunoblot analysis (western), fluorescence-activated cell sorting (FACS), immunoprecipitation analysis, ELISA, cytolytic T-cell response, ELISPOT, and chromium release assay. An immune response may also be "detected" through challenge of immunized animals with virulent SARS-CoV, either

before or after vaccination. ELISA assays are performed as described by Ausubel *et al.*, *Current Protocols in Molecular Biology,* John Wiley and Sons, Baltimore, Maryland (1989). Cytolytic T-cell responses are measured as described in Hartikka *et al.* "Vaxfectin Enhances the Humoral Response to Plasmid DNA-encoded Antigens." *Vaccine 19*: 1911-1923 (2001), which is hereby incorporated in its entirety by reference. Standard ELISPOT technology is used for the CD4+ and CD8+ T-cell assays as described in Example 6A. Standard chromium release assays are used to measure specific cytotoxic T lymphocyte (CTL) activity against the various SARS-CoV antigens.

[0239] As mentioned above, compositions of the present invention may be used both to prevent SARS-CoV infection, and also to therapeutically treat SARS-CoV infection. In individuals already exposed to SARS-CoV, or already suffering from SARS, the present invention is used to further stimulate the immune system of the vertebrate, thus reducing or eliminating the symptoms associated with that disease or disorder. As defined herein, "treatment " refers to the use of one or more compositions of the present invention to prevent, cure, retard, or reduce the severity of SARS symptoms in a vertebrate, and/or result in no worsening of SARS over a specified period of time in a vertebrate which has already been exposed to SARS-CoV and is thus in need of therapy. The term "prevention" refers to the use of one or more compositions of the present invention to generate immunity in a vertebrate which has not yet been exposed to a particular strain of SARS-CoV, thereby preventing or reducing disease symptoms if the vertebrate is later exposed to the particular strain of SARS-CoV. The methods of the present invention therefore may be referred to as therapeutic vaccination or preventative or prophylactic vaccination. It is not required that any composition of the present invention provide total immunity to SARS-CoV or totally cure or eliminate all SARS symptoms. As used herein, a "vertebrate in need of therapeutic and/or preventative immunity" refers to an individual for whom it is desirable to treat, *i.e.*, to prevent, cure, retard, or reduce the severity of SARS symptoms,

and/or result in no worsening of SARS over a specified period of time. Vertebrates to treat and/or vaccinate include humans, apes, monkeys (*e.g.*, owl, squirrel, cebus, rhesus, African green, patas, cynomolgus, and cercopithecus), orangutans, baboons, gibbons, and chimpanzees, dogs, wolves, cats, lions, and tigers, horses, donkeys, zebras, cows, pigs, sheep, deer, giraffes, bears, rabbits, mice, ferrets, seals, whales, ducks, geese, terns, shearwaters, gulls, turkeys, chickens, quail, pheasants, geese, starlings and budgerigars.

[0240] One or more compositions of the present invention are utilized in a "prime boost" regimen. An example of a "prime boost" regimen may be found in Yang, Z. *et al. J. Virol.* 77:799-803 (2002). In these embodiments, one or more polynucleotide vaccine compositions of the present invention are delivered to a vertebrate, thereby priming the immune response of the vertebrate to SARS-CoV, and then a second immunogenic composition is utilized as a boost vaccination. One or more compositions of the present invention are used to prime immunity, and then a second immunogenic composition, *e.g.*, a recombinant viral vaccine or vaccines, a different polynucleotide vaccine, or one or more purified subunit isolated SARS-CoV polypeptides or fragments, variants or derivatives thereof is used to boost the anti-SARS-CoV immune response.

[0241] In one embodiment, a priming composition and a boosting composition are delivered to a vertebrate in separate doses and vaccinations. For example, a single composition may comprise one or more polynucleotides encoding SARS-CoV protein(s), fragment(s), variant(s), or derivative(s) thereof and/or one or more isolated SARS-CoV polypeptide(s) or fragment(s), variant(s), or derivative(s) thereof as the priming component. The polynucleotides encoding the SARS-CoV polypeptides fragments, variants, or derivatives thereof may be contained in a single plasmid or viral vector or in multiple plasmids or viral vectors. At least one polynucleotide encoding a SARS-CoV protein and/or one or more SARS-CoV isolated polypeptide can serve as the boosting component. In this embodiment, the compositions of the priming component

and the compositions of the boosting component may be contained in separate vials. In one example, the boosting component is administered approximately 1 to 6 months after administration of the priming component.

[0242] In one embodiment, a priming composition and a boosting composition are combined in a single composition or single formulation. For example, a single composition may comprise an isolated SARS-CoV polypeptide or a fragment, variant, or derivative thereof as the priming component and a polynucleotide encoding an SARS-CoV protein as the boosting component. In this embodiment, the compositions may be contained in a single vial where the priming component and boosting component are mixed together. In general, because the peak levels of expression of protein from the polynucleotide does not occur until later (*e.g.*, 7-10 days) after administration, the polynucleotide component may provide a boost to the isolated protein component. Compositions comprising both a priming component and a boosting component are referred to herein as "combinatorial vaccine compositions" or "single formulation heterologous prime-boost vaccine compositions." In addition, the priming composition may be administered before the boosting composition, or even after the boosting composition, if the boosting composition is expected to take longer to act.

[0243] In another embodiment, the priming composition may be administered simultaneously with the boosting composition, but in separate formulations where the priming component and the boosting component are separated.

[0244] The terms "priming" or "primary" and "boost" or "boosting" as used herein may refer to the initial and subsequent immunizations, respectively, *i.e.*, in accordance with the definitions these terms normally have in immunology. However, in certain embodiments, *e.g.*, where the priming component and boosting component are in a single formulation, initial and subsequent immunizations may not be necessary as both the "prime" and the "boost" compositions are administered simultaneously.

[0245] In certain embodiments, one or more compositions of the present invention are delivered to a vertebrate by methods described herein, thereby

achieving an effective therapeutic and/or an effective preventative immune response. More specifically, the compositions of the present invention may be administered to any tissue of a vertebrate, including, but not limited to, muscle, skin, brain tissue, lung tissue, liver tissue, spleen tissue, bone marrow tissue, thymus tissue, heart tissue, *e.g.*, myocardium, endocardium, and pericardium, lymph tissue, blood tissue, bone tissue, pancreas tissue, kidney tissue, gall bladder tissue, stomach tissue, intestinal tissue, testicular tissue, ovarian tissue, uterine tissue, vaginal tissue, rectal tissue, nervous system tissue, eye tissue, glandular tissue, tongue tissue, and connective tissue, *e.g.*, cartilage.

[0246] Furthermore, the compositions of the present invention may be administered to any internal cavity of a vertebrate, including, but not limited to, the lungs, the mouth, the nasal cavity, the stomach, the peritoneal cavity, the intestine, any heart chamber, veins, arteries, capillaries, lymphatic cavities, the uterine cavity, the vaginal cavity, the rectal cavity, joint cavities, ventricles in brain, spinal canal in spinal cord, the ocular cavities, the lumen of a duct of a salivary gland or a liver. When the compositions of the present invention are administered to the lumen of a duct of a salivary gland or liver, the desired polypeptide is expressed in the salivary gland and the liver such that the polypeptide is delivered into the blood stream of the vertebrate from each of the salivary gland or the liver. Certain modes for administration to secretory organs of a gastrointestinal system using the salivary gland, liver and pancreas to release a desired polypeptide into the bloodstream are disclosed in U.S. Patent Nos. 5,837,693 and 6,004,944, both of which are incorporated herein by reference in their entireties.

[0247] In certain embodiments, the compositions are administered to muscle, either skeletal muscle or cardiac muscle, or to lung tissue. Specific, but non-limiting modes for administration to lung tissue are disclosed in Wheeler, C.J., *et al.*, *Proc. Natl. Acad. Sci. USA 93*:11454-11459 (1996), which is incorporated herein by reference in its entirety.

[0248] According to the disclosed methods, compositions of the present invention can be administered by intramuscular (i.m.), subcutaneous (s.c.), or intrapulmonary routes. Other suitable routes of administration include, but are not limited to intratracheal, transdermal, intraocular, intranasal, inhalation, intracavity, intravenous (i.v.), intraductal (*e.g.*, into the pancreas) and intraparenchymal (*i.e.*, into any tissue) administration. Transdermal delivery includes, but is not limited to intradermal (*e.g.*, into the dermis or epidermis), transdermal (*e.g.*, percutaneous) and transmucosal administration (*i.e.*, into or through skin or mucosal tissue). Intracavity administration includes, but is not limited to administration into oral, vaginal, rectal, nasal, peritoneal, or intestinal cavities as well as, intrathecal (*i.e.*, into spinal canal), intraventricular (*i.e.*, into the brain ventricles or the heart ventricles), inraatrial (*i.e.*, into the heart atrium) and sub arachnoid (*i.e.*, into the sub arachnoid spaces of the brain) administration.

[0249] Any mode of administration can be used so long as the mode results in the expression of the desired peptide or protein, in the desired tissue, in an amount sufficient to generate an immune response to SARS-CoV and/or to generate a prophylactically or therapeutically effective immune response to SARS-CoV in a vertebrate in need of such response. Administration means of the present invention include needle injection, catheter infusion, biolistic injectors, particle accelerators (*e.g.*, "gene guns" or pneumatic "needleless" injectors) Med-E-Jet (Vahlsing, H., *et al., J. Immunol. Methods 171*:11-22 (1994)), Pigjet (Schrijver, R., *et al., Vaccine 15*: 1908-1916 (1997)), Biojector (Davis, H., *et al., Vaccine 12*: 1503-1509 (1994); Gramzinski, R., *et al., Mol. Med. 4*: 109-118 (1998)), AdvantaJet (Linmayer, I., *et al., Diabetes Care 9:*294-297 (1986)), Medi-jector (Martins, J., and Roedl, E. J. *Occup. Med. 21:*821-824 (1979)), gelfoam sponge depots, other commercially available depot materials (*e.g.*, hydrogels), osmotic pumps (*e.g.*, Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, topical skin creams, and decanting, use of polynucleotide coated suture (Qin, Y., *et al., Life Sciences 65*: 2193-2203 (1999)) or topical applications during surgery.

Certain modes of administration are intramuscular needle-based injection and pulmonary application via catheter infusion. Energy-assisted plasmid delivery (EAPD) methods may also be employed to administer the compositions of the invention. One such method involves the application of brief electrical pulses to injected tissues, a procedure commonly known as electroporation. *See generally* Mir, L.M. *et al.*, *Proc. Natl. Acad. Sci USA 96*:4262-7 (1999); Hartikka, J. *et al.*, *Mol. Ther. 4*:407-15 (2001); Mathiesen, I., *Gene Ther. 6*:508-14(1999); Rizzuto G. *et al.*, *Hum. Gen. Ther. 11*:1891-900 (2000). Each of the references cited in this paragraph is incorporated herein by reference in its entirety.

[0250] Determining an effective amount of one or more compositions of the present invention depends upon a number of factors including, for example, the antigen being expressed or administered directly, (*e.g.*, S, N, E or M, or fragments, variants, or derivatives thereof), the age and weight of the subject, the precise condition requiring treatment and its severity, and the route of administration. Based on the above factors, determining the precise amount, number of doses, and timing of doses are within the ordinary skill in the art and will be readily determined by the attending physician or veterinarian.

[0251] Compositions of the present invention may include various salts, excipients, delivery vehicles and/or auxiliary agents as are disclosed, *e.g.*, in U.S. Patent Application Publication 2002/0019358, published February 14, 2002, which is incorporated herein by reference in its entirety.

[0252] Furthermore, compositions of the present invention may include one or more transfection facilitating compounds that facilitate delivery of polynucleotides to the interior of a cell, and/or to a desired location within a cell. As used herein, the terms "transfection facilitating compound," "transfection facilitating agent," and "transfection facilitating material" are synonymous, and may be used interchangeably. It should be noted that certain transfection facilitating compounds may also be "adjuvants" as described infra, *i.e.*, in addition to facilitating delivery of polynucleotides to the interior of a cell, the compound acts to alter or increase the immune response to the

antigen encoded by that polynucleotide. Examples of the transfection facilitating compounds include, but are not limited to inorganic materials such as calcium phosphate, alum (aluminum sulfate), and gold particles (*e.g.*, "powder" type delivery vehicles); peptides that are, for example, cationic, intercell targeting (for selective delivery to certain cell types), intracell targeting (for nuclear localization or endosomal escape), and ampipathic (helix forming or pore forming); proteins that are, for example, basic (*e.g.*, positively charged) such as histones, targeting (*e.g.*, asialoprotein), viral (*e.g.*, Sendai virus coat protein), and pore-forming; lipids that are, for example, cationic (*e.g.*, DMRIE, DOSPA, DC-Chol), basic (*e.g.*, steryl amine), neutral (*e.g.*, cholesterol), anionic (*e.g.*, phosphatidyl serine), and zwitterionic (*e.g.*, DOPE, DOPC); and polymers such as dendrimers, star-polymers, "homogenous" poly-amino acids (*e.g.*, poly-lysine, poly-arginine), "heterogeneous" poly-amino acids (*e.g.*, mixtures of lysine & glycine), co-polymers, polyvinylpyrrolidinone (PVP), poloxamers (*e.g.*, CRL 1005) and polyethylene glycol (PEG). A transfection facilitating material can be used alone or in combination with one or more other transfection facilitating materials. Two or more transfection facilitating materials can be combined by chemical bonding (*e.g.*, covalent and ionic such as in lipidated polylysine, PEGylated polylysine) (Toncheva, *et al., Biochim. Biophys. Acta 1380(3)*:354-368 (1988)), mechanical mixing (*e.g.*, free moving materials in liquid or solid phase such as "polylysine + cationic lipids") (Gao and Huang, *Biochemistry 35*:1027-1036 (1996); Trubetskoy, *et al., Biochem. Biophys. Acta 1131*:311-313 (1992)), and aggregation (*e.g.*, co-precipitation, gel forming such as in cationic lipids + poly-lactide, and polylysine + gelatin).

[0253] One category of transfection facilitating materials is cationic lipids. Examples of cationic lipids are 5-carboxyspermylglycine dioctadecylamide (DOGS) and dipalmitoyl-phophatidylethanolamine-5-carboxyspermylamide (DPPES). Cationic cholesterol derivatives are also useful, including {3ß-[N-N',N'-dimethylamino)ethane]-carbomoyl}-cholesterol (DC-Chol). Dimethyldioctdecyl-ammonium bromide (DDAB), N-(3-aminopropyl)-N,N-

(bis-(2-tetradecyloxyethyl))-N-methyl-ammonium bromide (PA-DEMO), N-(3-aminopropyl)-N,N-(bis-(2-dodecyloxyethyl))-N-methyl-ammonium bromide (PA-DELO), N,N,N-tris-(2-dodecyloxy)ethyl-N-(3-amino)propyl-ammonium bromide (PA-TELO), and N1-(3-aminopropyl)((2-dodecyloxy)ethyl)-N2-(2-dodecyloxy)ethyl-1-piperazinaminium bromide (GA-LOE-BP) can also be employed in the present invention.

[0254] Non-diether cationic lipids, such as DL-1,2-dioleoyl-3-dimethylaminopropyl-β-hydroxyethylammonium (DORI diester), 1-O-oleyl-2-oleoyl-3-dimethylaminopropyl-β-hydroxyethylammonium (DORI ester/ether), and their salts promote *in vivo* gene delivery. In some embodiments, cationic lipids comprise groups attached via a heteroatom attached to the quaternary ammonium moiety in the head group. A glycyl spacer can connect the linker to the hydroxyl group.

[0255] Specific, but non-limiting cationic lipids for use in certain embodiments of the present invention include DMRIE ((±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide), GAP-DMORIE ((±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-propanaminium bromide), and GAP-DLRIE ((±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-(bis-dodecyloxy)-1-propanaminium bromide).

[0256] Other specific but non-limiting cationic surfactants for use in certain embodiments of the present invention include Bn-DHRIE, DhxRIE, DhxRIE-OAc, DhxRIE-OBz and Pr-DOctRIE-OAc. These lipids are disclosed in copending U.S. patent application No. {Attorney Docket No. 1530.0610000}. In another aspect of the present invention, the cationic surfactant is Pr-DOctRIE-OAc.

[0257] Other cationic lipids include (±)-N,N-dimethyl-N-[2-(sperminecarboxamido) ethyl]-2,3-bis(dioleyloxy)-1-propaniminium pentahydrochloride (DOSPA), (±)-N-(2-aminoethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propaniminium bromide (β-aminoethyl-DMRIE or βAE-DMRIE) (Wheeler, *et al., Biochim. Biophys. Acta 1280*:1-11 (1996), and (±)-

N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-l-propaniminium bromide (GAP-DLRIE) (Wheeler, *et al., Proc. Natl. Acad. Sci. USA 93:*11454-11459 (1996)), which have been developed from DMRIE.

[0258] Other examples of DMRIE-derived cationic lipids that are useful for the present invention are (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-(bis-decyloxy)-1-propanaminium bromide (GAP-DDRIE), (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-(bis-tetradecyloxy)-1-propanaminium bromide (GAP-DMRIE), (±)-N-((N"-methyl)-N'-ureyl)propyl-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide (GMU-DMRIE), (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-1-propanaminium bromide (DLRIE), and (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis-([Z]-9-octadecenyloxy)propyl-1- propaniminium bromide (HP-DORIE).

[0259] In the embodiments where the immunogenic composition comprises a cationic lipid, the cationic lipid may be mixed with one or more co-lipids. For purposes of definition, the term "co-lipid" refers to any hydrophobic material which may be combined with the cationic lipid component and includes amphipathic lipids, such as phospholipids, and neutral lipids, such as cholesterol. Cationic lipids and co-lipids may be mixed or combined in a number of ways to produce a variety of non-covalently bonded macroscopic structures, including, for example, liposomes, multilamellar vesicles, unilamellar vesicles, micelles, and simple films. One non-limiting class of co-lipids are the zwitterionic phospholipids, which include the phosphatidylethanolamines and the phosphatidylcholines. Examples of phosphatidylethanolamines, include DOPE, DMPE and DPyPE. In certain embodiments, the co-lipid is DPyPE, which comprises two phytanoyl substituents incorporated into the diacylphosphatidylethanolamine skeleton. In other embodiments, the co-lipid is DOPE, CAS name 1,2-diolyeoyl-sn-glycero-3-phosphoethanolamine.

[0260] When a composition of the present invention comprises a cationic lipid and co-lipid, the cationic lipid:co-lipid molar ratio may be from about 9:1 to

about 1:9, from about 4:1 to about 1:4, from about 2:1 to about 1:2, or about 1:1.

[0261] In order to maximize homogeneity, the cationic lipid and co-lipid components may be dissolved in a solvent such as chloroform, followed by evaporation of the cationic lipid/co-lipid solution under vacuum to dryness as a film on the inner surface of a glass vessel (*e.g.*, a Rotovap round-bottomed flask). Upon suspension in an aqueous solvent, the amphipathic lipid component molecules self-assemble into homogenous lipid vesicles. These lipid vesicles may subsequently be processed to have a selected mean diameter of uniform size prior to complexing with, for example, a polynucleotide or a codon-optimized polynucleotide of the present invention, according to methods known to those skilled in the art. For example, the sonication of a lipid solution is described in Felgner *et al., Proc. Natl. Acad. Sci. USA 8:*,7413-7417 (1987) and in U.S. Pat. No. 5,264,618, the disclosures of which are incorporated herein by reference.

[0262] In those embodiments where the composition includes a cationic lipid, polynucleotides of the present invention are complexed with lipids by mixing, for example, a plasmid in aqueous solution and a solution of cationic lipid:co-lipid as prepared herein are mixed. The concentration of each of the constituent solutions can be adjusted prior to mixing such that the desired final plasmid/cationic lipid:co-lipid ratio and the desired plasmid final concentration will be obtained upon mixing the two solutions. The cationic lipid:co-lipid mixtures are suitably prepared by hydrating a thin film of the mixed lipid materials in an appropriate volume of aqueous solvent by vortex mixing at ambient temperatures for about 1 minute. The thin films are prepared by admixing chloroform solutions of the individual components to afford a desired molar solute ratio followed by aliquoting the desired volume of the solutions into a suitable container. The solvent is removed by evaporation, first with a stream of dry, inert gas (*e.g.,* argon) followed by high vacuum treatment.

[0263] Other hydrophobic and amphiphilic additives, such as, for example, sterols, fatty acids, gangliosides, glycolipids, lipopeptides, liposaccharides, neobees, niosomes, prostaglandins and sphingolipids, may also be included in compositions of the present invention. In such compositions, these additives may be included in an amount between about 0.1 mol % and about 99.9 mol % (relative to total lipid), about 1-50 mol %, or about 2-25 mol %.

[0264] Additional embodiments of the present invention are drawn to compositions comprising an auxiliary agent which is administered before, after, or concurrently with the polynucleotide. As used herein, an "auxiliary agent" is a substance included in a composition for its ability to enhance, relative to a composition which is identical *except* for the inclusion of the auxiliary agent, the entry of polynucleotides into vertebrate cells *in vivo*, and/or the *in vivo* expression of polypeptides encoded by such polynucleotides. Certain auxiliary agents may, in addition to enhancing entry of polynucleotides into cells, enhance an immune response to an immunogen encoded by the polynucleotide. Auxiliary agents of the present invention include nonionic, anionic, cationic, or zwitterionic surfactants or detergents, with nonionic surfactants or detergents being preferred, chelators, DNase inhibitors, poloxamers, agents that aggregate or condense nucleic acids, emulsifying or solubilizing agents, wetting agents, gel-forming agents, and buffers.

[0265] Auxiliary agents for use in compositions of the present invention include, but are not limited to non-ionic detergents and surfactants IGEPAL CA 630®, NONIDET NP-40, Nonidet® P40, Tween-20™, Tween-80™, Pluronic® F68 (ave. MW: 8400; approx. MW of hydrophobe, 1800; approx. wt. % of hydrophile, 80%), Pluronic F77® (ave. MW: 6600; approx. MW of hydrophobe, 2100; approx. wt. % of hydrophile, 70%), Pluronic P65®(ave. MW: 3400; approx. MW of hydrophobe, 1800; approx. wt. % of hydrophile, 50%), Triton X-100™, and Triton X-114™; the anionic detergent sodium dodecyl sulfate (SDS); the sugar stachyose; the condensing agent DMSO; and the chelator/DNAse inhibitor EDTA, CRL 1005 (12 kDa, 5% POE), and BAK

(Benzalkonium chloride 50% solution, available from Ruger Chemical Co. Inc.). In certain specific embodiments, the auxiliary agent is DMSO, Nonidet P40, Pluronic F68® (ave. MW: 8400; approx. MW of hydrophobe, 1800; approx. wt. % of hydrophile, 80%), Pluronic F77® (ave. MW: 6600; approx. MW of hydrophobe, 2100; approx. wt. % of hydrophile, 70%), Pluronic P65® (ave. MW: 3400; approx. MW of hydrophobe, 1800; approx. wt. % of hydrophile, 50%), Pluronic L64® (ave. MW: 2900; approx. MW of hydrophobe, 1800; approx. wt. % of hydrophile, 40%), and Pluronic F108® (ave. MW: 14600; approx. MW of hydrophobe, 3000; approx. wt. % of hydrophile, 80%). *See*, *e.g.*, U.S. Patent Application Publication No. 2002/0019358, published February 14, 2002, which is incorporated herein by reference in its entirety.

[0266] Certain compositions of the present invention may further include one or more adjuvants before, after, or concurrently with the polynucleotide. The term "adjuvant" refers to any material having the ability to (1) alter or increase the immune response to a particular antigen or (2) increase or aid an effect of a pharmacological agent. It should be noted, with respect to polynucleotide vaccines, that an "adjuvant," may be a transfection facilitating material. Similarly, certain "transfection facilitating materials" described *supra*, may also be an "adjuvant." An adjuvant may be used with a composition comprising a polynucleotide of the present invention. In a prime-boost regimen, as described herein, an adjuvant may be used with either the priming immunization, the booster immunization, or both. Suitable adjuvants include, but are not limited to, cytokines and growth factors; bacterial components (*e.g.*, endotoxins, in particular superantigens, exotoxins and cell wall components); aluminum-based salts; calcium-based salts; silica; polynucleotides; toxoids; serum proteins, viruses and virally-derived materials, poisons, venoms, imidazoquiniline compounds, poloxamers, and cationic lipids.

[0267] A great variety of materials have been shown to have adjuvant activity through a variety of mechanisms. Any compound which may increase the

expression, antigenicity or immunogenicity of the polypeptide is a potential adjuvant. The present invention provides an assay to screen for improved immune responses to potential adjuvants. Potential adjuvants which may be screened for their ability to enhance the immune response according to the present invention include, but are not limited to: inert carriers, such as alum, bentonite, latex, and acrylic particles; pluronic block polymers, such as TiterMax® (block copolymer CRL-8941, squalene (a metabolizable oil) and a microparticulate silica stabilizer), depot formers, such as Freunds adjuvant, surface active materials, such as saponin, lysolecithin, retinal, Quil A, liposomes, and pluronic polymer formulations; macrophage stimulators, such as bacterial lipopolysaccharide; alternate pathway complement activators, such as insulin, zymosan, endotoxin, and levamisole; and non-ionic surfactants, such as poloxamers, poly(oxyethylene)-poly(oxypropylene) tri-block copolymers. Also included as adjuvants are transfection-facilitating materials, such as those described above.

[0268] Poloxamers which may be screened for their ability to enhance the immune response according to the present invention include, but are not limited to, commercially available poloxamers such as Pluronic® surfactants, which are block copolymers of propylene oxide and ethylene oxide in which the propylene oxide block is sandwiched between two ethylene oxide blocks. Examples of Pluronic® surfactants include Pluronic® L121 (ave. MW: 4400; approx. MW of hydrophobe, 3600; approx. wt. % of hydrophile, 10%), Pluronic® L101 (ave. MW: 3800; approx. MW of hydrophobe, 3000; approx. wt. % of hydrophile, 10%), Pluronic® L81 (ave. MW: 2750; approx. MW of hydrophobe, 2400; approx. wt. % of hydrophile, 10%), Pluronic® L61 (ave. MW: 2000; approx. MW of hydrophobe, 1800; approx. wt. % of hydrophile, 10%), Pluronic® L31 (ave. MW: 1100; approx. MW of hydrophobe, 900; approx. wt. % of hydrophile, 10%), Pluronic® L122 (ave. MW: 5000; approx. MW of hydrophobe, 3600; approx. wt. % of hydrophile, 20%), Pluronic® L92 (ave. MW: 3650; approx. MW of hydrophobe, 2700; approx. wt. % of hydrophile, 20%), Pluronic® L72 (ave. MW: 2750; approx. MW of

hydrophobe, 2100; approx. wt. % of hydrophile, 20%), Pluronic® L62 (ave. MW: 2500; approx. MW of hydrophobe, 1800; approx. wt. % of hydrophile, 20%), Pluronic® L42 (ave. MW: 1630; approx. MW of hydrophobe, 1200; approx. wt. % of hydrophile, 20%), Pluronic® L63 (ave. MW: 2650; approx. MW of hydrophobe, 1800; approx. wt. % of hydrophile, 30%), Pluronic® L43 (ave. MW: 1850; approx. MW of hydrophobe, 1200; approx. wt. % of hydrophile, 30%), Pluronic® L64 (ave. MW: 2900; approx. MW of hydrophobe, 1800; approx. wt. % of hydrophile, 40%), Pluronic® L44 (ave. MW: 2200; approx. MW of hydrophobe, 1200; approx. wt. % of hydrophile, 40%), Pluronic® L35 (ave. MW: 1900; approx. MW of hydrophobe, 900; approx. wt. % of hydrophile, 50%), Pluronic® P123 (ave. MW: 5750; approx. MW of hydrophobe, 3600; approx. wt. % of hydrophile, 30%), Pluronic® P103 (ave. MW: 4950; approx. MW of hydrophobe, 3000; approx. wt. % of hydrophile, 30%), Pluronic® P104 (ave. MW: 5900; approx. MW of hydrophobe, 3000; approx. wt. % of hydrophile, 40%), Pluronic® P84 (ave. MW: 4200; approx. MW of hydrophobe, 2400; approx. wt. % of hydrophile, 40%), Pluronic® P105 (ave. MW: 6500; approx. MW of hydrophobe, 3000; approx. wt. % of hydrophile, 50%), Pluronic® P85 (ave. MW: 4600; approx. MW of hydrophobe, 2400; approx. wt. % of hydrophile, 50%), Pluronic® P75 (ave. MW: 4150; approx. MW of hydrophobe, 2100; approx. wt. % of hydrophile, 50%), Pluronic® P65 (ave. MW: 3400; approx. MW of hydrophobe, 1800; approx. wt. % of hydrophile, 50%), Pluronic® F127 (ave. MW: 12600; approx. MW of hydrophobe, 3600; approx. wt. % of hydrophile, 70%), Pluronic® F98 (ave. MW: 13000; approx. MW of hydrophobe, 2700; approx. wt. % of hydrophile, 80%), Pluronic® F87 (ave. MW: 7700; approx. MW of hydrophobe, 2400; approx. wt. % of hydrophile, 70%), Pluronic® F77 (ave. MW: 6600; approx. MW of hydrophobe, 2100; approx. wt. % of hydrophile, 70%), Pluronic® F108 (ave. MW: 14600; approx. MW of hydrophobe, 3000; approx. wt. % of hydrophile, 80%), Pluronic® F98 (ave. MW: 13000; approx. MW of hydrophobe, 2700; approx. wt. % of hydrophile, 80%), Pluronic® F88 (ave. MW: 11400; approx. MW of hydrophobe, 2400;

approx. wt. % of hydrophile, 80%), Pluronic® F68 (ave. MW: 8400; approx. MW of hydrophobe, 1800; approx. wt. % of hydrophile, 80%), Pluronic® F38 (ave. MW: 4700; approx. MW of hydrophobe, 900; approx. wt. % of hydrophile, 80%).

[0269] Reverse poloxamers which may be screened for their ability to enhance the immune response according to the present invention include, but are not limited to Pluronic® R 31R1 (ave. MW: 3250; approx. MW of hydrophobe, 3100; approx. wt. % of hydrophile, 10%), Pluronic® R 25R1 (ave. MW: 2700; approx. MW of hydrophobe, 2500; approx. wt. % of hydrophile, 10%), Pluronic® R 17R1 (ave. MW: 1900; approx. MW of hydrophobe, 1700; approx. wt. % of hydrophile, 10%), Pluronic® R 31R2 (ave. MW: 3300; approx. MW of hydrophobe, 3100; approx. wt. % of hydrophile, 20%), Pluronic® R 25R2 (ave. MW: 3100; approx. MW of hydrophobe, 2500; approx. wt. % of hydrophile, 20%), Pluronic® R 17R2 (ave. MW: 2150; approx. MW of hydrophobe, 1700; approx. wt. % of hydrophile, 20%), Pluronic® R 12R3 (ave. MW: 1800; approx. MW of hydrophobe, 1200; approx. wt. % of hydrophile, 30%), Pluronic® R 31R4 (ave. MW: 4150; approx. MW of hydrophobe, 3100; approx. wt. % of hydrophile, 40%), Pluronic® R 25R4 (ave. MW: 3600; approx. MW of hydrophobe, 2500; approx. wt. % of hydrophile, 40%), Pluronic® R 22R4 (ave. MW: 3350; approx. MW of hydrophobe, 2200; approx. wt. % of hydrophile, 40%), Pluronic® R 17R4 (ave. MW: 3650; approx. MW of hydrophobe, 1700; approx. wt. % of hydrophile, 40%), Pluronic® R 25R5 (ave. MW: 4320; approx. MW of hydrophobe, 2500; approx. wt. % of hydrophile, 50%), Pluronic® R 10R5 (ave. MW: 1950; approx. MW of hydrophobe, 1000; approx. wt. % of hydrophile, 50%), Pluronic® R 25R8 (ave. MW: 8550; approx. MW of hydrophobe, 2500; approx. wt. % of hydrophile, 80%), Pluronic® R 17R8 (ave. MW: 7000; approx. MW of hydrophobe, 1700; approx. wt. % of hydrophile, 80%), and Pluronic® R 10R8 (ave. MW: 4550; approx. MW of hydrophobe, 1000; approx. wt. % of hydrophile, 80%).

[0270] Other commercially available poloxamers which may be screened for their ability to enhance the immune response according to the present invention include compounds that are block copolymer of polyethylene and polypropylene glycol such as Synperonic® L121 (ave. MW: 4400), Synperonic® L122 (ave. MW: 5000), Synperonic® P104 (ave. MW: 5850), Synperonic® P105 (ave. MW: 6500), Synperonic® P123 (ave. MW: 5750), Synperonic® P85 (ave. MW: 4600) and Synperonic® P94 (ave. MW: 4600), in which L indicates that the surfactants are liquids, P that they are pastes, the first digit is a measure of the molecular weight of the polypropylene portion of the surfactant and the last digit of the number, multiplied by 10, gives the percent ethylene oxide content of the surfactant; and compounds that are nonylphenyl polyethylene glycol such as Synperonic® NP10 (nonylphenol ethoxylated surfactant - 10% solution), Synperonic® NP30 (condensate of 1 mole of nonylphenol with 30 moles of ethylene oxide) and Synperonic® NP5 (condensate of 1 mole of nonylphenol with 5.5 moles of naphthalene oxide).

[0271] Other poloxamers which may be screened for their ability to enhance the immune response according to the present invention include: (a) a polyether block copolymer comprising an A-type segment and a B-type segment, wherein the A-type segment comprises a linear polymeric segment of relatively hydrophilic character, the repeating units of which contribute an average Hansch-Leo fragmental constant of about –0.4 or less and have molecular weight contributions between about 30 and about 500, wherein the B-type segment comprises a linear polymeric segment of relatively hydrophobic character, the repeating units of which contribute an average Hansch-Leo fragmental constant of about –0.4 or more and have molecular weight contributions between about 30 and about 500, wherein at least about 80% of the linkages joining the repeating units for each of the polymeric segments comprise an ether linkage; (b) a block copolymer having a polyether segment and a polycation segment, wherein the polyether segment comprises at least an A-type block, and the polycation segment comprises a plurality of

cationic repeating units; and (c) a polyether-polycation copolymer comprising a polymer, a polyether segment and a polycationic segment comprising a plurality of cationic repeating units of formula —NH—R^0, wherein R^0 is a straight chain aliphatic group of 2 to 6 carbon atoms, which may be substituted, wherein said polyether segments comprise at least one of an A-type of B-type segment. *See* U.S. Patent No. 5,656,611, by Kabonov, *et al.*, which is incorporated herein by reference in its entirety. Other poloxamers of interest include CRL1005 (12 kDa, 5% POE), CRL8300 (11 kDa, 5% POE), CRL2690 (12 kDa, 10% POE), CRL4505 (15 kDa, 5% POE) and CRL1415 (9 kDa, 10% POE).

[0272] Other auxiliary agents which may be screened for their ability to enhance the immune response according to the present invention include, but are not limited to Acacia (gum arabic); the poloxyethylene ether R-O-$(C_2H_4O)_x$-H (BRIJ®), *e.g.*, polyethylene glycol dodecyl ether (BRIJ® 35, x=23), polyethylene glycol dodecyl ether (BRIJ® 30, x=4), polyethylene glycol hexadecyl ether (BRIJ® 52 x=2), polyethylene glycol hexadecyl ether (BRIJ® 56, x=10), polyethylene glycol hexadecyl ether (BRIJ® 58P, x=20), polyethylene glycol octadecyl ether (BRIJ® 72, x=2), polyethylene glycol octadecyl ether (BRIJ® 76, x=10), polyethylene glycol octadecyl ether (BRIJ® 78P, x=20), polyethylene glycol oleyl ether (BRIJ® 92V, x=2), and polyoxyl 10 oleyl ether (BRIJ® 97, x=10); poly-D-glucosamine (chitosan); chlorbutanol; cholesterol; diethanolamine; digitonin; dimethylsulfoxide (DMSO), ethylenediamine tetraacetic acid (EDTA); glyceryl monosterate; lanolin alcohols; mono- and di-glycerides; monoethanolamine; nonylphenol polyoxyethylene ether (NP-40®); octylphenoxypolyethoxyethanol (NONIDET NP-40 from Amresco); ethyl phenol poly (ethylene glycol ether)^n, n=11 (Nonidet® P40 from Roche); octyl phenol ethylene oxide condensate with about 9 ethylene oxide units (nonidet P40); IGEPAL CA 630® ((octyl phenoxy) polyethoxyethanol; structurally same as NONIDET NP-40); oleic acid; oleyl alcohol; polyethylene glycol 8000; polyoxyl 20 cetostearyl ether;

polyoxyl 35 castor oil; polyoxyl 40 hydrogenated castor oil; polyoxyl 40 stearate; polyoxyethylene sorbitan monolaurate (polysorbate 20, or TWEEN-20®; polyoxyethylene sorbitan monooleate (polysorbate 80, or TWEEN-80®); propylene glycol diacetate; propylene glycol monstearate; protamine sulfate; proteolytic enzymes; sodium dodecyl sulfate (SDS); sodium monolaurate; sodium stearate; sorbitan derivatives (SPAN®), *e.g.*, sorbitan monopalmitate (SPAN® 40), sorbitan monostearate (SPAN® 60), sorbitan tristearate (SPAN® 65), sorbitan monooleate (SPAN® 80), and sorbitan trioleate (SPAN® 85); 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosa-hexaene (squalene); stachyose; stearic acid; sucrose; surfactin (lipopeptide antibiotic from *Bacillus subtilis*); dodecylpoly(ethyleneglycolether)$_9$ (Thesit®) MW 582.9; octyl phenol ethylene oxide condensate with about 9-10 ethylene oxide units (Triton X-100™); octyl phenol ethylene oxide condensate with about 7-8 ethylene oxide units (Triton X-114™); tris(2-hydroxyethyl)amine (trolamine); and emulsifying wax.

[0273] In certain adjuvant compositions, the adjuvant is a cytokine. A composition of the present invention can comprise one or more cytokines, chemokines, or compounds that induce the production of cytokines and chemokines, or a polynucleotide encoding one or more cytokines, chemokines, or compounds that induce the production of cytokines and chemokines. Examples include, but are not limited to granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interleukin 15 (IL-15), interleukin 18 (IL-18), interferon alpha (IFNα), interferon beta (IFNβ), interferon gamma (IFNγ), interferon omega (IFNω), interferon tau (IFNϑ), interferon gamma inducing factor I (IGIF), transforming growth factor beta (TGF-β), RANTES (regulated upon

activation, normal T-cell expressed and presumably secreted), macrophage inflammatory proteins (*e.g.*, MIP-1 alpha and MIP-1 beta), *Leishmania* elongation initiating factor (LEIF), and Flt-3 ligand.

[0274] In certain compositions of the present invention, the polynucleotide construct may be complexed with an adjuvant composition comprising (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE). The composition may also comprise one or more co-lipids, *e.g.*, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and/or 1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE). An adjuvant composition comprising ;GAP-DMORIE and DPyPE at a 1:1 molar ratio is referred to herein as Vaxfectin™. *See, e.g.*, PCT Publication No. WO 00/57917, which is incorporated herein by reference in its entirety.

[0275] In other embodiments, the polynucleotide itself may function as an adjuvant as is the case when the polynucleotides of the invention are derived, in whole or in part, from bacterial DNA. Bacterial DNA containing motifs of unmethylated CpG-dinucleotides (CpG-DNA) triggers innate immune cells in vertebrates through a pattern recognition receptor (including toll receptors such as TLR 9) and thus possesses potent immunostimulatory effects on macrophages, dendritic cells and B-lymphocytes. *See, e.g.*, Wagner, H., *Curr. Opin. Microbiol. 5*:62-69 (2002); Jung, J. *et al.*, *J. Immunol. 169:* 2368-73 (2002); *see also* Klinman, D.M. *et al., Proc. Natl Acad. Sci. U.S.A. 93*:2879-83 (1996). Methods of using unmethylated CpG-dinucleotides as adjuvants are described in, for example, U.S. Patent Nos. 6,207,646, 6,406,705, and 6,429,199, the disclosures of which are herein incorporated by reference.

[0276] The ability of an adjuvant to increase the immune response to an antigen is typically manifested by a significant increase in immune-mediated protection. For example, an increase in humoral immunity is typically manifested by a significant increase in the titer of antibodies raised to the antigen, and an increase in T-cell activity is typically manifested in increased

cell proliferation, or cellular cytotoxicity, or cytokine secretion. An adjuvant may also alter an immune response, for example, by changing a primarily humoral or Th_2 response into a primarily cellular, or Th_1 response.

[0277] In certain embodiments, the compositions of the present invention may be administered in the absence of one or more transfection facilitating materials or auxiliary agents. It has been shown that, surprisingly, the cells of living vertebrates are capable of taking up and expressing polynucleotides that have been injected *in vivo*, even in the absence of any agent to facilitate transfection. Cohen, J., *Science* 259: 1691-1692; Felgner, P., *Scientific American* 276: 102-106 (1997). These references are hereby incorporated by reference in their entireties. Thus, by way of non-limiting examples, nucleic acid molecules and/or polynucleotides of the present invention (*e.g.*, plasmid DNA, mRNA, linear DNA, or oligonucleotides) may be administered in the absence of any one of, or any combination of more than one of the following transfection facilitating materials or auxiliary agents as described herein: inorganic materials including but not limited to calcium phosphate, alum, and/or gold particles; peptides including, but not limited to cationic peptides, amphipathic peptides, intercell targeting peptides, and/or intracell targetting peptides; proteins, including, but not limited to basic (*i.e.*, positively-charged) proteins, targeting proteins, viral proteins, and/or pore-forming proteins; lipids, including but not limited to cationic lipids, anionic lipids, basic lipids, neutral lipids, and/or zwitterionic lipids; polymers including but not limited to dendrimers, star-polymers, "homogeneous" poly-amino acids, "heterogenous" poly-amino acids, co-polymers, PVP, poloxamers, and/or PEG; surfactants, including but not limited to anionic surfactants, cationic surfactants, and zwitterionic surfactants; detergents, including but not limited to anionic detergents, cationic detergents, and zwitterionic detergents; chelators, including but not limited to EDTA; DNase inhibitors; condensing agents including, but not limited to DMSO; emulsifying or solublizing agents; gel-forming agents; buffers, and/or adjuvants.

[0278] Nucleic acid molecules and/or polynucleotides of the present invention, *e.g.*, plasmid DNA, mRNA, linear DNA or oligonucleotides, may be solubilized in any of various buffers. Suitable buffers include, for example, phosphate buffered saline (PBS), normal saline, Tris buffer, and sodium phosphate (*e.g.*, 150 mM sodium phosphate). Insoluble polynucleotides may be solubilized in a weak acid or weak base, and then diluted to the desired volume with a buffer. The pH of the buffer may be adjusted as appropriate. In addition, a pharmaceutically acceptable additive can be used to provide an appropriate osmolarity. Such additives are within the purview of one skilled in the art. For aqueous compositions used *in vivo*, sterile pyrogen-free water can be used. Such formulations will contain an effective amount of a polynucleotide together with a suitable amount of an aqueous solution in order to prepare pharmaceutically acceptable compositions suitable for administration to a human.

[0279] Compositions of the present invention can be formulated according to known methods. Suitable preparation methods are described, for example, in Remington's Pharmaceutical Sciences, 16th Edition, A. Osol, ed., Mack Publishing Co., Easton, PA (1980), and Remington's Pharmaceutical Sciences, 19th Edition, A.R. Gennaro, ed., Mack Publishing Co., Easton, PA (1995), both of which are incorporated herein by reference in their entireties. Although the composition may be administered as an aqueous solution, it can also be formulated as an emulsion, gel, solution, suspension, lyophilized form, or any other form known in the art. In addition, the composition may contain pharmaceutically acceptable additives including, for example, diluents, binders, stabilizers, and preservatives.

Passive Immunotherapy

[0280] Antibody therapy can be subdivided into two principally different activities: (i) passive immunotherapy using intact non-labeled antibodies or labeled antibodies and (ii) active immunotherapy using anti-idiotypes for re-establishment of network balance in autoimmunity

[0281] In passive immunotherapy, naked antibodies are administered to neutralize an antigen or to direct effector functions to targeted membrane associated antigens. Neutralization would be of a lymphokine, a hormone, or an anaphylatoxin, *i.e.*, C5a. Effector functions include complement fixation, macrophage activation and recruitment, and antibody-dependent cell-mediated cytotoxicity (ADCC). Naked antibodies have been used to treat leukemia (Ritz, S.F. *et al Blood*, 58:141-152 (1981)) and antibodies to GD2 have been used in treatments of neuroblastomas (Schulz *et al. Cancer Res.* 44:5914 (1984)) and melanomas (Irie *et al., Proc. Natl. Acad. Sci.* 83: 8694 (1986) One major advantage of passive antibody immunization is that it provides immediate immunity that can last for weeks and possibly months. Casadevall, A. "Passive Antibody Administration (Immediate Immunity) as a Specific Defense against Biological Weapons." *Emerging Infectious Diseases.* 8:833-841(2002).

[0282] The invention also provides for antibodies specifically reactive with SARS Co-V polypeptides which have been produced from an immune response elicited by the administration, to a vertebrate, of polynucleotide and polypeptides of the present invention. Anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols (See, for example, *Antibodies: A Laboratory Manual* ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). A vertebrate such as a mouse, a hamster, a rabbit, a horse, a human, or non-human primate can be immunized with an immunogenic form of a SARS Co-V polypeptide or polynucleotide, of the present invention, encoding an immunogenic form of a SARS-CoV polypeptide. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. An immunogenic portion of the SARS-CoV polypeptide can be administered in the presence of adjuvant and as part of compositions described herein. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibodies.

[0283] The antibodies of the invention are immunospecific for antigenic determinants of the SARS-CoV polypeptides of the invention, *e.g.*, antigenic determinants of a polypeptide of the invention or a closely related human or non-human mammalian homolog (*e.g.*, 90% homologous and at least about 95% homologous). In an alternative embodiment of the invention, the SARS Co-V antibodies do not substantially cross react (*i.e.*, react specifically) with a protein which is for example, less than 80% percent homologous to a sequence of the invention. By "not substantially cross react," is meant that the antibody has a binding affinity for a non-homologous protein which is less than 10 percent, less than 5 percent, or less than 1 percent, of the binding affinity for a protein of the invention. In an alternative embodiment, there is no cross-reactivity between viral and mammalian antigens.

[0284] In one embodiment, purified monoclonal antibodies or polyclonal antibodies containing the variable heavy and light sequences are used as therapeutic and prophylactic agents to treat or prevent SARS-CoV infection by passive antibody therapy. In general, this will comprise administering a therapeutically or prophylactically effective amount of the monoclonal or polyclonal antibodies to a susceptible vertebrate or one exhibiting SARS Co-V infection. A dosage effective amount will range from about 50 to 20,000 μg/Kg, and from about 100 to 5000 μg/Kg. However, suitable dosages will vary dependening on factors such as the condition of the treated host, weight, etc. Suitable effective dosages may be determined by those skilled in the art.

[0285] In an alternative embodiment, purified antibodies and the polynucleotides or polypeptides of the present invention are administered simultaneously (at the same time) or subsequent to the administration of the isolated antibodies, thereby providing both immediate and long lasting protection.

[0286] The monoclonal or polyclonal antibodies may be administered by any mode of administration suitable for administering antibodies. Typically, the subject antibodies will be administered by injection, *e.g.*, intravenous, intramuscular, or intraperitoneal injection (as described previously), or

aerosol. Aerosol administration is particularly preferred if the subjects treated comprise newborn infants.

[0287] Formulation of antibodies in pharmaceutically acceptable form may be effected by known methods, using known pharmaceutical carriers and excipients. Suitable carriers and excipients include by way of non-limiting example buffered saline, and bovine serum albumin.

[0288] Any polynucleotides or polypeptides, as described herein, can be used to produce the isolated antibodies of the invention. For example, SARS-CoV proteins S, N, M, and E, fragments, variants and derivatives thereof, are purified as described in Example 2. The purified protein then serves as an antigen for producing SARS-CoV specific monoclonal and polyclonal antibodies.

[0289] Any vertebrate can serve as a host for antibody production. Preferred hosts include, but are not limited to human, non-human primate, mouse, rabbit, horse, goat, donkey, cow, sheep, chickens, cat, dog. Alternatively, antibodies can be produced by cultivation ex vivo of lymphocytes from primed donors stimulated with CD40 resulting in expansion of human B cells Banchereau *et al.*, *Science* 251:70 (1991); Zhani *et al., J. Immunol.* 144:2955-2960, (1990); Tohma *et al.*, *J. Immunol.*146:2544-2552 (1991). Furthermore, an extra *in vitro* booster step can be used to obtain a higher yield of antibodies prior to immortalization of the cells. See Chaudhuri *et al.*, *Cancer Supplement* 73: 1098-1104 (1994); Steenbakkers *et al. Hum. Antibod. Hybridomas* 4: 166-173 (1993); Ferrarro *et al., Hum. Antibod. Hybridomas* 4:80-85 (1993); Kwekkeboom *et al., Immunol. Methods* 160:117-127 (1993), which are herein incorporated by reference.

[0290] An alternative to human primed donors, is to "recreate" or mimic splenic conditions in an immunocompromised animal host, such as the "Severe Combined Immune Deficient" (SCID) mouse. Human lymphocytes are readily adopted by the SCID mouse (hu-SCID) and produce high levels of immunoglobulins Mosier *et al, Nature* 335:256 (1988); McCune *et al, Science* 241:1632-1639 (1988). Moreover, if the donor used for reconstitution has been

exposed to a particular antigen, a strong secondary response to the same antigen can be elicited in such mice. Duchosal *et al. Nature* 355:258-262 (1992).

[0291] The term "antibody" as used herein is intended to include fragments thereof which are also specifically reactive with SARS-CoV polypeptides. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example. $F(ab')_2$ fragments can be generated by treating antibody with pepsin. The resulting $F(ab')_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments. The antibody of the invention is further intended to include bispecific and chimeric molecules having an anti-SARS-CoV portion.

[0292] Both monoclonal and polyclonal antibodies (Ab) directed against SARS-CoV polypeptides or SARS-CoV polypeptide variants, and antibody fragments such as Fab' and $F(ab')_2$, can be used to block the action of SARS-CoV polypeptides and allow the study of the role of a particular SARS-CoV polypeptide of the invention in the infectious life cycle of the virus and in pathogenesis.

[0293] Moreover, the antibodies possess utility as immunoprobes for diagnosis of SARS Co-V infection. This generally comprises taking a sample, *e.g.*, respiratory fluid, of a person suspected of having SARS-CoV infection and incubating the sample with the subject human monoclonal antibodies to detect the presence of SARS-CoV infected cells. This involves directly or indirectly labeling the subject human antibodies with a reporter molecule which provides for detection of human monoclonal antibody SARS-CoV immune complexes. Examples of known labels include by way of non-limiting example enzymes, *e.g.*,.β-lactamase, luciferase, and radiolabels. Methods for effecting immunodetection of antigens using monoclonal antibodies are well known in the art.

[0294] The following examples are included for purposes of illustration only and are not intended to limit the scope of the present invention, which is

defined by the appended claims. All references cited in the Examples are incorporated herein by reference in their entireties.

EXAMPLES

Materials and Methods

[0295] The following materials and methods apply generally to all the examples disclosed herein. Specific materials and methods are disclosed in each example, as necessary.

[0296] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology (including PCR), vaccinology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. *See*, for example, *Molecular Cloning A Laboratory Manual*, 2nd Ed., Sambrook *et al.*, ed., Cold Spring Harbor Laboratory Press: (1989); *DNA Cloning*, Volumes I and II (D. N. Glover ed., 1985); *Oligonucleotide Synthesis* (M. J. Gait ed., 1984); Mullis *et al.* U.S. Pat. No: 4,683,195; *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Transcription And Translation* (B. D. Hames & S. J. Higgins eds. 1984); *Culture Of Animal Cells* (R. I. Freshney, Alan R. Liss, Inc., 1987); *Immobilized Cells And Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); *Methods In Enzymology*, Vols. 154 and 155 (Wu *et al.* eds.), *Immunochemical Methods In Cell And Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); and in Ausubel *et al.*, *Current Protocols in Molecular Biology,* John Wiley and Sons, Baltimore, Maryland (1989).

Gene Construction

[0297] Constructs of the present invention are constructed based on the sequence information provided herein or in the art utilizing standard molecular biology techniques, including, but not limited to the following. First, a series complementary oligonucleotide pairs of 80-90 nucleotides each in length and spanning the length of the construct are synthesized by standard methods. These oligonucleotide pairs are synthesized such that upon annealing, they form double stranded fragments of 80-90 base pairs, containing cohesive ends. The single-stranded ends of each pair of oligonucleotides are designed to anneal with a single-stranded end of an adjacent oligonucleotide duplex. Several adjacent oligonucleotide pairs prepared in this manner are allowed to anneal, and approximately five to six adjacent oligonucleotide duplex fragments are then allowed to anneal together via the cohesive single stranded ends. This series of annealed oligonucleotide duplex fragments is then ligated together and cloned into a suitable plasmid, such as the TOPO® vector available from Invitrogen Corporation, Carlsbad, CA. The construct is then sequenced by standard methods. Constructs prepared in this manner, comprising 5 to 6 adjacent 80 to 90 base pair fragments ligated together, *i.e.*, fragments of about 500 base pairs, are prepared, such that the entire desired sequence of the construct is represented in a series of plasmid constructs. The inserts of these plasmids are then cut with appropriate restriction enzymes and ligated together to form the final construct. The final construct is then cloned into a standard bacterial cloning vector, and sequenced. The oligonucleotides and primers referred to herein can easily be designed by a person of skill in the art based on the sequence information provided herein and in the art, and such can be synthesized by any of a number of commercial nucleotide providers, for example Retrogen, San Diego, CA.

Plasmid Vector

[0298] Constructs of the present invention can be inserted, for example, into eukaryotic expression vectors VR1012 or VR10551. These vectors are built on a modified pUC18 background (*see* Yanisch-Perron, C., *et al. Gene 33*:103-119 (1985)), and contain a kanamycin resistance gene, the human cytomegalovirus immediate early promoter/enhancer and intron A, and the bovine growth hormone transcription termination signal, and a polylinker for inserting foreign genes. *See* Hartikka, J., *et al.*, *Hum. Gene Ther. 7*:1205-1217 (1996). However, other standard commercially available eukaryotic expression vectors may be used in the present invention, including, but not limited to: plasmids pcDNA3, pHCMV/Zeo, pCR3.1, pEF1/His, pIND/GS, pRc/HCMV2, pSV40/Zeo2, pTRACER-HCMV, pUB6/V5-His, pVAX1, and pZeoSV2 (available from Invitrogen, San Diego, CA), and plasmid pCI (available from Promega, Madison, WI).

[0299] An optimized backbone plasmid, termed VR-10551 has minor changes from the VR-1012 backbone described above. The VR-10551 vector is derived from and similar to VR-1012 in that it uses the human cytomegalovirus immediate early (hCMV-IE) gene enhancer/promoter and 5' untranslated region (UTR), including the hCMV-IE Intron A. The changes from the VR-1012 to the VR-10551 include some modifications to the multiple cloning site, and a modified rabbit ∃globin 3' untranslated region/polyadenylation signal sequence/transcriptional terminator has been substituted for the same functional domain derived from the bovine growth hormone gene.

Plasmid DNA Purification

[0300] Plasmid DNA may be transformed into competent cells of an appropriate *Escherichia coli* strain (including but not limited to the DH5α

strain) and highly purified covalently closed circular plasmid DNA may be isolated by a modified lysis procedure (Horn, N.A., *et al., Hum. Gene Ther.* *6*:565-573 (1995)) followed by standard double CsCl-ethidium bromide gradient ultracentrifugation (Sambrook, J., *et al., Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Plainview, New York (1989)). Alternatively, plasmid DNAs are purified using Giga columns from Qiagen (Valencia, CA) according to the kit instructions. All plasmid preparations are free of detectable chromosomal DNA, RNA and protein impurities based on gel analysis and the bicinchoninic protein assay (Pierce Chem. Co., Rockford IL). Endotoxin levels are measured using *Limulus* Amebocyte Lysate assay (LAL, Associates of Cape Cod, Falmouth, MA) in Endotoxin Units/mg of plasmid DNA. The spectrophotometric A_{260}/A_{280} ratios of the DNA solutions are also determined. Plasmids are ethanol precipitated and resuspended in an appropriate solution, *e.g.*, 150 mM sodium phosphate (for other appropriate excipients and auxiliary agents, see U.S. Patent Application Publication 20020019358, published February 14, 2002). DNA is stored at -20EC until use. DNA is diluted by mixing it with 300 mM salt solutions and by adding appropriate amount of USP water to obtain 1 mg/ml plasmid DNA in the desired salt at the desired molar concentration.

Injections of plasmid DNA

[0301] The quadriceps muscles of restrained awake mice (*e.g.*, female 6 - 12 week old BALB/c mice from Harlan Sprague Dawley, Indianapolis, IN) are injected bilaterally with 50 μg of DNA in 50 μl solution (100 μg in 100 μl total per mouse) using a disposable plastic insulin syringe and 28G 1/2 needle (Becton-Dickinson, Franklin Lakes, NJ, Cat. No. 329430) fitted with a plastic collar cut from a micropipette tip, as previously described (Hartikka, J., *et al.*, *Hum. Gene Ther.* *7*:1205-1217 (1996).

[0302] Animal care will comply with the "Guide for the Use and Care of Laboratory Animals," Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, D.C., 1996 as well as with Vical's Institutional Animal Care and Use Committee.

EXAMPLE 1

Construction of Expression Vectors

[0303] Plasmid constructs comprising the native coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg are constructed as follows. The S, S1, S2, N, M, or E genes from SARS-CoV Urbani or other strains (*e.g.,* CUKH-Su10, TOR2 and BJ01) are isolated from viral RNA by RT PCR, or prepared by direct synthesis if the wildtype sequence is known, by standard methods and are inserted into the vector VR-10551 via standard restriction sites, by standard methods.

[0304] Plasmid constructs comprising human codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, are prepared as follows. The codon-optimized coding regions are generated using the full, minimal, uniform, or other codon optimization methods described herein. The coding regions or codon optimized coding regions are constructed using standard PCR methods described herein, or are ordered commercially. The coding regions or codon-optimized coding regions are inserted into the vector VR-10551 via standard restriction sites, by standard methods.

[0305] Examples of constructs to be made are listed in Table 19.

TABLE 19

Gene	Strain	Backbone	Wild type/ Codon optimized
S	Urbani	10551	Wild type
S	Urbani	**10551**	**Codon optimized**
S1	Urbani	1012	Wild type
S1	Urbani	**10551**	**Codon optimized**
S2	Urbani	10551	Wild type
S2	Urbani	**10551**	**Codon optimized**
N	Urbani	10551	Wild type
N	Urbani	**10551**	**Codon optimized**
M	Urbani	10551	Wild type
M	Urbani	**10551**	**Codon optimized**
E	Urbani	10551	Wild type
E	Urbani	**10551**	**Codon optimized**

[0306] Plasmids constructed as above are propagated in *Escherichia coli* and purified by the alkaline lysis method (Sambrook, J., *et al., Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2 (1989)). CsCl-banded DNA are ethanol precipitated and resuspended in 0.9% saline to a final concentration of 2 mg/ml for injection. Alternately, plasmids are purified using any of a variety of commercial kits, or by other known procedures involving differential precipitation and/or chromatographic purification.

[0307] Expression is tested by formulating each of the plasmids in DMRIE/DOPE and transfecting cell lines including, but not limited to VM92 cells, fungal cells, including yeast cells such as *Saccharomyces* spp. cells; insect cells such as *Drosophila* S2, *Spodoptera* Sf9 or Sf21 cells and *Trichoplusa* High-Five cells; other animal cells (particularly mammalian cells and human cells) such as MDCK, CV1, 3T3, CPAE, A10, Sp2/0-Ag14, PC12, CHO, COS, VERO, HeLa, Bowes melanoma cells, SW-13, NCI-H295, RT4, HT-1376, UM-UC-3, IM-9, KG-1, R54;11, A-172, U-87MG, BT-20, MCF-7, SK-BR-3, ChaGo K-1, CCD-14Br, CaSki, ME-180, FHC, HT-29, Caco-2, SW480, HuTu80, Tera 1, NTERA-2, AN3 CA, KLE, RL95-2, Caki-1, ACHN, 769 P, CCRF-CEM, Hut 78, MOLT 4, HL-60, Hep-3B, HepG2, SK-HEP1, A-549, NCI-H146, NCI-H82, NCI-H82, SK-LU-1, WI-38, MRC-5,

HLF-a, CCD-19Lu, C39, Hs294T, SK-MEL5, COLO 829, U266B1, RPMI 2650, BeWo, JEG-3, JAR, SW 1353, MeKam, and SCC-4; and higher plant cells. Appropriate culture media and conditions for the above-described host cells are known in the art.

[0308] The supernatants are collected and the protein production tested by Western blot or ELISA. The relative expression of the wild type and codon optimized constructs are compared.

[0309] In addition to plasmids encoding single SARS-CoV proteins, single plasmids which contain a portion of a SARS-CoV coding region are constructed according to standard methods. For example, portions of a SARS-CoV coding region that is too large to be contained in a single plasmid may be inserted into two or more plasmids. Also, single plasmids which contain two or more SARS-CoV coding regions are constructed according to standard methods. For example, a polycistronic construct, where two or more SARS-CoV coding regions are transcribed as a single transcript in eukaryotic cells may be constructed by separating the various coding regions with IRES sequences (Jang *et al.* "A segment of the 5' nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation." *J. Virol.* 62: 2636-43 (1988); Jang *et al.* "Cap-independent Translation of Picornavirus RNAs: Structure and Function of the Internal Ribosomal Entry Site." *Enzyme* 44:292-309(1990)).

[0310] Alternatively, two or more coding regions may be inserted into a single plasmid, each with their own promoter sequence.

EXAMPLE 2

In vitro expression of SARS-CoV subunit proteins

[0311] Expression of SARS-CoV Nucleocapsid (N) and Spike (S) constructs were tested *in vitro* by transfection of a mouse melanoma cell line (VM92). The following expression constructs were transfected individually into VM92 cells and cultured for a period of time. All SARS-CoV sequences described

below, were cloned into the VR1012 expression vector. The VR9208 expression plasmid contains a nucleotide sequence encoding the SARS-CoV S1 domain which was codon-optimized according to the full optimization method described herein and is disclosed in SEQ ID NO:50. The VR9204 expression plasmid contains a nucleotide sequence encoding a fragment of the SARS-CoV S1 which corresponds to amino acids 1-417 of the SARS-CoV S1 protein. The coding sequence in VR9204 was also codon optimized according to the full optimization method described herein.

VR9219 - expressing full-length SARS-CoV N protein
VR9208 - expressing SARS-CoV S1 domain of the S protein (amino acids 1-683 of the S protein)
VR9204 - expressing a fragment of the SARS-CoV S1 domain (amino acids 1-417 of the S1 domain)
VR9209 - expressing SARS-CoV S2 domain of the S protein
VR9210 - expressing SARS-CoV secreted S protein

[0312] Both cell extracts and cell culture medium supernatants were analyzed by Western blot. The presence of the SARS-CoV N protein and S proteins were detected using commercial rabbit polyclonal antibodies which reconginze the N protein from SARS-CoV strain Urbani (IMG-543; Imgenex, San Diego, CA) and the S proteins from SARS-CoV strain Urbani (IMG-557, 542 and 541; Imgenex, Diego, CA). Western blot results are summarized below:

[0313] In both the supernantant and cell lystates from cells transfected with the VR9219 plasmid, protein bands of a molecular weight of between 37 and 50 kDa (as estimated by a protein molecular weight standard) were detectable. The SARS-CoV N protein has an expected molecule weight of 46 kDa. This result is consistent with efficient expression of the SARS-CoV N antigen.

[0314] The supernantant and cell lysates from cells transfected with four different SARS-CoV S antigen constructs were individually analyzed for the presence of the S antigen. The results are summarized below.

[0315] A protein band of 85-110 kDa (as estimated by a protein molecular weight standard) was detected by Western blot in both the lysate and

supernatant of cells transfected with the VR9204 plasmid (S1 domain - fragment).

[0316] A protein band of about 150 kDa (as estimated by a protein molecular weight standard) was detected by Western blot in both the lysate and supernatant of cells transfected with the VR9208 plasmid (S1 domain).

[0317] A protein band of approximately 111 kDa (as estimated by a protein molecular weight standard) was detected by Western blot in both the lysate and supernatant of cells transfected with the VR9209 plasmid (S2 domain).

[0318] A protein band of about 190 kDa (as estimated by a protein molecular weight standard) was detected by Western blot in both the lysate and supernatant of cells transfected with the VR9210 plasmid (secreted S).

[0319] These results are consistent with efficient expression and secretion of SARS-CoV Spike protein. Due to the presence of glycosylation sites in the S protein, the molecular weight is difficult to acurrately predict.

EXAMPLE 3

Preparation of SARS-CoV subunit proteins

[0320] Recombinantly prepared SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, for use as subunit proteins in the various combination therapies and compositions described herein, are prepared using the following procedure.

[0321] Eukaryotic cells transfected with expression plasmids such as those described in Example 1 are used to express SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg. Alternatively, a baculovirus system can

be used wherein insect cells such as, but not limited to, Sf9, Sf21, or D.Mel-2 cells are infected with recombinant baculoviruses which can express SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg. Other *in vitro* expression systems may be used, and are well known to those of ordinary skill in the art. For baculovirus expression of non-secreted forms of these proteins, cells which are infected with recombinant baculoviruses capable of expressing SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, are collected by knocking and scraping cells off the bottom of the flask in which they are grown. Cells infected with baculoviruses for 24 or 48 hours are less easy to detach from flask and may lyse, thus care must be taken with their removal. Eukaryotic cells which are transfected, either transiently or permanently, with expression plasmids encoding non-secreted forms of SARS-CoV proteins are gently scraped of the bottom of the flasks in which they are grown. Flasks containing the cells are then rinsed with PBS and the cells are transferred to 250 ml conical tubes. The tubes are spun at 1000 rpm in J-6 centrifuge (300 x g) for about 5-10 minutes. The cell pellets are washed two times with PBS and then resuspended in about 10-20 ml of PBS in order to count. The cells are finally resuspended at a concentration of about $2x10^7$ cells/ml in RSB (10mM Tris pH=7.5, 1.5mM $MgCl_2$, 10mM KCl).

[0322] At this point either a total cell lysate is prepared, or cytoplasmic and nuclear fractions are separated. Approximately 10^6 infected cells are used per lane of a standard SDS-PAGE mini-protein gel for gel analysis purposes. When separating cytoplasmic and nuclear fractions, 10% NP40 is added to the cells for a final concentration of 0.5%. The cell-NP40 mixture is vortexed and placed on ice for 10 minutes, vortexing occasionally. After ice incubation, the

cells are spun at 1500 rpm in a J-6 centrifuge (600 x 1) for 10 minutes. The supernantant is removed, which is the cytoplasmic fraction. The remaining pellet, containing the nuclei, is washed two times with buffer C (20 mM HEPES pH = 7.9, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM DTT) to remove cytoplasmic proteins. The nuclei are resuspended in buffer C to $5x10^7$ nuclei/ml. The nuclei are vortexed vigorously to break up particles and an aliquot is removed for the mini-protein gel, which is the nuclei fraction.

[0323] Whole cell lysates are prepared by simply resuspending the requisite number of cells in gel sample buffer.

[0324] For gel analysis, a small amount (about 10^6 nuclear equivalents) of the nuclear pellet is resuspended directly in gel sample buffer and run with equivalent amounts of whole cells, cytoplasm, and nuclei. Those fractions containing the SARS-CoV protein of interest are detected by Western blot analysis as described herein.

[0325] Following analysis as described above, larger quantities of crude subunit proteins are prepared from batch cell cultures by protein purification methods well known by those of ordinary skill in the art, *e.g.*, the use of HPLC.

[0326] Secreted versions of SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg are isolated from cell culture supernatants using various protein purification methods well known to those of ordinary skill in the art.

EXAMPLE 4

Preparation of Vaccine Formulations

[0327] Plasmid constructs comprising codon-optimized and non-codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S,

soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, as well as various controls, *e.g.*, empty vector, are formulated with the poloxamer CRL 1005 and BAK (Benzalkonium chloride 50% solution, available from Ruger Chemical Co. Inc.) by the following methods. Specific final concentrations of each component of the formulae are described in the following methods, but for any of these methods, the concentrations of each component may be varied by basic stoichiometric calculations known by those of ordinary skill in the art to make a final solution having the desired concentrations.

[0328] For example, the concentration of CRL 1005 is adjusted depending on, for example, transfection efficiency, expression efficiency, or immunogenicity, to achieve a final concentration of between about 1 mg/ml to about 75 mg/ml, for example, about 1 mg/ml, about 2 mg/ml, about 3 mg/ml, about 4 mg/ml, about 5 mg/ml, about 6.5 mg/ml, about 7 mg/ml, about 7.5 mg/ml, about 8 mg/ml, about 9 mg/ml, about 10 mg/ml, about 15 mg/ml, about 20 mg/ml, about 25 mg/ml, about 30 mg/ml, about 35 mg/ml, about 40 mg/ml, about 45 mg/ml, about 50 mg/ml, about 55 mg/ml, about 60 mg/ml, about 65 mg/ml, about 70 mg/ml, or about 75 mg/ml of CRL 1005.

[0329] Similarly, the concentration of DNA is adjusted depending on many factors, including the amount of a formulation to be delivered, the age and weight of the subject, the delivery method and route and the immunogenicity of the antigen being delivered. In general, formulations of the present invention are adjusted to have a final concentration from about 1 ng/ml to about 30 mg/ml of plasmid (or other polynucleotide). For example, a formulation of the present invention may have a final concentration of about 1 ng/ml, about 5 ng/ml, about 10 ng/ml, about 50 ng/ml, about 100 ng/ml, about 500 ng/ml, about 1 μg/ml, about 5 μg/ml, about 10 μg/ml, about 50 μg/ml, about 200 μg/ml, about 400 μg/ml, about 600 μg/ml, about 800 μg/ml, about 1 mg/ml, about 2 mg/ml, about 2.5, about 3 mg/ml, about 3.5, about 4 mg/ml, about 4.5, about 5 mg/ml, about 5.5 mg/ml, about 6 mg/ml, about 7 mg/ml,

about 8 mg/ml, about 9 mg/ml, about 10 mg/ml, about 20 mg/ml, or about 30 mg/ml of a plasmid.

[0330] Certain formulations of the present invention include a cocktail of plasmids (*see, e,g,*, Example 1 *supra*) of the present invention, *e.g.*, comprising coding regions encoding SARS-CoV proteins, for example SARS-CoV S, S1, S2, N, M, or E and optionally, plasmids encoding immunity enhancing proteins, *e.g.*, cytokines. Various plasmids desired in a cocktail are combined together in PBS or other diluent prior to the addition to the other ingredients. Furthermore, plasmids may be present in a cocktail at equal proportions, or the ratios may be adjusted based on, for example, relative expression levels of the antigens or the relative immunogenicity of the encoded antigens. Thus, various plasmids in the cocktail may be present in equal proportions, or up to twice or three times as much of one plasmid may be included relative to other plasmids in the cocktail.

[0331] Additionally, the concentration of BAK may be adjusted depending on, for example, a desired particle size and improved stability. Indeed, in certain embodiments, formulations of the present invention include CRL 1005 and DNA, but are free of BAK. In general BAK-containing formulations of the present invention are adjusted to have a final concentration of BAK from about 0.05 mM to about 0.5 mM. For example, a formulation of the present invention may have a final BAK concentration of about 0.05 mM, 0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM, or 0.5 mM.

[0332] The total volume of the formulations produced by the methods below may be scaled up or down, by choosing apparatus of proportional size. Finally, in carrying out any of the methods described below, the three components of the formulation, BAK, CRL 1005, and plasmid DNA, may be added in any order. In each of these methods described below the term "cloud point" refers to the point in a temperature shift, or other titration, at which a clear solution becomes cloudy, *i.e.*, when a component dissolved in a solution begins to precipitate out of solution.

Thermal Cycling of a Pre-Mixed Formulation

[0333] This example describes the preparation of a formulation comprising 0.3 mM BAK, 7.5 mg/ml CRL 1005, and 5 mg/ml of DNA in a total volume of 3.6 ml. The ingredients are combined together at a temperature below the cloud point and then the formulation is thermally cycled to room temperature (above the cloud point) several times, according to the protocol outlined in Figure 2.

[0334] A 1.28 mM solution of BAK is prepared in PBS, 846 µl of the solution is placed into a 15 ml round bottom flask fitted with a magnetic stirring bar, and the solution is stirred with moderate speed, in an ice bath on top of a stirrer/hotplate (hotplate off) for 10 minutes. CRL 1005 (27 µl) is then added using a 100 µl positive displacement pipette and the solution is stirred for a further 60 minutes on ice. Plasmids comprising coding regions or codon-optimized coding regions encoding SARS-CoV proteins, for example, S, S1, S2, N, M, or E, as described herein, and optionally, additional plasmids comprising codon-optimized or non-codon-optimized coding regions encoding, *e.g.*, additional SARS-CoV proteins, and or other proteins, *e.g.*, cytokines, are mixed together at desired proportions in PBS to achieve 6.4 mg/ml total DNA. This plasmid cocktail is added dropwise, slowly, to the stirring solution over 1 min using a 5 ml pipette. The solution at this point (on ice) is clear since it is below the cloud point of the poloxamer and is further stirred on ice for 15 min. The ice bath is then removed, and the solution is stirred at ambient temperature for 15 minutes to produce a cloudy solution as the poloxamer passes through the cloud point.

[0335] The flask is then placed back into the ice bath and stirred for a further 15 minutes to produce a clear solution as the mixture is cooled below the poloxamer cloud point. The ice bath is again removed and the solution stirred at ambient temperature for a further 15 minutes. Stirring for 15 minutes above and below the cloud point (total of 30 minutes), is defined as one thermal cycle. The mixture is cycled six more times. The resulting formulation may

be used immediately, or may be placed in a glass vial, cooled below the cloud point, and frozen at -80 °C for use at a later time.

Thermal Cycling, Dilution and Filtration of a Pre-mixed Formulation, Using Increased Concentrations of CRL 1005

[0336] This example describes the preparation of a formulation comprising 0.3 mM BAK, 34 mg/ml or 50 mg/ml CRL 1005, and 2.5 mg/ml of DNA in a final volume of 4.0 ml. The ingredients are combined together at a temperature below the cloud point, then the formulation is thermally cycled to room temperature (above the cloud point) several times, diluted, and filtered according to the protocol outlined in Figure 3.

[0337] Plasmids comprising wild-type or codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, and or other proteins, *e.g.*, cytokines, are mixed together at desired proportions in PBS to achieve 6.4 mg/ml total DNA. This plasmid cocktail is placed into the 15 ml round bottom flask fitted with a magnetic stirring bar, and for the formulation containing 50 mg/ml CRL 1005, 3.13 ml of a solution containing about 3.2 mg/ml of *e.g.*, S1 encoding plasmid and about 3.2 mg/ml S2 encoding plasmid (about 6.4 mg/ml total DNA) is placed into the 15 ml round bottom flask fitted with a magnetic stirring bar, and the solutions are stirred with moderate speed, in an ice bath on top of a stirrer/hotplate (hotplate off) for 10 minutes. CRL 1005 (136 µl for 34 mg/ml final concentration, and 100 µl for 50 mg/ml final concentration) is then added using a 200 µl positive displacement pipette and the solution is stirred for a further 30 minutes on ice. Solutions of 1.6 mM and 1.8 mM BAK are prepared in PBS, and 739 µl of 1.6 mM and 675µl of 1.8 mM are then added dropwise, slowly, to the stirring poloxamer solutions with concentrations of 34 mg/ml or 50 mg/ml mixtures,

respectively, over 1 min using a 1ml pipette. The solutions at this point are clear since they are below the cloud point of the poloxamer and are stirred on ice for 30 min. The ice baths are then removed; the solutions stirred at ambient temperature for 15 minutes to produce cloudy solutions as the poloxamer passes through the cloud point.

[0338] The flasks are then placed back into the ice baths and stirred for a further 15 minutes to produce clear solutions as the mixtures cooled below the poloxamer cloud point. The ice baths are again removed and the solutions stirred for a further 15 minutes. Stirring for 15 minutes above and below the cloud point (total of 30 minutes), is defined as one thermal cycle. The mixtures are cycled two more times.

[0339] In the meantime, two Steriflip® 50 ml disposable vacuum filtration devices, each with a 0.22 μm Millipore Express® membrane (available from Millipore, cat # SCGP00525) are placed in an ice bucket, with a vacuum line attached and left for 1 hour to allow the devices to equilibrate to the temperature of the ice. The poloxamer formulations are then diluted to 2.5 mg/ml DNA with PBS and filtered under vacuum.

[0340] The resulting formulations may be used immediately, or may be transferred to glass vials, cooled below the cloud point, and frozen at -80 °C for use at a later time.

A Simplified Method Without Thermal Cycling

[0341] This example describes a simplified preparation of a formulation comprising 0.3 mM BAK, 7.5 mg/ml CRL 1005, and 5 mg/ml of DNA in a total volume of 2.0 ml. The ingredients are combined together at a temperature below the cloud point and then the formulation is simply filtered and then used or stored, according to the protocol outlined in Figure 4.

[0342] A 0.77 mM solution of BAK is prepared in PBS, and 780 μl of the solution is placed into a 15 ml round bottom flask fitted with a magnetic stirring bar, and the solution is stirred with moderate speed, in an ice bath on

top of a stirrer/hotplate (hotplate off) for 15 minutes. CRL 1005 (15 µl) is then added using a 100 µl positive displacement pipette and the solution is stirred for a further 60 minutes on ice. Plasmids comprising coding regions or codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, and or other proteins, *e.g.*, cytokines, are mixed together at desired proportions in PBS to achieve a final concentration of about 8.3 mg/ml total DNA. This plasmid cocktail is added dropwise, slowly, to the stirring solution over 1 min using a 5 ml pipette. The solution at this point (on ice) is clear since it is below the cloud point of the poloxamer and is further stirred on ice for 15 min.

[0343] In the meantime, one Steriflip® 50 ml disposable vacuum filtration device, with a 0.22 µm Millipore Express® membrane (available from Millipore, cat # SCGP00525) is placed in an ice bucket, with a vacuum line attached and left for 1 hour to allow the device to equilibrate to the temperature of the ice. The poloxamer formulation is then filtered under vacuum, below the cloud point and then allowed to warm above the cloud point. The resulting formulations may be used immediately, or may be transferred to glass vials, cooled below the cloud point and then frozen at -80°C for use at a later time.

EXAMPLE 5

Animal Immunizations

[0344] The immunogenicity of the various SARS-CoV expression products encoded polynucleotides and codon-optimized polynucleotides described herein are initially evaluated based on each plasmid's ability to mount an immune response *in vivo*. Plasmids are tested individually and in

combinations by injecting single constructs as well as multiple constructs. Immunizations are initially carried out in animals, such as mice, rabbits, goats, sheep, domestic cats, non-human primates, or other suitable animal, by intramuscular (IM) injections. Serum is collected from immunized animals, and the antigen specific antibody response is quantified by ELISA assay using purified immobilized antigen proteins in a protein – immunized subject antibody – anti-species antibody type assay, according to standard protocols. The tests of immunogenicity further include measuring antibody titer, neutralizing antibody titer, T-cell proliferation, T-cell secretion of cytokines, and cytolytic T cell responses. Correlation to protective levels of the immune responses in humans are made according to methods well known by those of ordinary skill in the art. *See* above.

A. DNA formulations

[0345] Plasmid DNA is formulated with a poloxamer by any of the methods described in Example 3. Alternatively, plasmid DNA is prepared as described above and dissolved at a concentration of about 0.1 mg/ml to about 10 mg/ml, preferably about 1 mg/ml, in PBS with or without transfection-facilitating cationic lipids, *e.g.*, DMRIE/DOPE at a 4:1 DNA:lipid mass ratio. Alternative DNA formulations include 150 mM sodium phosphate instead of PBS, adjuvants, *e.g.*, Vaxfectin™ at a 4:1 DNA: Vaxfectin™ mass ratio, mono-phosphoryl lipid A (detoxified endotoxin) from *S. minnesota* (MPL) and trehalosedicorynomycolateAF (TDM), in 2% oil (squalene)-Tween 80-water (MPL + TDM, available from Sigma/Aldrich, St. Louis, MO, (catalog # M6536)), a solubilized mono-phosphoryl lipid A formulation (AF, available from Corixa), or (±)-N-(3-Acetoxypropyl)-N,N-dimethyl-2,3-bis(octyloxy)-1-propanaminium chloride (compound # VC1240) (*see* Shriver, J.W. *et al.*, *Nature 415*:331-335 (2002), and P.C.T. Publication No. WO 02/00844 A2, each of which is incorporated herein by reference in its entirety).

B. Animal immunizations

[0346] Plasmid constructs comprising codon-optimized or non-codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, as well as various controls, *e.g.*, empty vector, are injected into BALB/c mice as single plasmids or as cocktails of two or more plasmids, as either DNA in PBS or formulated with the poloxamer-based delivery system: 2 mg/ml DNA, 3 mg/ml CRL 1005, and 0.1 mM BAK. Groups of 10 mice are immunized three times, at biweekly intervals, and serum is obtained to determine antibody titers to each of the antigens. Groups are also included in which mice are immunized with a trivalent preparation, containing each of three plasmid constructs expressing any of the SARS Co-V polypeptides, *e.g.*, soluble, extracellular S1, M, and N polypeptides, in equal mass.

An example of an immunization schedule is as follows:

Day –3	Pre-bleed
Day 0	Plasmid injections, intramuscular, bilateral in rectus femoris, 5- 50 μg/leg
Day 20	Serum Collection
Day 21	Plasmid injections, intramuscular, bilateral in rectus femoris, 5-50 μg/leg
Day 48	Serum Collection
Day 49	Plasmid injections, intramuscular, bilateral in rectus femoris, 5-50 μg/leg
Day 59	Serum collection

[0347] Serum antibody titers, at the various time points are determined by ELISA, using as the antigen SARS-CoV protein preparations including, but

not limited to, purified recombinant proteins, transfection supernatants and lysates from mammalian or insect cells transfected with the various plasmids described herein, or live, inactivated, or lysed SARS-CoV virus.

C. Immunization of Mice with Vaccine Formulations Using a VAXFECTIN™ Adjuvant

[0348] VAXFECTIN™ (a 1:1 molar ratio of the cationic lipid VC1052 and the neutral co-lipid DPyPE) is a synthetic cationic lipid formulation which has shown promise for its ability to enhance antibody titers against an antigen when administered with DNA encoding the antigen intramuscularly to mice. *See* Hartikka *et al.* "Vaxfectin Enhances the Humoral Response to Plasmid DNA-encoded Antigens." *Vaccine 19*: 1911-1923 (2001).

[0349] In mice, intramuscular injection of VAXFECTIN™ formulated with, for example, DNA encoding the IAV NP protein increased antibody titers to NP up to 20-fold to levels that could not be reached with DNA alone. In rabbits, complexing DNA with VAXFECTIN™ enhanced antibody titers up to 50-fold. Thus, VAXFECTIN™ shows promise as a delivery system and as an adjuvant in a DNA vaccine.

[0350] Vaxfectin mixtures are prepared by mixing chloroform solutions of VC1052 cationic lipid with chloroform solutions of DpyPE neutral co-lipid. Dried films are prepared in 2 ml sterile glass vials by evaporating the chloroform under a stream of nitrogen, and placing the vials under vacuum overnight to remove solvent traces. Each vial contains 1.5 μmole each of VC1052 and DPyPE. Liposomes are prepared by adding sterile water followed by vortexing. The resulting liposome solution is mixed with DNA at a phosphate mole:cationic lipid mole ratio of 4:1.

[0351] Plasmid constructs comprising codon-optimized and non-codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments,

variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, as well as various controls, *e.g.*, empty vector, are mixed together at desired proportions in PBS to achieve a final concentration of at 1.0 mg/ml. The plasmid cocktail, as well as the controls, are formulated with VAXFECTIN™. Groups of 5 Balb/c female mice are injected bilaterally in the rectus femoris muscle with 50 µl of DNA solution (100 µl total/mouse), on days 1 and 21 and 49 with each formulation. Mice are bled for serum on days 0 (prebleed), 20 (bleed 1), and 41 (bleed 2), and 62 (bleed 3), and up to 40 weeks post-injection. Antibody titers to the various SARS CoV proteins encoded by the plasmid DNAs are measured by ELISA as described elsewhere herein.

[0352] Cytolytic T-cell responses are measured as described in Hartikka *et al.* "Vaxfectin Enhances the Humoral Response to Plasmid DNA-encoded Antigens." *Vaccine 19*: 1911-1923 (2001) and is incorporated herein in its entirety by reference. Standard ELISPOT technology is used for the CD4+ and CD8+ T-cell assays as described in Example 6, part A.

D. Production of SARS-CoV antisera in animals

[0353] Plasmid constructs comprising codon-optimized and non-codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, as well as various controls, *e.g.*, empty vector, are prepared according to the immunization scheme described above and injected into a suitable animal for generating polyclonal antibodies. Serum is collected and the antibody titered as above.

[0354] Monoclonal antibodies are also produced using hybridoma technology. Kohler, *et al., Nature 256*:495 (1975); Kohler, *et al., Eur. J. Immunol. 6*:511 (1976); Kohler, *et al., Eur. J. Immunol. 6*:292 (1976); Hammerling, *et al.*, in

Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., (1981), pp. 563-681, each of which is incorporated herein by reference in its entirety. In general, such procedures involve immunizing an animal (preferably a mouse) as described above. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (Sp2/0), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands *et al., Gastroenterology 80*:225-232 (1981), incorporated herein by reference in its entirety. The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the various SARS-CoV proteins.

[0355] Alternatively, additional antibodies capable of binding to SARS-CoV proteins described herein may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, various SARS-CoV-specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the SARS-CoV protein-specific antibody can be blocked by the cognate SARS-CoV protein. Such antibodies comprise anti-idiotypic antibodies to the SARS-CoV protein-specific antibody and can be used to immunize an animal to induce formation of further SARS-CoV-specific antibodies.

[0356] It will be appreciated that Fab and $F(ab')_2$ and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). Alternatively, SARS-CoV polypeptide binding

fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

[0357] It may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science 229*:1202 (1985); Oi, *et al., BioTechniques 4*:214 (1986); Cabilly, *et al.*, U.S. Patent No. 4,816,567; Taniguchi, *et al.*, EP 171496; Morrison, *et al.*, EP 173494; Neuberger, *et al.*, WO 8601533; Robinson, *et al.*, WO 8702671; Boulianne, *et al., Nature 312*:643 (1984); Neuberger, *et al., Nature 314*:268 (1985).

[0358] These antibodies are used, for example, in diagnostic assays, as a research reagent, or to further immunize animals to generate SARS-CoV-specific anti-idiotypic antibodies. Non-limiting examples of uses for anti-SARS-CoV antibodies include use in Western blots, ELISA (competitive, sandwich, and direct), immunofluorescence, immunoelectron microscopy, radioimmunoassay, immunoprecipitation, agglutination assays, immunodiffusion, immunoelectrophoresis, and epitope mapping. Weir, D. *Ed. Handbook of Experimental Immunology*, 4th ed. Vols. I and II, Blackwell Scientific Publications (1986).

EXAMPLE 6

Mouse and Rabbit Immunogenicity Studies to SARS-CoV antigens

[0359] Balb/c mice were injected intramuscularly bilaterally with 100 μg of SARS-CoV antigen expressing plasmid. VR9204, VR9208, VR9209, VR9210, VR9219 plasmids were formulated in PBS and DMRIE:DOPE at a 4:1 DNA:lipid mass ratio.

[0360] New Zealand white rabbits were injected intramuscularly bilaterally with 1 mg of SARS-CoV antigen expressing plasmid (VR9219 (N antigen) or VR9204 (S1 fragment antigen), formulated with DMRIE:DOPE, on days 1, 28

and 56. Rabbit sera anti-antigen titers were determined by ELISA assay. The ELISA assay was performed according to standard protocols. ELISA plates used in the assay were coated with cell culture supernatants, from cells transfected with the a SARS-CoV antigen plasmid. Sera from rabbits which had been injected with the corresponding plasmid was then applied to the plates. Bound rabbit antibodies were detected using an alkaline phosphatase-modified donkey anti-rabbit IgG monoclonal antibody (Jackson Immuno Research; Cat No. 711-055-152). Bound antibodies were detected by standard colorimetric method after 2.5 hours of incubation with chromogenic substrates. Optical Density was determined at a wavelength of 405nm. The results of the ELISA assay are summarized below.

[0361] Data shown in Table 20 demonstrate the presence of anti-nucleocapsid antibodies at day 21 in rabbits injected with plasmid VR9219 expressing full-length SARS-CoV nucleocapsid antigen. The antibody titers reach a plateau at day 42 (1:400 dilution).

[0362] In another experiment, rabbits were injected with plasmid VR9204, which expresses a fragment of the SARS-CoV Spike S1 domain. ELISA plates were coated with *in vitro*-produced full length-secreted Spike protein from cells transfected with plasmid VR9210. Antibodies IMG-542 and IMG-557, which recognize amino acids 288-303 and 1124-1140 of the SARS-CoV spike protein respectively (available from Imgenex, San Diego, CA), were used as positive controls in the ELISA assay. An ELISA plate coated with supernatant from VR1012-transfected VM92 cells was used as a negative control in the ELISA assay. The data shown in Table 20 demonstrate the presence of anti-Spike antibodies at days 42 and 50 after injection.

TABLE 20

Anti-SARS CoV Antigen Titers (Rabbits)

	Nucleocapsid Plamsid - VR9219 1/400 sera dilution	S1 fragment Plasmid - VR9204 1/200 sera dilution
Day 21	0.92	0.22
Day 42	3.9	0.74
Day 50	NA	0.51
Day 80	4	NA
Pre-bleed	0.13	0.19
IMG-542	NA	0.44
IMG-557	NA	2.41
VR1012	0.15	0.21

EXAMPLE 7

Mucosal Vaccination and Electrically Assisted Plasmid Delivery

A. Mucosal DNA Vaccination

[0363] Plasmid constructs comprising codon-optimized and non-codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, as well as various controls, *e.g.*, empty vector, (100 μg/50 μl total DNA) are delivered to BALB/c mice at 0, 2 and 4 weeks via i.m., intranasal (i.n.), intravenous (i.v.), intravaginal (i.vag.), intrarectal (i.r.) or oral routes. The DNA is delivered unformulated, formulated with the cationic lipids DMRIE/DOPE (DD) or GAP-DLRIE/DOPE (GD), or formulatated with a poloxamer as described in Example 3. As endpoints, serum IgG titers against the various SARS-CoV antigens are measured by ELISA and splenic T-cell

responses are measured by antigen-specific production of IFN-gamma and IL-4 in ELISPOT assays. Standard chromium release assays are used to measure specific cytotoxic T lymphocyte (CTL) activity against the various SARS-CoV antigens. In addition, IgG and IgA responses against the various SARS-CoV antigens are analyzed by ELISA of vaginal washes.

B. Electrically-assisted plasmid delivery

[0364] *In vivo* gene delivery may be enhanced through the application of brief electrical pulses to injected tissues, a procedure referred to herein as electrically-assisted plasmid delivery. See, *e.g.*, Aihara, H. & Miyazaki, J. *Nat. Biotechnol. 16:*867-70 (1998); Mir, L.M. *et al.*, *Proc. Natl Acad. Sci.* USA *96:*4262-67 (1999); Hartikka, J. *et al.*, *Mol. Ther. 4:*407-15 (2001); and Mir, L.M. *et al.*; Rizzuto, G. *et al.*, *Hum Gene Ther 11:*1891-900 (2000); Widera, G. *et al, J. of Immuno. 164*: 4635-4640 (2000). The use of electrical pulses for cell electropermeabilization has been used to introduce foreign DNA into prokaryotic and eukaryotic cells *in vitro*. Cell permeabilization can also be achieved locally, *in vivo*, using electrodes and optimal electrical parameters that are compatible with cell survival.

[0365] The electroporation procedure can be performed with various electroporation devices. These devices include external plate type electrodes or invasive needle/rod electrodes and can possess two electrodes or multiple electrodes placed in an array. Distances between the plate or needle electrodes can vary depending upon the number of electrodes, size of target area and treatment subject.

[0366] The TriGrid needle array, used in examples described herein, is a three electrode array comprising three elongate electrodes in the approximate shape of a geometric triangle. Needle arrays may include single, double, three, four, five, six or more needles arranged in various array formations. The electrodes are connected through conductive cables to a high voltage switching device that is connected to a power supply.

[0367] The electrode array is placed into the muscle tissue, around the site of nucleic acid injection, to a depth of approximately 3 mm to 3 cm. The depth of insertion varies depending upon the target tissue and the size of the patient receiving electroporation. After injection of foreign nucleic acid, such as plasmid DNA, and a period of time sufficient for distribution of the nucleic acid, square wave electrical pulses are applied to the tissue. The amplitude of each pulse ranges from about 100 volts to about 1500 volts, *e.g.*, about 100 volts, about 200 volts, about 300 volts, about 400 volts, about 500 volts, about 600 volts, about 700 volts, about 800 volts, about 900 volts, about 1000 volts, about 1100 volts, about 1200 volts, about 1300 volts, about 1400 volts, or about 1500 volts or about 1-1.5kV/cm, based on the spacing between electrodes. Each pulse has a duration of about 1μs to about 1000μs, *e.g.*, about 1μs, about 10μs, about 50μs, about 100μs, about 200μs, about 300μs, about 400μs, about 500μs, about 600μs, about 700μs, about 800μs, about 900μs, or about 1000μs, and a pulse frequency on the order of about 1-10 Hz. The polarity of the pulses may be reversed during the electroporation procedure by switching the connectors to the pulse generator. Pulses are repeated multiple times. The electroporation parameters (*e.g.*, voltage amplitude, duration of pulse, number of pulses, depth of electrode insertion and frequency) will vary based on target tissue type, number of electrodes used and distance of electrode spacing, as would be understood by one of ordinary skill in the art.

[0368] Immediately after completion of the pulse regimen, subjects receiving electroporation can be optionally treated with membrane stabilizing agents to prolong cell membrane permeability as a result of the electroporation. Examples of membrane stabilizing agents include, but are not limited to, steroids (*e.g.*, dexamethasone, methylprednisone and progesterone), angiotensin II and vitamin E. A single dose of dexamethasone, approximately 0.1 mg per kilogram of body weight, should be sufficient to achieve a beneficial affect.

[0369] EAPD techniques such as electroporation can also be used for plasmids contained in liposome formulations. The liposome – plasmid suspension is administered to the animal or patient and the site of injection is treated with a safe but effective electrical field generated, for example, by a TriGrid needle array. The electroporation may aid in plasmid delivery to the cell by destabilizing the liposome bilayer so that membrane fusion between the liposome and the target cellular structure occurs. Electroporation may also aid in plasmid delivery to the cell by triggering the release of the plasmid, in high concentrations, from the liposome at the surface of the target cell so that the plasmid is driven across the cell membrane by a concentration gradient via the pores created in the cell membrane as a result of the electroporation.

[0370] Female BALB/c mice aged 8-10 weeks are anesthetized with inhalant isoflurane and maintained under anesthesia for the duration of the electroporation procedure. The legs are shaved prior to treatment. Plasmid constructs comprising codon-optimized and non-codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, as well as various controls, *e.g.*, empty vector, are administered to BALB/c mice (n = 10) via unilateral injection in the quadriceps with 25 μg total of a plasmid DNA per mouse using an 0.3 cc insulin syringe and a 26 gauge, 1/2 length needle fitted with a plastic collar to regulate injection depth. Approximately one minute after injection, electrodes are applied. Modified caliper electrodes are used to apply the electrical pulse. *See* Hartikka J. *et al. Mol Ther 188*:407-415 (2001). The caliper electrode plates are coated with conductivity gel and applied to the sides of the injected muscle before closing to a gap of 3 mm for administration of pulses. EAPD is applied using a square pulse type at 1-10 Hz with a field strength of 100-500 V/cm, 1-10 pulses, of 10-100 ms each.

[0371] Mice are vaccinated ± EAPD at 0, 2 and 4 weeks. As endpoints, serum IgG titers against the various SARS-CoV antigens are measured by ELISA and splenic T-cell responses are measured by antigen-specific production of IFN-gamma and IL-4 in ELISPOT assays. Standard chromium release assays are used to measure specific cytotoxic T lymphocyte (CTL) activity against the various SARS-CoV antigens.

[0372] Rabbits (n = 3) are given bilateral injections in the quadriceps muscle with plasmid constructs comprising codon-optimized and non-codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, as well as various controls, *e.g.*, empty vector. The implantation area is shaved and the TriGrid electrode array is implanted into the target region of the muscle. 3.0 mg of plasmid DNA is administered per dose through the injection port of the electrode array. An injection collar is used to control the depth of injection. Electroporation begins approximately one minute after injection of the plasmid DNA is complete. Electroporation is administered with a TriGrid needle array, with eletrodes evenly spaced 7mm apart, using an Ichor TGP-2 pulse generator. The array is inserted into the target muscle to a depth of about 1 to 2 cm. 4-8 pulses are administered. Each pulse has a duration of about 50-100 μs, an amplitude of about 1-1.2kV/cm and a pulse frequency of 1 Hz. The injection and electroporation may be repeated.

[0373] Sera are collected from vaccinated rabbits at various time points. As endpoints, serum IgG titers against the various SARS-CoV antigens are measured by ELISA and PBMC T-cell proliferative responses are measured by antigen-specific production of IFN-gamma and IL-4 in ELISPOT assays or by quantification of intracellular cytokine staining. Standard chromium release assays are used to measure specific cytotoxic T lymphocyte (CTL) activity against the various SARS-CoV antigens.

[0374] To test the effect of electroporation on therapeutic protein expression in non-human primates, male or female rhesus monkeys are given either 2 or 6 EAPD-assisted i.m. injections of plasmid constructs comprising codon-optimized and/or non-codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, as well as various controls, *e.g.*, empty vector, (0.1 to 10 mg DNA total per animal). Target muscle groups include, but are not limited to, bilateral rectus fermoris, cranial tibialis, biceps, gastrocenemius or deltoid muscles. The target area is shaved and a needle array, comprising between 4 and 10 electrodes, spaced between 0.5-1.5 cm apart, is implanted into the target muscle. Once injections are complete, a sequence of brief electrical pulses is applied to the electrodes implanted in the target muscle using an Ichor TGP-2 pulse generator. The pulses have an amplitude of approximately 120 – 200V. The pulse sequence is completed within one second. During this time, the target muscle may make brief contractions or twitches. The injection and electroporation may be repeated.

[0375] Sera are collected from vaccinated monkeys at various time points. As endpoints, serum IgG titers against the various SARS-CoV antigens are measured by ELISA and PBMC T-cell proliferative responses are measured by antigen-specific production of IFN-gamma and IL-4 in ELISPOT assays or by quantification of intracellular cytokine staining Standard chromium release assays are used to measure specific cytotoxic T lymphocyte (CTL) activity against the various SARS-CoV antigens.

EXAMPLE 8

Combinatorial DNA Vaccine Using Heterologous Prime-Boost Vaccination

[0376] This Example describes vaccination with a combinatorial formulation including one or more polynucleotides comprising at least one codon-optimized or non-codon optimized coding regions encoding a SARS-CoV protein or fragment, variant, or derivative thereof prepared with an adjuvant and/or transfection facilitating agent; and also an isolated SARS-CoV protein or fragment, variant, or derivative thereof. Thus, antigen is provided in two forms. The exogenous isolated protein stimulates antigen specific antibody and CD4+ T-cell responses, while the polynucleotide-encoded protein, produced as a result of cellular uptake and expression of the coding region, stimulates a CD8+ T-cell response. Unlike conventional "prime-boost" vaccination strategies, this approach provides different forms of antigen in the same formulation. Because antigen expression from the DNA vaccine doesn't peak until 7-10 days after injection, the DNA vaccine provides a boost for the protein component. Furthermore, the formulation takes advantage of the immunostimulatory properties of the bacterial plasmid DNA.

A. Formulation determinations for SARS-CoV proteins

[0377] This example mainly describes this procedure using an S2 subunit protein; however, the methods described herein are applicable to any SARS-CoV subunit protein combined with any polynucleotide vaccine formulation. For example any polynucleotide comprising a codon-optimized or non-codon-optimized coding region encoding any SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg may be combined with any subunit SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble

S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg. Because only a small amount of protein is needed in this method, it is conceivable that the approach could be used to reduce the dose of other types of protein or antibody based vaccines, not described herein, when administered in combination with the polynucleotides and polypeptides of the present invention. The decreased dosing of other vaccines would allow for the increased availability of scarce or expensive vaccines. This feature would be particularly important for vaccines against pandemic SARS or biological warfare agents.

[0378] In this example, an injection dose of 10 μg SARS-CoV S protein, subunit 2 (S2) DNA per mouse, prepared essentially as described in Example 2 and in Ulmer, J.B., *et al.*, *Science 259:*1745-49 (1993) and Ulmer, J.B. *et al.*, *J Virol. 72:*5648-53 (1998) is pre-determined in dose response studies to induce T cell and antibody responses in the linear range of the dose response and results in a response rate of greater than 95% of mice injected. Each formulation, either a plasmid comprising a codon-optimized or non-codon-optimized coding region encoding S2 alone ("S2 DNA"), or S2 DNA +/- S2 protein formulated with Ribi I or the cationic lipids, DMRIE:DOPE or Vaxfectin, is prepared in the recommended buffer for that vaccine modality. For injections with S2 DNA formulated with cationic lipid, the DNA is diluted in 2X PBS to 0.2 mg/ml +/- purified recombinant S2 protein (produced in baculovirus as described in Example 2) at 0.08 mg/ml. Each cationic lipid is reconstituted from a dried film by adding 1 ml of sterile water for injection (SWFI) to each vial and vortexing continuously for 2 min., then diluted with SWFI to a final concentration of 0.15 mM. Equal volumes of S2 DNA (+/- S2 protein) and cationic lipid are mixed to obtain a DNA to cationic lipid molar ratio of 4:1. For injections with DNA containing Ribi I adjuvant (Sigma), Ribi I is reconstituted with saline to twice the final concentration. Ribi I (2X) is mixed with an equal volume of S2 DNA at 0.2 mg/ml in saline +/- S2 protein at 0.08 mg/ml. For immunizations without cationic lipid or Ribi, S2 DNA is

prepared in 150 mM sodium phosphate buffer, pH 7.2. For each experiment, groups of 9 BALB/c female mice at 7-9 weeks of age are injected with 50 µl of S2 DNA +/- S2 protein, cationic lipid or Ribi I. Injections are given bilaterally in each rectus femoris at day 0 and day 21. The mice are bled by OSP on day 20 and day 33 and serum titers of individual mice are measured.

[0379] S2 specific serum antibody titers are determined by indirect binding ELISA using 96 well ELISA plates coated overnight at 4°C with purified recombinant S2 protein at 0.5 µg per well in BBS buffer pH 8.3. S2-coated wells are blocked with 1% bovine serum albumin in BBS for 1 h at room temperature. Two-fold serial dilutions of sera in blocking buffer are incubated for 2 h at room temperature and detected by incubating with alkaline phosphatase conjugated (AP) goat anti-mouse IgG-Fc (Jackson Immunoresearch, West Grove, PA) at 1:5000 for 2 h at room temperature. Color is developed with 1 mg/ml para-nitrophenyl phosphate (Calbiochem, La Jolla, CA) in 50 mM sodium bicarbonate buffer, pH 9.8 and 1 mM $MgCl_2$ and the absorbance read at 405 nm. The titer is the reciprocal of the last dilution exhibiting an absorbance value 2 times that of pre-bleed samples.

[0380] Standard ELISPOT technology, used to identify the number of interferon gamma (IFN-γ) secreting cells after stimulation with specific antigen (spot forming cells per million splenocytes, expressed as SFU/million), is used for the CD4+ and CD8+ T-cell assays. For the screening assays, 3 mice from each group are sacrificed on day 34, 35, and 36. At the time of collection, spleens from each group are pooled, and single cell suspensions made in cell culture media using a dounce homogenizer. Red blood cells are lysed, and cells washed and counted. For the CD4+ and CD8+ assays, cells are serially diluted 3- fold, starting at 10^6 cells per well and transferred to 96 well ELISPOT plates pre-coated with anti-murine IFN-γ monoclonal antibody. Spleen cells are stimulated with the H-$2K^d$ binding peptide, TYQRTRALV (SEQ ID NO: 55) at 1 µg/ml and recombinant murine IL-2 at 1 U/ml for the CD8+ assay and with purified recombinant S2 protein at 20 µg/ml for the CD4+ assay. Cells are stimulated for 20-24 hours at 37°C in

5% CO_2, then the cells are washed out and biotin labeled anti-IFN-γ monoclonal antibody added for a 2 hour incubation at room temperature. Plates are washed and horseradish peroxidase-labeled avidin is added. After a 1-hour incubation at room temperature, AEC substrate is added and "spots" developed for 15 min. Spots are counted using the Immunospot automated spot counter (C.T.L. Inc., Cleveland OH). Thus, CD4+ and CD8+ responses are measured in three separate assays, using spleens collected on each of three consecutive days.

B. Determining combinatorial formulations with SARS-CoV polynucleotide constructs

[0381] Plasmid constructs comprising codon-optimized or non-codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, as well as various controls, *e.g.*, empty vector, are used in the prime-boost compositions described herein. For the prime-boost modalities, the same protein may be used for the boost, *e.g.*, DNA encoding S2 with S2 protein, or a heterologous boost may be used, *e.g.*, DNA encoding S2 with an M protein boost. Each formulation, the plasmid comprising a coding region for the SARS-CoV protein alone, or the plasmid comprising a coding region for the SARS-CoV protein plus the isolated protein, is formulated with Ribi I or the cationic lipids, DMRIE:DOPE or Vaxfectin. The formulations are prepared in the recommended buffer for that vaccine modality. Exemplary formulations, using S2 as an example, are described herein. Other plasmid/protein formulations, including multivalent formulations, can be easily prepared by one of ordinary skill in the art by following this example. For injections with DNA formulated with cationic lipid, the DNA is diluted in 2X PBS to 0.2 mg/ml +/- purified recombinant

SARS-CoV protein at 0.08 mg/ml. Each cationic lipid is reconstituted from a dried film by adding 1 ml of sterile water for injection (SWFI) to each vial and vortexing continuously for 2 min., then diluted with SWFI to a final concentration of 0.15 mM. Equal volumes of S2 DNA (+/- S2 protein) and cationic lipid are mixed to obtain a DNA to cationic lipid molar ratio of 4:1. For injections with DNA containing Ribi I adjuvant (Sigma), Ribi I is reconstituted with saline to twice the final concentration. Ribi I (2X) is mixed with an equal volume of S2 DNA at 0.2 mg/ml in saline +/- S2 protein at 0.08 mg/ml. For immunizations without cationic lipid or Ribi, S2 DNA is prepared in 150 mM sodium phosphate buffer, pH 7.2. For each experiment, groups of 9 BALB/c female mice at 7-9 weeks of age are injected with 50 µl of S2 DNA +/- S2 protein, cationic lipid or Ribi I. The formulations are administered to BALB/c mice (n = 10) via bilateral injection in each rectus femoris at day 0 and day 21.

[0382] The mice are bled on day 20 and day 33, and serum titers of individual mice to the various SARS-CoV antigens are measured. Serum antibody titers specific for the various SARS-CoV antigens are determined by ELISA. Standard ELISPOT technology, used to identify the number of interferon gamma (IFN-γ) secreting cells after stimulation with specific antigen (spot forming cells per million splenocytes, expressed as SFU/million), is used for the CD4+ and CD8+ T-cell assays using 3 mice from each group vaccinated as above, sacrificed on day 34, 35, and 36, post vaccination.

EXAMPLE 9

Challenge in Non-Human Primates

[0383] The purpose of these studies is to evaluate three or more of the optimal plasmid DNA vaccine formulations for immunogenicity in non-human primates. Prelmimary challenge experiments may be carried out in toher suitable animal modes, for example birds as described below, or in domestic cats. Rhesus or cynomologus monkeys (6/group) are vaccinated with plasmid

constructs comprising codon-optimized and non-codon-optimized coding regions encoding SARS-CoV proteins, for example, SARS-CoV S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2 proteins, fusions thereof, or fragments, variants or derivatives of such proteins either alone or as fusions with a carrier protein, *e.g.*, HBcAg, as well as various controls, *e.g.*, empty vector, intramuscularly 0.1 to 2 mg DNA combined with cationic lipid, and/or poloxamer and/or aluminum phosphate based or other adjuvants at 0, 1 and 4 months.

[0384] Blood is drawn twice at baseline and then again at the time of and two weeks following each vaccination, and then again 4 months following the last vaccination. At 2 weeks post-vaccination, plasma is analyzed for humoral response and PBMCs are monitored for cellular responses, by standard methods described herein. Animals are monitored for 4 months following the final vaccination to determine the durability of the immune response.

[0385] Animals are challenged within 2-4 weeks following the final vaccination. Animals are challenged intratracheally with the suitable dose of virus based on preliminary challege studies. Nasal swabs, pharyngeal swabs and lung lavages are collected at days 0, 2, 4, 6, 8 and 11 post-challenge and will be assayed for cell-free virus titers on monkey kidney cells. After challenge, animals are monitored for clinical symptoms, *e.g.*, rectal temperature, body weight, leukocyte counts, and in addition, hematocrit and respiratory rate. Oropharyngeal swab samples are taken to allow determination of the length of viral shedding. Illness is scored using a variety of conventional illness scoring methods such as the system developed by Berendt & Hall (*Infect Immun 16*:476-479 (1977)), and will be analyzed by analysis of variance and the method of least significant difference.

EXAMPLE 10

Challenge in Birds

In this example, various vaccine formulations of the present invention are tested in a chicken SARS-CoV model. For these studies a SARS-CoV is used for the challenge. Plasmid constructs comprising codon-optimized and non-codon-optimized coding regions encoding S, S1, S2, N, M, E, soluble S, soluble S1, soluble S2, soluble TPA-S, soluble TPA-S1, and soluble TPA-S2, as described herein, fusions; or alternatively, coding regions (either codon-optimized or non-codon optimized) encoding various SARS-CoV proteins or fragments, variants or derivatives, either alone or as fusions with a carrier protein, *e.g.*, HBcAg, as well as various controls, *e.g.*, empty vector, are formulated with cationic lipid, and/or poloxamer and/or aluminum phosphate based or other adjuvants. The vaccine formulations are delivered at a dose of about 1-10 μg, delivered IM into the defeathered breast area, at 0 and 1 month. The animals are bled for antibody results 3 weeks following the second vaccine. Antibody titers against the various SARS-CoV antigens are determined using techniques described in the literature. See, *e.g.*, Kodihalli S. *et al.*, *Vaccine* *18*:2592-9 (2000). The birds are challenged intranasally with 0.1 mL containing 100 LD_{50} 3 weeks post second vaccination. The birds are monitored daily for 10 days for disease symptoms, which include gasping, coughing and nasal discharge, wet eyes and swollen sinuses, reduced food consumption and weight loss. Tracheal and cloacal swabs are taken 4 days following challenge for virus titration.

* * *

[0386] The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and any compositions or methods which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description

and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

[0387] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

WHAT IS CLAIMED IS:

1. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:2, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

2. The method of claim 1, wherein said polypeptide comprises SEQ ID NO: 2, with the exception that the amino acid at position 577 can be A or S.

3. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:4, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

4. The method of claim 3, wherein said polypeptide comprises SEQ ID NO: 4.

5. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:6, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

6. The method of claim 5, wherein said polypeptide comprises SEQ ID NO:6.

7. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:8, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

8. The method of claim 7, wherein said polypeptide comprises SEQ ID NO: 8.

9. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:10, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

10. The method of claim 9, wherein said polypeptide comprises SEQ ID NO: 10.

11. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:12, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

12. The method of claim 11, wherein said polypeptide comprises SEQ ID NO:12.

13. A method for raising a detectable immune response to the SARS-CoV Nucleocapsid protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:14, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

14. The method of claim 13, wherein said polypeptide comprises SEQ ID NO:14.

15. A method for raising a detectable immune response to the SARS-CoV Nucleocapsid protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:16, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

16. The method of claim 15, wherein said polypeptide comprises SEQ ID NO:16.

17. A method for raising a detectable immune response to the SARS-CoV Membrane protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:19, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

18. The method of claim 17, wherein said polypeptide comprises SEQ ID NO:19.

19. The method of any one of claims 1-18 wherein said polynucleotide is DNA.

20. The method of any one of claims 1-18 wherein said polynucleotide is a DNA sequence incorporated into a vector.

21. The method of claim 20, wherein said vector is a plasmid.

22. The method of claim 21, wherein said plasmid vector further comprises a eukaryotic expression vector.

23. The method of any one of claims 1-18, wherein said polynucleotide contains a promoter sequence.

24. The method of claim 23, wherein said promoter sequence is a cell specific promoter that directs transcription of said polynucleotide in specific cell types.

25. The method of any one of claims 1-24, wherein said polynucleotide contains a sequence operatively coding for the secretion of said polypeptide.

26. The method of any one of claims 1-25, further comprising administering said polynucleotide with a carrier.

27. The method of claim 26, further comprising administering said polynucleotide with a component selected from the group consisting of an adjuvant, and a transfection facilitation compound.

28. The method of claim 27, wherein said adjuvant is selected from the group consisting of

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) and a neutral lipid;

a cytokine;

mono-phosphoryl lipid A and trehalosedicorynomycolateAF (MPL + TDM);

a solubilized mono-phosphoryl lipid A formulation; and

CRL1005/BAK.

29. The method of claim 28, wherein said adjuvant comprises (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE), and wherein said neutral lipid is selected from the group consisting of:

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE),

1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and

1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE).

30. The method of claim 29, wherein said neutral lipid is DPyPE.

31. The method of claim 27, comprising the transfection facilitating compound (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) (DMRIE).

32. The method of claim 27, wherein said adjuvant or said transfecting facilitating compound comprises CRL 1005 and benzalkonium chloride (BAK).

33. The composition administered in claim 32, wherein said CRL1005 and BAK are present at concentrations selected from the group consisting of:

0.3 mM BAK and 7.5 mg/ml CRL 1005,

0.3 mM BAK and 34 mg/ml CRL 1005, and

0.3 mM BAK and 50 mg/ml CRL 1005.

[33.5]. The method of any one of claims 1-25, wherein said polynucleotide is administered free from lipids and poloxamers.

34. The method of any one of claims 1 to 33.5, wherein said encoded polypeptide is fused to a small assembly polypeptide and wherein said polypeptide fusion can self-assemble into multimers.

35. The method of any one of claims 1-18, wherein said immune response is therapeutic or prophylactic.

36. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising at least 20 contiguous amino acids of SEQ ID NO:2, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

37. The method of claim 36, wherein said polynucleotide encodes a polypeptide comprising at least 50 contiguous amino acids of SEQ ID NO: 2.

38. The method of claim 36, wherein said polynucleotide encodes a polypeptide comprising at least 100 contiguous amino acids of SEQ ID NO: 2.

39. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising at least 20 contiguous amino acids of SEQ ID NO:4, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

40. The method of claim 39, wherein said polynucleotide encodes a polypeptide comprising at least 50 contiguous amino acids of SEQ ID NO: 4.

41. The method of claim 39, wherein said polynucleotide encodes a polypeptide comprising at least 100 contiguous amino acids of SEQ ID NO: 4.

42. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising at least 20 contiguous amino acids of SEQ ID NO:6, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

43. The method of claim 42, wherein said polynucleotide encodes a polypeptide comprising at least 50 contiguous amino acids of SEQ ID NO: 6.

44. The method of claim 42, wherein said polynucleotide encodes a polypeptide comprising at least 100 contiguous amino acids of SEQ ID NO: 6.

45. A method for raising a detectable immune response to the SARS-CoV Nucleocapsid protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising at least 20 contiguous amino acids of SEQ ID NO:14, wherein said polynucleotide is

administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

46. The method of claim 45, wherein said polynucleotide encodes a polypeptide comprising at least 50 contiguous amino acids of SEQ ID NO: 14.

47. The method of claim 45, wherein said polynucleotide encodes a polypeptide comprising at least 100 contiguous amino acids of SEQ ID NO: 14.

48. A method for raising a detectable immune response to the SARS-CoV Nucleocapsid protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising at least 20 contiguous amino acids of SEQ ID NO:16, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

49. The method of claim 48, wherein said polynucleotide encodes a polypeptide comprising at least 50 contiguous amino acids of SEQ ID NO: 16.

50. The method of claim 48, wherein said polynucleotide encodes a polypeptide comprising at least 100 contiguous amino acids of SEQ ID NO: 16.

51. A method for raising a detectable immune response to the SARS-CoV Membrane protein, comprising administering to a vertebrate a polynucleotide which operably encodes a polypeptide comprising at least 20 contiguous amino acids of SEQ ID NO:19, wherein said polynucleotide is administered in an amount sufficient to elicit a detectable immune response to the encoded polypeptide.

52. The method of claim 51, wherein said polynucleotide encodes a polypeptide comprising at least 50 contiguous amino acids of SEQ ID NO: 19.

53. The method of claim 51, wherein said polynucleotide encodes a polypeptide comprising at least 100 contiguous amino acids of SEQ ID NO: 19.

54. The method of any one of claims 36-53 wherein said polynucleotide is DNA.

55. The method of any one of claims 36-53 wherein said polynucleotide is a DNA sequence incorporated into a vector.

56. The method of claim 55, wherein said vector is a plasmid.

57. The method of claim 56, wherein said plasmid vector further comprises a eukaryotic expression vector.

58. The method of any one of claims 36-57, wherein said polynucleotide contains a promoter sequence.

59. The method of claim 58, wherein said promoter sequence is a cell specific promoter that directs transcription of said polynucleotide in specific cell types.

60. The method of any one of claims 36-59, wherein said polynucleotide contains a sequence operatively coding for the secretion of said polypeptide.

61. The method of any one of claims 36-60, further comprising administering said polynucleotide with a carrier.

62. The method of claim 58, further comprising administering said polynucleotide with a component selected from the group consisting of an adjuvant, and a transfection facilitation compound.

63. The method of claim 62, wherein said adjuvant is selected from the group consisting of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) and a neutral lipid;

a cytokine;

mono-phosphoryl lipid A and trehalosedicorynomycolateAF (MPL + TDM);

a solubilized mono-phosphoryl lipid A formulation; and

CRL1005/BAK.

64. The method of claim 63, wherein said adjuvant comprises(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE), and wherein said neutral lipid is selected from the group consisting of:

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE),

1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and

1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE).

65. The method of claim 64, wherein said neutral lipid is DPyPE.

66. The method of claim 62, comprising the transfection facilitating compound (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) (DMRIE).

67. The method of claim method of claim 62, wherein said adjuvant if said transfecting facilitating compound comprises CRL 1005 and benzalkonium chloride (BAK).

68. The method of claim 67, wherein said CRL1005 and BAK are present at concentrations selected from the group consisting of:

0.3 mM BAK and 7.5 mg/ml CRL 1005,

0.3 mM BAK and 34 mg/ml CRL 1005, and

0.3 mM BAK and 50 mg/ml CRL 1005.

69. The method of any one of claims 36-60, wherein said polynucleotide is administered free from lipids and poloxamers.

70. The method of any one of claims 36-69, wherein said immune response is therapeutic or prophylactic.

71. The method of any one of claims 36-69, wherein said encoded polypeptide is fused to a small assembly polypeptide and wherein said polypeptide fusion can self-assemble into multimers.

72. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:2, wherein said polypeptide is administered in an amount sufficient to elicit a detectable immune response to said polypeptide

73. The method of claim 72, wherein said polypeptide is SEQ ID NO: 2, with the exception that the amino acid a position 577 can be A or S.

74. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a

polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:4, wherein said polypeptide is administered in an amount sufficient to elicit a detectable immune response to said polypeptide.

75. The method of claim 74, wherein said polypeptide comprises SEQ ID NO: 4.

76. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:6, wherein said polypeptide is administered in an amount sufficient to elicit a detectable immune response to said polypeptide.

77. The method of claim 76, wherein said polypeptide comprises SEQ ID NO:6.

78. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:8, wherein said polypeptide is administered in an amount sufficient to elicit a detectable immune response to said polypeptide.

79. The method of claim 78, wherein said polypeptide comprises SEQ ID NO: 8.

80. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:10, wherein said polypeptide is administered in an amount sufficient to elicit a detectable immune response to said polypeptide.

81. The method of claim 80, wherein said polypeptide comprises SEQ ID NO: 10.

82. A method for raising a detectable immune response to the SARS-CoV Spike protein, comprising administering to a vertebrate a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:12, wherein said polypeptide is administered in an amount sufficient to elicit a detectable immune response to said polypeptide.

83. The method of claim 82, wherein said polypeptide comprises SEQ ID NO:12.

84. A method for raising a detectable immune response to the SARS-CoV Nucleocapsid protein, comprising administering to a vertebrate a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:14, wherein said polypeptide is administered in an amount sufficient to elicit a detectable immune response to said polypeptide.

85. The method of claim 84, wherein said polypeptide is SEQ ID NO:14.

86. A method for raising a detectable immune response to the SARS-CoV Nucleocapsid protein, comprising administering to a vertebrate a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:16, wherein said polypeptide is administered in an amount sufficient to elicit a detectable immune response to said polypeptide.

87. The method of claim 86, wherein said polypeptide comprises SEQ ID NO:16.

88. A method for raising a detectable immune response to the SARS-CoV Membrane protein, comprising administering to a vertebrate a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO:19, wherein said polypeptide is administered in an amount sufficient to elicit a detectable immune response to said polypeptide.

89. The method of claim 88, wherein said polypeptide comprises SEQ ID NO:19.

90. The method of any one of claims 72-89, further comprising administering said polypeptide with a carrier.

91. The method of claim 90, further comprising administering said polypeptide with a component selected from the group consisting of an adjuvant.

92. The method of any one of claims 72-90, wherein immune response is therapeutic or prophylactic.

93. The method of any one of claims 72-92, wherein said encoded polypeptide is fused to a small assembly polypeptide and wherein said polypeptide fusion can self-assemble into multimers.

94. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO: 2, wherein said nucleic acid fragment is a fragment of a codon-optimized region for the polypeptide of SEQ ID NO:2;

wherein about 37 of the 81 phenylalanine codons in said coding region are TTT and about 44 of the phenylalanine codons are TTC;

wherein about 7 of the 92 leucine codons in said coding region are TTA, about 12 of the leucine codons are TTG, about 12 of the leucine

codons are CTT, about 18 of the leucine codons are CTC, about 7 of the leucine codons are CTA, and about 36 of the leucine codons are CTG;

wherein about 26 of the 74 isoleucine codons in said coding region are ATT, about 35 of the isoleucine codons are ATC, and about 13 of the isoleucine codons are ATA;

wherein about 18 of the methionine codons in said coding region are ATG;

wherein about 15 of the 86 valine codons in said coding region are GTT, about 40 of the valine codons are GTG, about 10 of the valine codons are GTA, and about 21 of the valine codons are GTC;

wherein about 17 of the 91 serine codons in said coding region are TCT, about 20 of the serine codons are TCC, about 14 of the serine codons are TCA, about 5 of the serine codons are TCG, about 13 of the serine codons are AGT, and about 22 of the serine codons are AGC;

wherein about 16 of the 56 proline codons in said coding region are CCT, about 18 of the proline codons are CCC, about 16 of the proline codons are CCA, and about 6 of the proline codons are CCG;

wherein about 23 of the 96 threonine codons in said coding region are ACT, about 35 of the threonine codons are ACC, about 27 of the threonine codons are ACA, and about 11 of the threonine codons are ACG;

wherein about 21 of the 81 alanine codons in said coding region are GCT, about 33 of the alanine codons are GCC, about 18 of the alanine codons are GCA, and about 9 of the alanine codons are GCG;

wherein about 23 of the 52 tyrosine codons in said coding region are TAT and about 29 of the tyrosine codons are TAC;

wherein about 6 of the 14 histidine codons in said coding region are CAT and about 8 of the histidine codons are CAC;

wherein about 14 of the 55 glutamine codons in said coding region are CAA and about 41 of the glutamine codons are CAG;

wherein about 37 of the 81 asparagine codons in said coding region are AAT and about 44 of the asparagine codons are AAC;

wherein about 24 of the 56 lysine codons in said coding region are AAA and about 32 of the lysine codons are AAG;

wherein about 32 of the 70 aspartic acid codons in said coding region are GAT and about 38 of the aspartic acid codons are GAC;

wherein about 17 of the 40 glutamic acid codons in said coding region are GAA and about 23 of the glutamic acid codons are GAG;

wherein about 14 of the 30 cysteine codons in said coding region are TGT and about 16 of the cysteine codons are TGC;

wherein about 10 of the tryptophan codons in said coding region are TGG;

wherein about 3 of the 39 arginine codons in said coding region are CGT, about 7 of the arginine codons are CGC, about 4 of the arginine codons are CGA, about 8 of the arginine codons are CGG, about 9 of the arginine codons are AGA, and about 8 of the arginine codons are AGG; and

wherein about 12 of the 74 glycine codons in said coding region are GGT, about 25 of the glycine codons are GGC, about 19 of the glycine codons are GGA, and about 18 of the glycine codons are GGG and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

95. The polynucleotide of claim 94, wherein said codon optimized coding region comprises SEQ ID NO:24.

96. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:2, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:2;

wherein the 81 phenylalanine codons in said coding region are TTC, the 92 leucine codons in said coding region are CTG, the 74 isoleucine codons in said coding region are ATC, the 18 methionine codons in said

coding region are ATG, the 86 valine codons in said coding region are GTG, the 91 serine codons in said coding region are AGC, the 56 proline codons in said coding region are CCC, the 96 threonine codons in said coding region are ACC, the 81 alanine codons in said coding region are GCC, the 52 tyrosine codons in said coding region are TAC, the 14 histidine codons in said coding region are CAC, the 55 glutamine codons in said coding region are CAG, the 84 asparagine codons in said coding region are AAC, the 56 lysine codons in said coding region are AAG, the 70 aspartic acid codons in said coding region are GAC, the 40 glutamic acid codons in said coding region are GAG, the 30 cysteine codons in said coding region are TGC, the 10 tryptophan codon in said coding region is TGG, the 39 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 74 glycine codons in said coding region are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

97. The polynucleotide of claim 96, wherein said codon optimized coding region comprises SEQ ID NO:25.

98. The polynucleotide of any one of claims 94-97, wherein said nucleic acid fragment encodes at least 50 contiguous amino acids of SEQ ID NO:2.

99. The polynucleotide of any one of claims 94-97, wherein said nucleic acid fragment encodes at least 100 contiguous amino acids of SEQ ID NO:2.

100. The polynucleotide of claim 99, wherein said nucleic acid fragment encodes SEQ ID NO:2.

101. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO: 2, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:2;

wherein about 37 of the 81 phenylalanine codons in said coding region are TTT and about 44 of the phenylalanine codons are TTC;

wherein about 7 of the 92 leucine codons in said coding region are TTA, about 12 of the leucine codons are TTG, about 12 of the leucine codons are CTT, about 18 of the leucine codons are CTC, about 7 of the leucine codons are CTA, and about 36 of the leucine codons are CTG;

wherein about 26 of the 74 isoleucine codons in said coding region are ATT, about 35 of the isoleucine codons are ATC, and about 13 of the isoleucine codons are ATA;

wherein about 18 of the methionine codons in said coding region are ATG;

wherein about 15 of the 86 valine codons in said coding region are GTT, about 40 of the valine codons are GTG, about 10 of the valine codons are GTA, and about 21 of the valine codons are GTC;

wherein about 17 of the 91 serine codons in said coding region are TCT, about 20 of the serine codons are TCC, about 14 of the serine codons are TCA, about 5 of the serine codons are TCG, about 13 of the serine codons are AGT, and about 22 of the serine codons are AGC;

wherein about 16 of the 56 proline codons in said coding region are CCT, about 18 of the proline codons are CCC, about 16 of the proline codons are CCA, and about 6 of the proline codons are CCG;

wherin about 23 of the 96 threonine codons in said coding region are ACT, about 35 of the threonine codons are ACC, about 27 of the threonine codons are ACA, and about 11 of the threonine codons are ACG;

wherein about 21 of the 81 alanine codons in said coding region are GCT, about 33 of the alanine codons are GCC, about 18 of the alanine codons are GCA, and about 9 of the alanine codons are GCG;

wherein about 23 of the 52 tyrosine codons in said coding region are TAT and about 29 of the tyrosine codons are TAC;

wherein about 6 of the 14 histidine codons in said coding region are CAT and about 8 of the histidine codons are CAC;

wherein about 14 of the 55 glutamine codons in said coding region are CAA and about 41 of the glutamine codons are CAG;

wherein about 37 of the 81 asparagine codons in said coding region are AAT and about 44 of the asparagine codons are AAC;

wherein about 24 of the 56 lysine codons in said coding region are AAA and about 32 of the lysine codons are AAG;

wherein about 32 of the 70 aspartic acid codons in said coding region are GAT and about 38 of the aspartic acid codons are GAC;

wherein about 17 of the 40 glutamic acid codons in said coding region are GAA and about 23 of the glutamic acid codons are GAG;

wherein about 14 of the 30 cysteine codons in said coding region are TGT and about 16 of the cysteine codons are TGC;

wherein about 10 of the tryptophan codons in said coding region are TGG;

wherein about 3 of the 39 arginine codons in said coding region are CGT, about 7 of the arginine codons are CGC, about 4 of the arginine codons are CGA, about 8 of the arginine codons are CGG, about 9 of the arginine codons are AGA, and about 8 of the arginine codons are AGG; and

wherein about 12 of the 74 glycine codons in said coding region are GGT, about 25 of the glycine codons are GGC, about 19 of the glycine codons are GGA, and about 18 of the glycine codons are GGG and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

102. The polynucleotide of claim 101, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO:2.

103. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO:2, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:2;

wherein the 81 phenylalanine codons in said coding region are TTC, the 92 leucine codons in said coding region are CTG, the 74 isoleucine codons in said coding region are ATC, the 18 methionine codons in said coding region are ATG, the 86 valine codons in said coding region are GTG, the 91 serine codons in said coding region are AGC, the 56 proline codons in said coding region are CCC, the 96 threonine codons in said coding region are ACC, the 81 alanine codons in said coding region are GCC, the 52 tyrosine codons in said coding region are TAC, the 14 histidine codons in said coding region are CAC, the 55 glutamine codons in said coding region are CAG, the 84 asparagine codons in said coding region are AAC, the 56 lysine codons in said coding region are AAG, the 70 aspartic acid codons in said coding region are GAC, the 40 glutamic acid codons in said coding region are GAG, the 30 cysteine codons in said coding region are TGC, the 10 tryptophan codon in said coding region is TGG, the 39 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 74 glycine codons in said coding region are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

104. The polynucleotide of claim 103, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO: 2.

105. The polynucleotide of claim 103, wherein said nucleic acid fragment encodes SEQ ID NO: 2.

106. The polynucleotide of any one of claims 94 to 105, wherein said nucleic acid fragment is ligated to a heterologous nucleic acid.

107. The polynucleotide of claim 106, wherein said heterologous nucleic acid encodes a heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment.

108. The polynucleotide of claim 107, wherein said heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment comprises a small self-assembly polypeptide, wherein said heterologous polypeptide self-assemble into multimers.

109. The polynucleotide of any one of claim 94 to 108, wherein said nucleic acid fragment is ligated to a nucleic acid operatively encoding for the secretion of said polypeptide.

110. The polynucleotide of any one of claims 94 to 109, which is DNA, and wherein said nucleic acid fragment is operably associated with a promoter.

111. The polynucleotide of any one of claims 94 to 109, which is RNA.

112. The polynucleotide of claim 111, which is messenger RNA (mRNA).

113. A vector comprising the polynucleotide of any one of claims 94 to 111.

114. The vector of claim 113, which is a plasmid.

115. A composition comprising the polynucleotide of any one of claims 94 to 112, and a carrier.

116. The composition of claim 115, further comprising a component selected from the group consisting of an adjuvant, and a transfection facilitating compound.

117. The composition of claim 116, wherein said adjuvant is selected from the group consisting of:

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) and a neutral lipid;

a cytokine;

mono-phosphoryl lipid A and trehalosedicorynomycolateAF (MPL + TDM);

a solubilized mono-phosphoryl lipid A formulation; and

CRL1005/BAK.

118. The composition of claim 117, wherein said adjuvant comprises(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE), and wherein said neutral lipid is selected from the group consisting of:

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE),

1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and

1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE).

119. The composition of claim 118, wherein said neutral lipid is DPyPE.

120. The composition of claim 115, comprising the transfection facilitating compound (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) (DMRIE).

121. The composition of claim 115, wherein said adjuvant or said transfection facilitating compound comprises CRL1005 and benzalkonium chloride (BAK).

122. The composition of claim 118, wherein said CRL1005 and BAK are present at concentrations selected from the group consisting of:

0.3 mM BAK and 7.5 mg/ml CRL 1005,

0.3 mM BAK and 34 mg/ml CRL 1005, and

0.3 mM BAK and 50 mg/ml CRL 1005.

123. The composition of claim 121 or claim 122, which is formulated by combining together the CRL1005, BAK, and polynucleotide at a temperature below the cloud point, without thermal cycling to a temperature above the cloud point..

124. A method to treat or prevent SARS-CoV infection in a vertebrate comprising: administering to a vertebrate in need thereof the polynucleotide of any one of claims 94 to 112 or the composition of any one of claims 115 to 123.

125. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:4, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:4;

wherein about 24 of the 53 phenylalanine codons in said coding region are TTT, and about 29 of the phenylalanine codons are TTC;

wherein about 3 of the 46 leucine codons in said coding region are TTA, about 6 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 9 of the leucine codons are CTC, about 4 of the leucine codons are CTA, and about 18 of the leucine codons are CTG;

wherein about 13 of the 38 isoleucine codons in said coding region are ATT, about 18 of the isoleucine codons are ATC, and about 7 of the isoleucine codons are ATA;

wherein about 8 of the methionine codons in said coding region are ATG;

wherein about 10 of the 53 valine codons in said coding region are GTT, about 13 of the valine codons are GTC, about 5 of the valine codons are GTA, and about 25 of the valine codons are GTG;

wherein about 10 of the 56 serine codons in said coding region are TCT, about 12 of the serine codons are TCC, about 8 of the serine codons are TCA, about 3 of the serine codons are TCG, about 9 of the serine codons are AGT, and about 14 of the serine codons are AGC;

wherein about 10 of the 37 proline codons in said coding region are CCT, about 12 of the proline codons are CCC, about 11 of the proline codons are CCA, and about 4 of the proline codons are CCG;

wherein about 14 of the 58 threonine codons in said coding region are ACT, about 21 of the threonine codons are ACC, about 16 of the threonine codons are ACA, and about 7 of the threonine codons are ACG;

wherein about 10 of the 38 alanine codons in said coding region are GCT, about 15 of the alanine codons are GCC, about 9 of the alanine codons are GCA, and about 4 of the alanine codons are GCG;

wherein about 15 of the 35 tyrosine codons in said coding region are TAT and about 20 of the tyrosine codons are TAC;

wherein about 4 of the 9 histidine codons in said coding region are CAT and about 5 of the histidine codons are CAC;

wherein about 5 of the 21 glutamine codons in said coding region are CAA and about 16 of the glutamine codons are CAG;

wherein about 21 of the 46 asparagine codons in said coding region are AAT and about 25 of the asparagine codons are AAC;

wherein about 13 of the 31 lysine codons in said coding region are AAA and about 18 of the lysine codons are AAG;

wherein about 20 of the 44 aspartic acid codons in said coding region are GAT and about 24 of the aspartic acid codons are GAC;

wherein about 7 of the 17 glutamic acid codons in said coding region are GAA and about 10 of the glutamic acid codons are GAG;

wherein about 9 of the 20 cysteine codons in said coding region are TGT and about 11 of the cysteine codons are TGC;

wherein about 6 of the tryptophan codons in said coding region are TGG;

wherein about 2 of the 23 arginine codons in said coding region are CGT, about 4 of the arginine codons are CGC, about 3 of the arginine codons are CGA, about 5 of the arginine codons are CGG, about 4 of the arginine codons are AGA, and about 5 of the arginine codons are AGG;

wherein and about 7 of the 44 glycine codons in said coding region are GGT, about 15 of the glycine codons are GGC, about 11 of the glycine codons are GGA, and about 11 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

126. The polynucleotide of claim 125, wherein said codon optimized coding region comprises SEQ ID NO: 26.

127. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:4, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:4;

wherein the 53 phenylalanine codons in said coding region are TTC, the 46 leucine codons in said coding region are CTG, the 38 isoleucine codons in said coding region are ATC, the 8 methionine codons in said coding region are ATG, the 53 valine codons in said coding region are GTG, the 56 serine codons in said coding region are AGC, the 37 proline codons in said coding region are CCC, the 58 threonine codons in said coding region are ACC, the 38 alanine codons in said coding region are GCC, the 35 tyrosine codons in said coding region are TAC, the 9 histidine codons in said coding region are CAC, the 21 glutamine codons in said coding region are CAG, the 46 asparagine codons in said coding region are AAC, the 31 lysine codons in said coding region are AAG, the 44 aspartic acid codons in said coding region are GAC, the 17 glutamic acid codons in said coding region are GAG, the 20 cysteine codons in said coding region are TGC; the 6 tryptophan codons in said coding region are TGG, the 23 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 44 glycine codons are GGC.; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

128. The polynucleotide of claim 127, wherein said codon optimized coding region comprises SEQ ID NO:27.

129. The polynucleotide of any one of claims 125-128, wherein said nucleic acid fragment encodes at least 50 contiguous amino acids of SEQ ID NO:4.

130. The polynucleotide of any one of claims 125-128, wherein said nucleic acid fragment encodes at least 100 contiguous amino acids of SEQ ID NO:4.

131. The polynucleotide of claim 127, wherein said nucleic acid fragment encodes SEQ ID NO:4.

132. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO: 4, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:4;

wherein about 24 of the 53 phenylalanine codons in said coding region are TTT, and about 29 of the phenylalanine codons are TTC;

wherein about 3 of the 46 leucine codons in said coding region are TTA, about 6 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 9 of the leucine codons are CTC, about 4 of the leucine codons are CTA, and about 18 of the leucine codons are CTG;

wherein about 13 of the 38 isoleucine codons in said coding region are ATT, about 18 of the isoleucine codons are ATC, and about 7 of the isoleucine codons are ATA;

wherein about 8 of the methionine codons in said coding region are ATG;

wherein about 10 of the 53 valine codons in said coding region are GTT, about 13 of the valine codons are GTC, about 5 of the valine codons are GTA, and about 25 of the valine codons are GTG;

wherein about 10 of the 56 serine codons in said coding region are TCT, about 12 of the serine codons are TCC, about 8 of the serine codons are TCA, about 3 of the serine codons are TCG, about 9 of the serine codons are AGT, and about 14 of the serine codons are AGC;

wherein about 10 of the 37 proline codons in said coding region are CCT, about 12 of the proline codons are CCC, about 11 of the proline codons are CCA, and about 4 of the proline codons are CCG;

wherein about 14 of the 58 threonine codons in said coding region are ACT, about 21 of the threonine codons are ACC, about 16 of the threonine codons are ACA, and about 7 of the threonine codons are ACG;

wherein about 10 of the 38 alanine codons in said coding region are GCT, about 15 of the alanine codons are GCC, about 9 of the alanine codons are GCA, and about 4 of the alanine codons are GCG;

wherein about 15 of the 35 tyrosine codons in said coding region are TAT and about 20 of the tyrosine codons are TAC;

wherein about 4 of the 9 histidine codons in said coding region are CAT and about 5 of the histidine codons are CAC;

wherein about 5 of the 21 glutamine codons in said coding region are CAA and about 16 of the glutamine codons are CAG;

wherein about 21 of the 46 asparagine codons in said coding region are AAT and about 25 of the asparagine codons are AAC;

wherein about 13 of the 31 lysine codons in said coding region are AAA and about 18 of the lysine codons are AAG;

wherein about 20 of the 44 aspartic acid codons in said coding region are GAT and about 24 of the aspartic acid codons are GAC;

wherein about 7 of the 17 glutamic acid codons in said coding region are GAA and about 10 of the glutamic acid codons are GAG;

wherein about 9 of the 20 cysteine codons in said coding region are TGT and about 11 of the cysteine codons are TGC;

wherein about 6 of the tryptophan codons in said coding region are TGG;

wherein about 2 of the 23 arginine codons in said coding region are CGT, about 4 of the arginine codons are CGC, about 3 of the arginine codons are CGA, about 5 of the arginine codons are CGG, about 4 of the arginine codons are AGA, and about 5 of the arginine codons are AGG;

wherein and about 7 of the 44 glycine codons in said coding region are GGT, about 15 of the glycine codons are GGC, about 11 of the glycine codons are GGA, and about 11 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

133. The polynucleotide of claim 132, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO:4.

134. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO:4, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:4;

wherein the 53 phenylalanine codons in said coding region are TTC, the 46 leucine codons in said coding region are CTG, the 38 isoleucine codons in said coding region are ATC, the 8 methionine codons in said coding region are ATG, the 53 valine codons in said coding region are GTG, the 56 serine codons in said coding region are AGC, the 37 proline codons in said coding region are CCC, the 58 threonine codons in said coding region are ACC, the 38 alanine codons in said coding region are GCC, the 35 tyrosine codons in said coding region are TAC, the 9 histidine codons in said coding region are CAC, the 21 glutamine codons in said coding region are CAG, the 46 asparagine codons in said coding region are AAC, the 31 lysine codons in said coding region are AAG, the 44 aspartic acid codons in said coding region are GAC, the 17 glutamic acid codons in said coding region are GAG, the 20 cysteine codons in said coding region are TGC; the 6 tryptophan codons in said coding region are TGG, the 23 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 44 glycine codons are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

135. The polynucleotide of claim 134, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO: 4.

136. The polynucleotide of claim 135, wherein said nucleic acid fragment encodes SEQ ID NO: 4.

137. The polynucleotide of any one of claims 125 to 136, wherein said nucleic acid fragment is ligated to a heterologous nucleic acid.

138. The polynucleotide of claim 137, wherein said heterologous nucleic acid encodes a heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment.

139. The polynucleotide of claim 138, wherein said heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment comprises a small self-assembly polypeptide, wherein said heterologous polypeptide self-assemble into multimers.

140. The polynucleotide of any one of claim 125 to 139, wherein said nucleic acid fragment is ligated to a nucleic acid operatively encoding for the secretion of said polypeptide.

141. The polynucleotide of any one of claims 125 to 140, which is DNA, and wherein said nucleic acid fragment is operably associated with a promoter.

142. The polynucleotide of any one of claims 125 to 140, which is RNA.

143. The polynucleotide of claim 142, which is messenger RNA (mRNA).

144. A vector comprising the polynucleotide of any one of claims 125 to 142.

145. The vector of claim 144, which is a plasmid.

146. A composition comprising the polynucleotide of any one of claims 125 to 143, and a carrier.

147. The composition of claim 146, further comprising a component selected from the group consisting of an adjuvant, and a transfection facilitating compound.

148. The composition of claim 147, wherein said adjuvant is selected from the group consisting of:

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) and a neutral lipid;

a cytokine;

mono-phosphoryl lipid A and trehalosedicorynomycolateAF (MPL + TDM);

a solubilized mono-phosphoryl lipid A formulation; and

CRL1005/BAK.

149. The composition of claim 148, wherein said adjuvant comprises(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE), and wherein said neutral lipid is selected from the group consisting of:

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE),

1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and

1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE).

150. The composition of claim 149, wherein said neutral lipid is DPyPE.

151. The composition of claim 147, comprising the transfection facilitating compound (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) (DMRIE).

152. The composition of claim 147, wherein said adjuvant or said transfection facilitating compound comprises CRL1005 and benzalkonium chloride (BAK).

153. The composition of claim 152, wherein said CRL1005 and BAK are present at concentrations selected from the group consisting of:

0.3 mM BAK and 7.5 mg/ml CRL 1005,

0.3 mM BAK and 34 mg/ml CRL 1005, and

0.3 mM BAK and 50 mg/ml CRL 1005.

154. The composition of claim 152 or claim 153, which is formulated by combining together the CRL1005, BAK, and polynucleotide at a temperature below the cloud point, without thermal cycling to a temperature above the cloud point..

155. A method to treat or prevent SARS-CoV infection in a vertebrate comprising: administering to a vertebrate in need thereof the polynucleotide of any one of claims 125 to 143 or the composition of any one of claims 146 to 154.

156. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:6, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:6;

wherein about 13 of the 28 phenylalanine codons in said coding region are TTT, and about 15 of the phenylalanine codons are TTC;

wherein about 3 of the 46 leucine codons in said coding region are TTA, about 6 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 9 of the leucine codons are CTC, about 4 of the leucine codons are CTA, and about 18 of the leucine codons are CTG;

wherein about 13 of the 36 isoleucine codons in said coding region are ATT, about 17 of the isoleucine codons are ATC, and about 6 of the isoleucine codons are ATA;

wherein about 10 of the methionine codons in said coding region are ATG;

wherein about 6 of the 33 valine codons in said coding region are GTT, about 15 of the valine codons are GTG, about 4 of the valine codons are GTA, and about 8 of the valine codons are GTC;

wherein about 6 of the 35 serine codons in said coding region are TCT, about 8 of the serine codons are TCC, about 5 of the serine codons are TCA, about 2 of the serine codons are TCG, about 6 of the serine codons are AGT, and about 8 of the serine codons are AGC;

wherein about 5 of the 19 proline codons in said coding region are CCT, about 6 of the proline codons are CCC, about 6 of the proline codons are CCA, and about 2 of the proline codons are CCG;

wherein about 9 of the 38 threonine codons in said coding region are ACT, about 14 of the threonine codons are ACC, about 11 of the threonine codons are ACA, and about 4 of the threonine codons are ACG;

wherein about 11 of the 43 alanine codons in said coding region are GCT, about 17 of the alanine codons are GCC, about 10 of the alanine codons are GCA, and about 5 of the alanine codons are GCG;

wherein about 7 of the 17 tyrosine codons in said coding region are TAT and about 10 of the tyrosine codons are TAC;

wherein about 2 of the 5 histidine codons in said coding region are CAT and about 3 of the histidine codons are CAC;

wherein about 9 of the 34 glutamine codons in said coding region are CAA and about 25 of the glutamine codons are CAG;

wherein about 16 of the 35 asparagine codons in said coding region are AAT and about 19 of the asparagine codons are AAC;

wherein about 11 of the 25 lysine codons in said coding region are AAA and about 14 of the lysine codons are AAG;

wherein about 12 of the 26 aspartic acid codons in said coding region are GAT and about 14 of the aspartic acid codons are GAC;

wherein about 10 of the 23 glutamic acid codons in said coding region are GAA and about 13 of the glutamic acid codons are GAG;

wherein about 5 of the 10 cysteine codons in said coding region are TGT and about 5 of the cysteine codons are TGC;

wherein about 4 of the tryptophan codons in said coding region are TGG;

wherein about 1 of the 16 arginine codons in said coding region is CGT, about 3 of the arginine codons are CGC, about 2 of the arginine codons are CGA, about 3 of the arginine codons are CGG, about 4 of the arginine codons are AGA, and about 3 of the arginine codons are AGG; and

wherein about 5 of the 30 glycine codons in said coding region are GGT, about 10 of the glycine codons are GGC, about 8 of the glycine codons are GGA, and about 7 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

157. The polynucleotide of claim 156, wherein said codon optimized coding region comprises SEQ ID NO:28.

158. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:6, wherein

said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:6;

wherein the 28 phenylalanine codons in said coding region are TTC, the 46 leucine codons in said coding region are CTG, the 36 isoleucine codons in said coding region are ATC, the 10 methionine codons in said coding region are ATG, the 33 valine codons in said coding region are GTG, the 35 serine codons in said coding region are AGC, the 19 proline codons in said coding region are CCC, the 38 threonine codons in said coding region are ACC, the 43 alanine codons in said coding region are GCC, the 17 tyrosine codons in said coding region are TAC, the 5 histidine codons in said coding region are CAC, the 34 glutamine codons in said coding region are CAG, the 35 asparagine codons in said coding region are AAC, the 25 lysine codons in said coding region are AAG, the 26 aspartic acid codons in said coding region are GAC, the 23 glutamic acid codons in said coding region are GAG, the 10 cysteine codons in said coding region are TGC, the 4 tryptophan codons in said coding regions are TGG, the 16 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 30 glycine codons are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

159. The polynucleotide of claim 158, wherein said codon optimized coding region comprises SEQ ID NO:29.

160. The polynucleotide of any one of claims 156-159, wherein said nucleic acid fragment encodes at least 50 contiguous amino acids of SEQ ID NO:6.

161. The polynucleotide of claim 160, wherein said nucleic acid fragment encodes at least 100 contiguous amino acids of SEQ ID NO:6.

162. The polynucleotide of claim 161, wherein said nucleic acid fragment encodes SEQ ID NO:6.

163. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO: 6, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:6;

wherein about 13 of the 28 phenylalanine codons in said coding region are TTT, and about 15 of the phenylalanine codons are TTC;

wherein about 3 of the 46 leucine codons in said coding region are TTA, about 6 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 9 of the leucine codons are CTC, about 4 of the leucine codons are CTA, and about 18 of the leucine codons are CTG;

wherein about 13 of the 36 isoleucine codons in said coding region are ATT, about 17 of the isoleucine codons are ATC, and about 6 of the isoleucine codons are ATA;

wherein about 10 of the methionine codons in said coding region are ATG;

wherein about 6 of the 33 valine codons in said coding region are GTT, about 15 of the valine codons are GTG, about 4 of the valine codons are GTA, and about 8 of the valine codons are GTC;

wherein about 6 of the 35 serine codons in said coding region are TCT, about 8 of the serine codons are TCC, about 5 of the serine codons are TCA, about 2 of the serine codons are TCG, about 6 of the serine codons are AGT, and about 8 of the serine codons are AGC;

wherein about 5 of the 19 proline codons in said coding region are CCT, about 6 of the proline codons are CCC, about 6 of the proline codons are CCA, and about 2 of the proline codons are CCG;

wherein about 9 of the 38 threonine codons in said coding region are ACT, about 14 of the threonine codons are ACC, about 11 of the threonine codons are ACA, and about 4 of the threonine codons are ACG;

wherein about 11 of the 43 alanine codons in said coding region are GCT, about 17 of the alanine codons are GCC, about 10 of the alanine codons are GCA, and about 5 of the alanine codons are GCG;

wherein about 7 of the 17 tyrosine codons in said coding region are TAT and about 10 of the tyrosine codons are TAC;

wherein about 2 of the 5 histidine codons in said coding region are CAT and about 3 of the histidine codons are CAC;

wherein about 9 of the 34 glutamine codons in said coding region are CAA and about 25 of the glutamine codons are CAG;

wherein about 16 of the 35 asparagine codons in said coding region are AAT and about 19 of the asparagine codons are AAC;

wherein about 11 of the 25 lysine codons in said coding region are AAA and about 14 of the lysine codons are AAG;

wherein about 12 of the 26 aspartic acid codons in said coding region are GAT and about 14 of the aspartic acid codons are GAC;

wherein about 10 of the 23 glutamic acid codons in said coding region are GAA and about 13 of the glutamic acid codons are GAG;

wherein about 5 of the 10 cysteine codons in said coding region are TGT and about 5 of the cysteine codons are TGC;

wherein about 4 of the tryptophan codons in said coding region are TGG;

wherein about 1 of the 16 arginine codons in said coding region is CGT, about 3 of the arginine codons are CGC, about 2 of the arginine codons are CGA, about 3 of the arginine codons are CGG, about 4 of the arginine codons are AGA, and about 3 of the arginine codons are AGG; and

wherein about 5 of the 30 glycine codons in said coding region are GGT, about 10 of the glycine codons are GGC, about 8 of the glycine codons are GGA, and about 7 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

164. The polynucleotide of claim 163, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO:6.

165. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO:6, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:6;

wherein the 28 phenylalanine codons in said coding region are TTC, the 46 leucine codons in said coding region are CTG, the 36 isoleucine codons in said coding region are ATC, the 10 methionine codons in said coding region are ATG, the 33 valine codons in said coding region are GTG, the 35 serine codons in said coding region are AGC, the 19 proline codons in said coding region are CCC, the 38 threonine codons in said coding region are ACC, the 43 alanine codons in said coding region are GCC, the 17 tyrosine codons in said coding region are TAC, the 5 histidine codons in said coding region are CAC, the 34 glutamine codons in said coding region are CAG, the 35 asparagine codons in said coding region are AAC, the 25 lysine codons in said coding region are AAG, the 26 aspartic acid codons in said coding region are GAC, the 23 glutamic acid codons in said coding region are GAG, the 10 cysteine codons in said coding region are TGC, the 4 tryptophan codons in said coding regions are TGG, the 16 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 30 glycine codons are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

166. The polynucleotide of claim 165, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO: 6.

167. The polynucleotide of claim 166, wherein said nucleic acid fragment encodes SEQ ID NO: 6.

168. The polynucleotide of any one of claims 156 to 167, wherein said nucleic acid fragment is ligated to a heterologous nucleic acid.

169. The polynucleotide of claim 168, wherein said heterologous nucleic acid encodes a heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment.

170. The polynucleotide of claim 169, wherein said heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment comprises a small self-assembly polypeptide, wherein said heterologous polypeptide self-assemble into multimers

171. The polynucleotide of any one of claim 156 to 168, wherein said nucleic acid fragment is ligated to a nucleic acid operatively encoding for the secretion of said polypeptide.

172. The polynucleotide of any one of claims 156 to 171, which is DNA, and wherein said nucleic acid fragment is operably associated with a promoter.

173. The polynucleotide of any one of claims 156 to 171, which is RNA.

174. The polynucleotide of claim 173, which is messenger RNA (mRNA).

175. A vector comprising the polynucleotide of any one of claims 156 to 173.

176. The vector of claim 175, which is a plasmid.

177. A composition comprising the polynucleotide of any one of claims 156 to 174, and a carrier.

178. The composition of claim 177, further comprising a component selected from the group consisting of an adjuvant, and a transfection facilitating compound.

179. The composition of claim 178, wherein said adjuvant is selected from the group consisting of:

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) and a neutral lipid;

a cytokine;

mono-phosphoryl lipid A and trehalosedicorynomycolateAF (MPL + TDM);

a solubilized mono-phosphoryl lipid A formulation; and

CRL1005/BAK.

180. The composition of claim 179, wherein said adjuvant comprises(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE), and wherein said neutral lipid is selected from the group consisting of:

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE),

1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and

1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE).

181. The composition of claim 180, wherein said neutral lipid is DPyPE.

182. The composition of claim 178, comprising the transfection facilitating compound (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) (DMRIE).

183. The composition of claim 178, wherein said adjuvant or said transfection facilitating compound comprises CRL1005 and benzalkonium chloride (BAK).

184. The composition of claim 183, wherein said CRL1005 and BAK are present at concentrations selected from the group consisting of:

0.3 mM BAK and 7.5 mg/ml CRL 1005,

0.3 mM BAK and 34 mg/ml CRL 1005, and

0.3 mM BAK and 50 mg/ml CRL 1005.

185. The composition of claim 183 or claim 184, which is formulated by combining together the CRL1005, BAK, and polynucleotide at a temperature below the cloud point, without thermal cycling to a temperature above the cloud point.

186. A method to treat or prevent SARS-CoV infection in a vertebrate comprising: administering to a vertebrate in need thereof the polynucleotide of any one of claims 156 to 174 or the composition of any one of claims 177 to 185.

187. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:8, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:8;

wherein about 36 of the 79 phenylalanine codons in said coding region are TTT, and about 43 of the phenylalanine codons are TTC;

wherein about 7 of the 92 leucine codons in said coding region are TTA, about 12 of the leucine codons are TTG, about 12 of the leucine codons are CTT, about 18 of the leucine codons are CTC, about 7 of the leucine codons are CTA, and about 36 of the leucine codons are CTG;

wherein about 26 of the 73 isoleucine codons in said coding region are ATT, about 35 of the isoleucine codons are ATC, and about 12 of the isoleucine codons are ATA;

wherein about 19 methionine codons in said coding region are ATG;

wherein about 16 of the 89 valine codons in said coding region are GTT, about 41 of the valine codons are GTG, about 11 of the valine codons are GTA, and about 21 of the valine codons are GTC;

wherein about 17 of the 91 serine codons in said coding region are TCT, about 20 of the serine codons are TCC, about 14 of the serine codons are TCA, about 5 of the serine codons are TCG, about 15 of the serine codons are AGT, and about 22 of the serine codons are AGC;

wherein about 16 of the 57 proline codons in said coding region are CCT, about 19 of the proline codons are CCC, about 16 of the proline codons are CCA, and about 6 of the proline codons are CCG;

wherein about 23 of the 94 threonine codons in said coding region are ACT, about 34 of the threonine codons are ACC, about 26 of the threonine codons are ACA, and about 11 of the threonine codons are ACG;

wherein about 22 of the 84 alanine codons in said coding region are GCT, about 34 of the alanine codons are GCC, about 19 of the alanine codons are GCA, and about 9 of the alanine codons are GCG;

wherein about 23 of the 52 tyrosine codons in said coding region are TAT and about 29 of the tyrosine codons are TAC;

wherein about 6 of the 14 histidine codons in said coding region are CAT and about 8 of the histidine codons are CAC;

wherein about 14 of the 55 glutamine codons in said coding region are CAA and about 41 of the glutamine codons are CAG;

wherein about 37 of the 81 asparagine codons in said coding region are AAT and about 44 of the asparagine codons are AAC;

wherein about 24 of the 57 lysine codons in said coding region are AAA and about 33 of the lysine codons are AAG;

wherein about 33 of the 71 aspartic acid codons in said coding region are GAT and about 38 of the aspartic acid codons are GAC;

wherein about 17 of the 40 glutamic acid codons in said coding region are GAA and about 23 of the glutamic acid codons are GAG;

wherein about 15 of the 33 cysteine codons in said coding region are TGT and about 18 of the cysteine codons are TGC;

wherein about 10 tryptophan codons in said coding region are TGG;

wherein about 3 of the 41 arginine codons in said coding region are CGT, about 8 of the arginine codons are CGC, about 5 of the arginine codons are CGA, about 8 of the arginine codons are CGG, about 9 of the arginine codons are AGA, and about 8 of the arginine codons are AGG; and

wherein about 13 of the 77 glycine codons in said coding region are GGT, about 26 of the glycine codons are GGC, about 19 of the glycine codons are GGA, and about 19 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

188. The polynucleotide of claim 187, wherein said codon optimized coding region comprises SEQ ID NO:30.

189. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:8, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:8;

wherein the 79 phenylalanine codons in said coding region are TTC, the 92 leucine codons in said coding region are CTG, the 73 isoleucine codons in said coding region are ATC, the 19 methionine codons in said coding region are ATG, the 89 valine codons in said coding region are GTG, the 93 serine codons in said coding region are AGC, the 57 proline codons in said coding region are CCC, the 94 threonine codons in said coding region are ACC, the 84 alanine codons in said coding region are GCC, the 52 tyrosine codons in said coding region are TAC, the 14 histidine codons in said coding region are CAC, the 55 glutamine codons in said coding region are CAG, the 81 asparagine codons in said coding region are AAC, the 57 lysine codons in said coding region are AAG, the 71 aspartic acid codons in said coding region are GAC, the 40 glutamic acid codons in said coding region are GAG, the 33 cysteine codons in said coding region are TGC, the 10 tryptophan codons in said coding region is TGG, the 41 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 77 glycine codons in said coding region are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

190. The polynucleotide of claim 189, wherein said codon optimized coding region comprises SEQ ID NO:31.

191. The polynucleotide of any one of claims 187-190, wherein said nucleic acid fragment encodes at least 50 contiguous amino acids of SEQ ID NO:8.

192. The polynucleotide of any one of claims 191, wherein said nucleic acid fragment encodes at least 100 contiguous amino acids of SEQ ID NO:8.

193. The polynucleotide of claim 192, wherein said nucleic acid fragment encodes SEQ ID NO:8.

194. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO: 8, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:8;

wherein about 36 of the 79 phenylalanine codons in said coding region are TTT, and about 43 of the phenylalanine codons are TTC;

wherein about 7 of the 92 leucine codons in said coding region are TTA, about 12 of the leucine codons are TTG, about 12 of the leucine codons are CTT, about 18 of the leucine codons are CTC, about 7 of the leucine codons are CTA, and about 36 of the leucine codons are CTG;

wherein about 26 of the 73 isoleucine codons in said coding region are ATT, about 35 of the isoleucine codons are ATC, and about 12 of the isoleucine codons are ATA;

wherein about 19 methionine codons in said coding region are ATG;

wherein about 16 of the 89 valine codons in said coding region are GTT, about 41 of the valine codons are GTG, about 11 of the valine codons are GTA, and about 21 of the valine codons are GTC;

wherein about 17 of the 91 serine codons in said coding region are TCT, about 20 of the serine codons are TCC, about 14 of the serine codons are TCA, about 5 of the serine codons are TCG, about 15 of the serine codons are AGT, and about 22 of the serine codons are AGC;

wherein about 16 of the 57 proline codons in said coding region are CCT, about 19 of the proline codons are CCC, about 16 of the proline codons are CCA, and about 6 of the proline codons are CCG;

wherein about 23 of the 94 threonine codons in said coding region are ACT, about 34 of the threonine codons are ACC, about 26 of the threonine codons are ACA, and about 11 of the threonine codons are ACG;

wherein about 22 of the 84 alanine codons in said coding region are GCT, about 34 of the alanine codons are GCC, about 19 of the alanine codons are GCA, and about 9 of the alanine codons are GCG;

wherein about 23 of the 52 tyrosine codons in said coding region are TAT and about 29 of the tyrosine codons are TAC;

wherein about 6 of the 14 histidine codons in said coding region are CAT and about 8 of the histidine codons are CAC;

wherein about 14 of the 55 glutamine codons in said coding region are CAA and about 41 of the glutamine codons are CAG;

wherein about 37 of the 81 asparagine codons in said coding region are AAT and about 44 of the asparagine codons are AAC;

wherein about 24 of the 57 lysine codons in said coding region are AAA and about 33 of the lysine codons are AAG;

wherein about 33 of the 71 aspartic acid codons in said coding region are GAT and about 38 of the aspartic acid codons are GAC;

wherein about 17 of the 40 glutamic acid codons in said coding region are GAA and about 23 of the glutamic acid codons are GAG;

wherein about 15 of the 33 cysteine codons in said coding region are TGT and about 18 of the cysteine codons are TGC;

wherein about 10 tryptophan codons in said coding region are TGG;

wherein about 3 of the 41 arginine codons in said coding region are CGT, about 8 of the arginine codons are CGC, about 5 of the arginine codons are CGA, about 8 of the arginine codons are CGG, about 9 of the arginine codons are AGA, and about 8 of the arginine codons are AGG; and

wherein about 13 of the 77 glycine codons in said coding region are GGT, about 26 of the glycine codons are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

195. The polynucleotide of claim 194, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO:8.

196. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO:8, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:8;

wherein the 79 phenylalanine codons in said coding region are TTC, the 92 leucine codons in said coding region are CTG, the 73 isoleucine codons in said coding region are ATC, the 19 methionine codons in said coding region are ATG, the 89 valine codons in said coding region are GTG, the 93 serine codons in said coding region are AGC, the 57 proline codons in said coding region are CCC, the 94 threonine codons in said coding region are ACC, the 84 alanine codons in said coding region are GCC, the 52 tyrosine codons in said coding region are TAC, the 14 histidine codons in said coding region are CAC, the 55 glutamine codons in said coding region are CAG, the 81 asparagine codons in said coding region are AAC, the 57 lysine codons in said coding region are AAG, the 71 aspartic acid codons in said coding region are GAC, the 40 glutamic acid codons in said coding region are GAG, the 33 cysteine codons in said coding region are TGC, the 10 tryptophan codons in said coding region is TGG, the 41 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 77 glycine codons in said coding region are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

197. The polynucleotide of claim 196, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO: 8.

198. The polynucleotide of claim 197, wherein said nucleic acid fragment encodes SEQ ID NO: 8.

199. The polynucleotide of any one of claims 187 to 198, wherein said nucleic acid fragment is ligated to a heterologous nucleic acid.

200. The polynucleotide of claim 199, wherein said heterologous nucleic acid encodes a heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment.

201. The polynucleotide of claim 200, wherein said heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment comprises a small self-assembly polypeptide, wherein said heterologous polypeptide self-assemble into multimers.

202. The polynucleotide of any one of claim 187 to 199, wherein said nucleic acid fragment is ligated to a nucleic acid operatively encoding for the secretion of said polypeptide.

203. The polynucleotide of any one of claims 187 to 202, which is DNA, and wherein said nucleic acid fragment is operably associated with a promoter.

204. The polynucleotide of any one of claims 187 to 202, which is RNA.

205. The polynucleotide of claim 204, which is messenger RNA (mRNA).

206. A vector comprising the polynucleotide of any one of claims 187 to 204.

207. The vector of claim 206, which is a plasmid.

208. A composition comprising the polynucleotide of any one of claims 187 to 205, and a carrier.

209. The composition of claim 208, further comprising a component selected from the group consisting of an adjuvant, and a transfection facilitating compound.

210. The composition of claim 209, wherein said adjuvant is selected from the group consisting of:

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) and a neutral lipid;

a cytokine;

mono-phosphoryl lipid A and trehalosedicorynomycolateAF (MPL + TDM);

a solubilized mono-phosphoryl lipid A formulation; and

CRL1005/BAK.

211. The composition of claim 210, wherein said adjuvant comprises(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-

tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE), and wherein said neutral lipid is selected from the group consisting of:

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE),

1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and

1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE).

212. The composition of claim 211, wherein said neutral lipid is DPyPE.

213. The composition of claim 209, comprising the transfection facilitating compound (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) (DMRIE).

214. The composition of claim 209, wherein said adjuvant or said transfection facilitating compound comprises CRL1005 and benzalkonium chloride (BAK).

215. The composition of claim 214, wherein said CRL1005 and BAK are present at concentrations selected from the group consisting of:

0.3 mM BAK and 7.5 mg/ml CRL 1005,

0.3 mM BAK and 34 mg/ml CRL 1005, and

0.3 mM BAK and 50 mg/ml CRL 1005.

216. The composition of claim 214 or claim 215, which is formulated by combining together the CRL1005, BAK, and polynucleotide at a temperature below the cloud point, without thermal cycling to a temperature above the cloud point..

217. A method to treat or prevent SARS-CoV infection in a vertebrate comprising: administering to a vertebrate in need thereof the

polynucleotide of any one of claims 187 to 205 or the composition of any one of claims 208 to 216.

218. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:10, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:10;

wherein about 23 of the 51 phenylalanine codons in said coding region are TTT, and about 28 of the phenylalanine codons are TTC;

wherein about 3 of the 46 leucine codons in said coding region are TTA, about 6 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 9 of the leucine codons are CTC, about 4 of the leucine codons are CTA, and about 18 of the leucine codons are CTG;

wherein about 13 of the 37 isoleucine codons in said coding region are ATT, about 18 of the isoleucine codons are ATC, and about 6 of the isoleucine codons are ATA;

wherein about 9 methionine codons in said coding region are ATG;

wherein about 10 of the 56 valine codons in said coding region are GTT, about 26 of the valine codons are GTG, about 7 of the valine codons are GTA, and about 13 of the valine codons are GTC;

wherein about 11 of the 58 serine codons in said coding region are TCT, about 13 of the serine codons are TCC, about 9 of the serine codons are TCA, about 3 of the serine codons are TCG, about 8 of the serine codons are AGT, and about 14 of the serine codons are AGC;

wherein about 11 of the 38 proline codons in said coding region are CCT, about 13 of the proline codons are CCC, about 10 of the proline codons are CCA, and about 4 of the proline codons are CCG;

wherein about 14 of the 56 threonine codons in said coding region are ACT, about 20 of the threonine codons are ACC, about 16 of the threonine codons are ACA, and about 6 of the threonine codons are ACG;

wherein about 11 of the 41 alanine codons in said coding region are GCT, about 16 of the alanine codons are GCC, about 10 of the alanine codons are GCA, and about 4 of the alanine codons are GCG;

wherein about 15 of the 35 tyrosine codons in said coding region are TAT and about 20 of the tyrosine codons are TAC;

wherein about 4 of the 9 histidine codons in said coding region are CAT and about 5 of the histidine codons are CAC;

wherein about 5 of the 21 glutamine codons in said coding region are CAA and about 16 of the glutamine codons are CAG;

wherein about 21 of the 46 asparagine codons in said coding region are AAT and about 25 of the asparagine codons are AAC;

wherein about 14 of the 32 lysine codons in said coding region are AAA and about 18 of the lysine codons are AAG;

wherein about 21 of the 45 aspartic acid codons in said coding region are GAT and about 24 of the aspartic acid codons are GAC;

wherein about 7 of the 17 glutamic acid codons in said coding region are GAA and about 10 of the glutamic acid codons are GAG;

wherein about 10 of the 23 cysteine codons in said coding region are TGT and about 13 of the cysteine codons are TGC;

wherein about 6 tryptophan codons in said coding region are TGG;

wherein about 2 of the 25 arginine codons in said coding region are CGT, about 5 of the arginine codons are CGC, about 3 of the arginine codons are CGA, about 5 of the arginine codons are CGG, about 5 of the arginine codons are AGA, and about 5 of the arginine codons are AGG;

wherein and about 8 of the 47 glycine codons in said coding region are GGT, about 16 of the glycine codons are GGC, about 11 of the glycine codons are GGA, and about 12 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

219. The polynucleotide of claim 218, wherein said codon optimized coding region comprises SEQ ID NO: 32.

220. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:10, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:10;

wherein the 51 phenylalanine codons in said coding region are TTC, the 46 leucine codons in said coding region are CTG, the 37 isoleucine codons in said coding region are ATC, the 9 methionine codons in said coding region are ATG, the 56 valine codons in said coding region are GTG, the 58 serine codons in said coding region are AGC, the 38 proline codons in said coding region are CCC, the 56 threonine codons in said coding region are ACC, the 41 alanine codons in said coding region are GCC, the 35 tyrosine codons in said coding region are TAC, the 9 histidine codons in said coding region are CAC, the 21 glutamine codons in said coding region are CAG, the 46 asparagine codons in said coding region are AAC, the 32 lysine codons in said coding region are AAG, the 45 aspartic acid codons in said coding region are GAC, the 17 glutamic acid codons in said coding region are GAG, the 23 cysteine codons in said coding region are TGC, the 6 tryptophan codons in said coding region are TGG, the 25 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 47 glycine codons in said coding region are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

221. The polynucleotide of claim 220, wherein said codon optimized coding region comprises SEQ ID NO:33.

222. The polynucleotide of any one of claims 218 to 221, wherein said nucleic acid fragment encodes at least 50 contiguous amino acids of SEQ ID NO:10.

223. The polynucleotide of claim 222, wherein said nucleic acid fragment encodes at least 100 contiguous amino acids of SEQ ID NO:10.

224. The polynucleotide of claim 223, wherein said nucleic acid fragment encodes SEQ ID NO:8.

225. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO: 10, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:10;

wherein about 23 of the 51 phenylalanine codons in said coding region are TTT, and about 28 of the phenylalanine codons are TTC;

wherein about 3 of the 46 leucine codons in said coding region are TTA, about 6 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 9 of the leucine codons are CTC, about 4 of the leucine codons are CTA, and about 18 of the leucine codons are CTG;

wherein about 13 of the 37 isoleucine codons in said coding region are ATT, about 18 of the isoleucine codons are ATC, and about 6 of the isoleucine codons are ATA;

wherein about 9 methionine codons in said coding region are ATG;

wherein about 10 of the 56 valine codons in said coding region are GTT, about 26 of the valine codons are GTG, about 7 of the valine codons are GTA, and about 13 of the valine codons are GTC;

wherein about 11 of the 58 serine codons in said coding region are TCT, about 13 of the serine codons are TCC, about 9 of the serine codons

are TCA, about 3 of the serine codons are TCG, about 8 of the serine codons are AGT, and about 14 of the serine codons are AGC;

wherein about 11 of the 38 proline codons in said coding region are CCT, about 13 of the proline codons are CCC, about 10 of the proline codons are CCA, and about 4 of the proline codons are CCG;

wherein about 14 of the 56 threonine codons in said coding region are ACT, about 20 of the threonine codons are ACC, about 16 of the threonine codons are ACA, and about 6 of the threonine codons are ACG;

wherein about 11 of the 41 alanine codons in said coding region are GCT, about 16 of the alanine codons are GCC, about 10 of the alanine codons are GCA, and about 4 of the alanine codons are GCG;

wherein about 15 of the 35 tyrosine codons in said coding region are TAT and about 20 of the tyrosine codons are TAC;

wherein about 4 of the 9 histidine codons in said coding region are CAT and about 5 of the histidine codons are CAC;

wherein about 5 of the 21 glutamine codons in said coding region are CAA and about 16 of the glutamine codons are CAG;

wherein about 21 of the 46 asparagine codons in said coding region are AAT and about 25 of the asparagine codons are AAC;

wherein about 14 of the 32 lysine codons in said coding region are AAA and about 18 of the lysine codons are AAG;

wherein about 21 of the 45 aspartic acid codons in said coding region are GAT and about 24 of the aspartic acid codons are GAC;

wherein about 7 of the 17 glutamic acid codons in said coding region are GAA and about 10 of the glutamic acid codons are GAG;

wherein about 10 of the 23 cysteine codons in said coding region are TGT and about 13 of the cysteine codons are TGC;

wherein about 6 tryptophan codons in said coding region are TGG;

wherein about 2 of the 25 arginine codons in said coding region are CGT, about 5 of the arginine codons are CGC, about 3 of the arginine

codons are CGA, about 5 of the arginine codons are CGG, about 5 of the arginine codons are AGA, and about 5 of the arginine codons are AGG;

wherein and about 8 of the 47 glycine codons in said coding region are GGT, about 16 of the glycine codons are GGC, about 11 of the glycine codons are GGA, and about 12 of the glycine codons are GGG; and;

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

226. The polynucleotide of claim 225, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO:10.

227. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO:10, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:10;

wherein the 51 phenylalanine codons in said coding region are TTC, the 46 leucine codons in said coding region are CTG, the 37 isoleucine codons in said coding region are ATC, the 9 methionine codons in said coding region are ATG, the 56 valine codons in said coding region are GTG, the 58 serine codons in said coding region are AGC, the 38 proline codons in said coding region are CCC, the 56 threonine codons in said coding region are ACC, the 41 alanine codons in said coding region are GCC, the 35 tyrosine codons in said coding region are TAC, the 9 histidine codons in said coding region are CAC, the 21 glutamine codons in said coding region are CAG, the 46 asparagine codons in said coding region are AAC, the 32 lysine codons in said coding region are AAG, the 45 aspartic acid codons in said coding region are GAC, the 17 glutamic acid codons in said coding region are GAG, the 23 cysteine codons in said coding region are TGC, the 6 tryptophan codons in said coding region are TGG, the 25 arginine codons in said coding region are

selected from the group consisting of CGG, AGA, or AGG, and the 47 glycine codons in said coding region are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

228. The polynucleotide of claim 227, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO: 10.

229. The polynucleotide of claim 228, wherein said nucleic acid fragment encodes SEQ ID NO: 10.

230. The polynucleotide of any one of claims 218 to 229, wherein said nucleic acid fragment is ligated to a heterologous nucleic acid.

231. The polynucleotide of claim 230, wherein said heterologous nucleic acid encodes a heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment.

232. The polynucleotide of claim 231, wherein said heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment comprises a small self-assembly polypeptide, wherein said heterologous polypeptide self-assemble into multimers

233. The polynucleotide of any one of claim 218 to 229, wherein said nucleic acid fragment is ligated to a nucleic acid operatively encoding for the secretion of said polypeptide.

234. The polynucleotide of any one of claims 218 to 233, which is DNA, and wherein said nucleic acid fragment is operably associated with a promoter.

235. The polynucleotide of any one of claims 218 to 233, which is RNA.

236. The polynucleotide of claim 235, which is messenger RNA (mRNA).

237. A vector comprising the polynucleotide of any one of claims 218 to 235.

238. The vector of claim 237, which is a plasmid.

239. A composition comprising the polynucleotide of any one of claims 218 to 236, and a carrier.

240. The composition of claim 239, further comprising a component selected from the group consisting of an adjuvant, and a transfection facilitating compound.

241. The composition of claim 240, wherein said adjuvant is selected from the group consisting of:

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) and a neutral lipid;

a cytokine;

mono-phosphoryl lipid A and trehalosedicorynomycolateAF (MPL + TDM);

a solubilized mono-phosphoryl lipid A formulation; and

CRL1005/BAK.

242. The composition of claim 241, wherein said adjuvant comprises(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE), and wherein said neutral lipid is selected from the group consisting of:

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE),

1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and

1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE).

243. The composition of claim 242, wherein said neutral lipid is DPyPE.

244. The composition of claim 240, comprising the transfection facilitating compound (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) (DMRIE).

245. The composition of claim 240, wherein said adjuvant or said transfection facilitating compound comprises CRL1005 and benzalkonium chloride (BAK).

246. The composition of claim 245, wherein said CRL1005 and BAK are present at concentrations selected from the group consisting of:

0.3 mM BAK and 7.5 mg/ml CRL 1005,

0.3 mM BAK and 34 mg/ml CRL 1005, and

0.3 mM BAK and 50 mg/ml CRL 1005.

247. The composition of claim 245 or claim 246, which is formulated by combining together the CRL1005, BAK, and polynucleotide at a temperature below the cloud point, without thermal cycling to a temperature above the cloud point..

248. A method to treat or prevent SARS-CoV infection in a vertebrate comprising: administering to a vertebrate in need thereof the polynucleotide of any one of claims 218 to 236 or the composition of any one of claims 239 to 247.

249. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:12, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:12;

wherein about 13 of the 29 phenylalanine codons in said coding region are TTT, and about 16 of the phenylalanine codons are TTC;

wherein about 4 of the 50 leucine codons are TTA, in said coding region about 6 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 10 of the leucine codons are CTC, about 4 of the leucine codons are CTA, and about 20 of the leucine codons are CTG;

wherein about 13 of the 36 isoleucine codons in said coding region are ATT, about 17 of the isoleucine codons are ATC, and about 6 of the isoleucine codons are ATA;

wherein about 12 methionine codons in said coding region are ATG;

wherein about 6 of the 36 valine codons in said coding region are GTT, about 9 of the valine codons are GTG, about 4 of the valine codons are GTA, and about 17 of the valine codons are GTG;

wherein about 7 of the 38 serine codons in said coding region are TCT, about 8 of the serine codons are TCC, about 6 of the serine codons are TCA, about 2 of the serine codons are TCG, about 6 of the serine codons are AGT, and about 9 of the serine codons are AGC;

wherein about 6 of the 20 proline codons in said coding region are CCT, about 7 of the proline codons are CCC, about 5 of the proline codons are CCA, and about 2 of the proline codons are CCG;

wherein about 9 of the 38 threonine codons in said coding region are ACT, about 14 of the threonine codons are ACC, about 11 of the threonine codons are ACA, and about 4 of the threonine codons are ACG;

wherein about 12 of the 46 alanine codons in said coding region are GCT, about 19 of the alanine codons are GCC, about 10 of the alanine codons are GCA, and about 5 of the alanine codons are GCG;

wherein about 7 of the 17 tyrosine codons in said coding region are TAT and about 10 of the tyrosine codons are TAC;

wherein about 2 of the 5 histidine codons in said coding region are CAT and about 3 of the histidine codons are CAC;

wherein about 9 of the 34 glutamine codons in said coding region are CAA and about 25 of the glutamine codons are CAG;

wherein about 16 of the 35 asparagine codons in said coding region are AAT and about 19 of the asparagine codons are AAC;

wherein about 11 of the 26 lysine codons in said coding region are AAA and about 15 of the lysine codons are AAG;

wherein about 12 of the 27 aspartic acid codons in said coding region are GAT and about 15 of the aspartic acid codons are GAC;

wherein about 16 of the 23 glutamic acid codons in said coding region are GAA and about 13 of the glutamic acid codons are GAG;

wherein about 6 of the 13 cysteine codons in said coding region are TGT and about 7 of the cysteine codons are TGC;

wherein about 4 tryptophan codons in said coding region are TGG;

wherein about 1 of the 18 arginine codons in said coding region are CGT;

wherein about 3 of the arginine codons in said coding region are CGC, about 2 of the arginine codons are CGA, about 4 of the arginine codons are CGG, about 4 of the arginine codons are AGA, and about 4 of the arginine codons are AGG;

wherein and about 6 of the 34 glycine codons in said coding region are GGT, about 12 of the glycine codons are GGC, about 8 of the glycine codons are GGA, and about 8 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

250. The polynucleotide of claim 249, wherein said codon optimized coding region comprises SEQ ID NO:34.

251. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:12, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:12;

wherein the 29 phenylalanine codons in said coding region are TTC, the 50 leucine codons in said coding region are CTG, the 36 isoleucine codons in said coding region are ATC, the 12 methionine codons in said coding region are ATG, the 36 valine codons in said coding region are GTG, the 38 serine codons in said coding region are AGC, the 20 proline codons in said coding region are CCC, the 38 threonine codons in said coding region are ACC, the 46 alanine codons in said coding region are GCC, the 17 tyrosine codons in said coding region are TAC, the 5 histidine codons in said coding region are CAC, the 34 glutamine codons in said coding region are CAG, the 35 asparagine codons in said coding region are AAC, the 26 lysine codons in said coding region are AAG, the 35 aspartic acid codons in said coding region are GAC, the 23 glutamic acid codons in said coding region are GAG, the 13 cysteine codons in said coding region are TGC, the 4 tryptophan codons in said coding region are TGG, the 18 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 34 glycine codons are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

252. The polynucleotide of claim 251, wherein said codon optimized coding region comprises SEQ ID NO:35.

253. The polynucleotide of any one of claims 249 to 252, wherein said nucleic acid fragment encodes at least 50 contiguous amino acids of SEQ ID NO:12.

254. The polynucleotide of claim 253, wherein said nucleic acid fragment encodes at least 100 contiguous amino acids of SEQ ID NO:12.

255. The polynucleotide of claim 254, wherein said nucleic acid fragment encodes SEQ ID NO:12.

256. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO: 12, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:12;

wherein about 13 of the 29 phenylalanine codons in said coding region are TTT, and about 16 of the phenylalanine codons are TTC;

wherein about 4 of the 50 leucine codons are TTA, in said coding region about 6 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 10 of the leucine codons are CTC, about 4 of the leucine codons are CTA, and about 20 of the leucine codons are CTG;

wherein about 13 of the 36 isoleucine codons in said coding region are ATT, about 17 of the isoleucine codons are ATC, and about 6 of the isoleucine codons are ATA;

wherein about 12 methionine codons in said coding region are ATG;

wherein about 6 of the 36 valine codons in said coding region are GTT, about 9 of the valine codons are GTG, about 4 of the valine codons are GTA, and about 17 of the valine codons are GTG;

wherein about 7 of the 38 serine codons in said coding region are TCT, about 8 of the serine codons are TCC, about 6 of the serine codons are TCA, about 2 of the serine codons are TCG, about 6 of the serine codons are AGT, and about 9 of the serine codons are AGC;

wherein about 6 of the 20 proline codons in said coding region are CCT, about 7 of the proline codons are CCC, about 5 of the proline codons are CCA, and about 2 of the proline codons are CCG;

wherein about 9 of the 38 threonine codons in said coding region are ACT, about 14 of the threonine codons are ACC, about 11 of the threonine codons are ACA, and about 4 of the threonine codons are ACG;

wherein about 12 of the 46 alanine codons in said coding region are GCT, about 19 of the alanine codons are GCC, about 10 of the alanine codons are GCA, and about 5 of the alanine codons are GCG;

wherein about 7 of the 17 tyrosine codons in said coding region are TAT and about 10 of the tyrosine codons are TAC;

wherein about 2 of the 5 histidine codons in said coding region are CAT and about 3 of the histidine codons are CAC;

wherein about 9 of the 34 glutamine codons in said coding region are CAA and about 25 of the glutamine codons are CAG;

wherein about 16 of the 35 asparagine codons in said coding region are AAT and about 19 of the asparagine codons are AAC;

wherein about 11 of the 26 lysine codons in said coding region are AAA and about 15 of the lysine codons are AAG;

wherein about 12 of the 27 aspartic acid codons in said coding region are GAT and about 15 of the aspartic acid codons are GAC;

wherein about 16 of the 23 glutamic acid codons in said coding region are GAA and about 13 of the glutamic acid codons are GAG;

wherein about 6 of the 13 cysteine codons in said coding region are TGT and about 7 of the cysteine codons are TGC;

wherein about 4 tryptophan codons in said coding region are TGG;

wherein about 1 of the 18 arginine codons in said coding region are CGT;

wherein about 3 of the arginine codons in said coding region are CGC, about 2 of the arginine codons are CGA, about 4 of the arginine codons are CGG, about 4 of the arginine codons are AGA, and about 4 of the arginine codons are AGG;

wherein and about 6 of the 34 glycine codons in said coding region are GGT, about 12 of the glycine codons are GGC, about 8 of the glycine codons are GGA, and about 8 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

257. The polynucleotide of claim 256, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO:12.

258. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO:12, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:12;

wherein the 29 phenylalanine codons in said coding region are TTC, the 50 leucine codons in said coding region are CTG, the 36 isoleucine codons in said coding region are ATC, the 12 methionine codons in said coding region are ATG, the 36 valine codons in said coding region are GTG, the 38 serine codons in said coding region are AGC, the 20 proline codons in

said coding region are CCC, the 38 threonine codons in said coding region are ACC, the 46 alanine codons in said coding region are GCC, the 17 tyrosine codons in said coding region are TAC, the 5 histidine codons in said coding region are CAC, the 34 glutamine codons in said coding region are CAG, the 35 asparagine codons in said coding region are AAC, the 26 lysine codons in said coding region are AAG, the 35 aspartic acid codons in said coding region are GAC, the 23 glutamic acid codons in said coding region are GAG, the 13 cysteine codons in said coding region are TGC, the 4 tryptophan codons in said coding region are TGG, the 18 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 34 glycine codons are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

259. The polynucleotide of claim 258, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO: 12.

260. The polynucleotide of claim 259, wherein said nucleic acid fragment encodes SEQ ID NO: 12.

261. The polynucleotide of any one of claims 249 to 260, wherein said nucleic acid fragment is ligated to a heterologous nucleic acid.

262. The polynucleotide of claim 261, wherein said heterologous nucleic acid encodes a heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment.

263. The polynucleotide of claim 262, wherein said heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment

comprises a small self-assembly polypeptide, wherein said heterologous polypeptide self-assemble into multimers.

264. The polynucleotide of any one of claim 249 to 262, wherein said nucleic acid fragment is ligated to a nucleic acid operatively encoding for the secretion of said polypeptide.

265. The polynucleotide of any one of claims 249 to 264, which is DNA, and wherein said nucleic acid fragment is operably associated with a promoter.

266. The polynucleotide of any one of claims 249 to 264, which is RNA.

267. The polynucleotide of claim 266, which is messenger RNA (mRNA).

268. A vector comprising the polynucleotide of any one of claims 249 to 266.

269. The vector of claim 268, which is a plasmid.

270. A composition comprising the polynucleotide of any one of claims 249 to 267, and a carrier.

271. The composition of claim 270, further comprising a component selected from the group consisting of an adjuvant, and a transfection facilitating compound.

272. The composition of claim 271, wherein said adjuvant is selected from the group consisting of:

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) and a neutral lipid;

a cytokine;

mono-phosphoryl lipid A and trehalosedicorynomycolateAF (MPL + TDM);

a solubilized mono-phosphoryl lipid A formulation; and

CRL1005/BAK.

273. The composition of claim 272, wherein said adjuvant comprises(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE), and wherein said neutral lipid is selected from the group consisting of:

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE),

1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and

1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE).

274. The composition of claim 273, wherein said neutral lipid is DPyPE.

275. The composition of claim 271, comprising the transfection facilitating compound (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) (DMRIE).

276. The composition of claim 271, wherein said adjuvant or said transfection facilitating compound comprises CRL1005 and benzalkonium chloride (BAK).

277. The composition of claim 276, wherein said CRL1005 and BAK are present at concentrations selected from the group consisting of:

0.3 mM BAK and 7.5 mg/ml CRL 1005,
0.3 mM BAK and 34 mg/ml CRL 1005, and
0.3 mM BAK and 50 mg/ml CRL 1005.

278. The composition of claim 276 or claim 277, which is formulated by combining together the CRL1005, BAK, and polynucleotide at a temperature below the cloud point, without thermal cycling to a temperature above the cloud point..

279. A method to treat or prevent SARS-CoV infection in a vertebrate comprising: administering to a vertebrate in need thereof the polynucleotide of any one of claims 249 to 267 or the composition of any one of claims 270 to 278.

280. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:14, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:14;

wherein about 4 of the 13 phenylalanine codons in said coding region are TTT, and about 9 of the phenylalanine codons are TTC;

wherein about 1 of the 26 leucine codons in said coding region are TTA, about 6 of the leucine codons are TTG, about 7 of the leucine codons are CTT, about 3 of the leucine codons are CTC, about 5 of the leucine codons are CTA, and about 4 of the leucine codons are CTG;

wherein about 7 of the 11 isoleucine codons in said coding region are ATT, about 3 of the isoleucine codons are ATC, and about 1 of the isoleucine codons are ATA;

wherein the 7 methionine codons in said coding region are ATG;

wherein about 4 of the 11 valine codons in said coding region are GTT, about 4 of the valine codons are GTC, about 1 of the valine codons is GTA, and about 2 of the valine codons are GTG;

wherein about 10 of the 35 serine codons in said coding region are TCT, about 3 of the serine codons are TCC, about 9 of the serine codons are TCA, about 1 of the serine codons is TCG, about 7 of the serine codons are AGT, and about 5 of the serine codons are AGC;

wherein about 10 of the 31 proline codons in said coding region are CCT, about 9 of the proline codons are CCC, about 10 of the proline codons are CCA, and about 2 of the proline codons are CCG;

wherein about 17 of the 33 threonine codons in said coding region are ACT, about 5 of the threonine codons are ACC, about 11 of the threonine codons are ACA, and about 0 of the threonine codons is ACG;

wherein about 14 of the 34 alanine codons in said coding region are GCT, about 8 of the alanine codons are GCC, about 9 of the alanine codons are GCA, and about 3 of the alanine codons are GCG;

wherein about 2 of the 11 tyrosine codons in said coding region are TAT and about 9 of the tyrosine codons are TAC;

wherein about 3 of the 5 histidine codons in said coding region are CAT and about 2 of the histidine codons are CAC;

wherein about 24 of the 34 glutamine codons in said coding region are CAA and about 10 of the glutamine codons are CAG;

wherein about 16 of the 25 asparagine codons in said coding region are AAT and about 9 of the asparagine codons are AAC;

wherein about 20 of the 29 lysine codons in said coding region are AAA and about 9 of the lysine codons are AAG;

wherein about 10 of the 22 aspartic acid codons in said coding region are GAT and about 12 of the aspartic acid codons are GAC;

wherein about 7 of the 14 glutamic acid codons in said coding region are GAA and about 7 of the glutamic acid codons are GAG;

wherein about 5 tryptophan codons in said coding region are TGG;

wherein about 5 of the 31 arginine codons in said coding region are CGT, about 8 of the arginine codons are CGC, about 6 of the arginine codons are CGA, about 0 of the arginine codons are CGG, about 10 of the arginine codons are AGA, and about 2 of the arginine codons are AGG;

wherein and about 10 of the 45 glycine codons in said coding region are GGT, about 16 of the glycine codons are GGC, about 16 of the glycine codons are GGA, and about 3 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

281. The polynucleotide of claim 280, wherein said codon optimized coding region comprises SEQ ID NO:36.

282. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:14, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:14;

wherein the 13 phenylalanine codons in said coding region are TTC, the 26 leucine codons in said coding region are CTG, the 11 isoleucine codons in said coding region are ATC, the 7 methionine codons in said coding region are ATG, the 11 valine codons in said coding region are GTG, the 35 serine codons in said coding region are AGC, the 31 proline codons in said coding region are CCC, the 33 threonine codons in said coding region are ACC, the 34 alanine codons in said coding region are GCC, the 11 tyrosine codons in said coding region are TAC, the 5 histidine codons in said coding region are CAC, the 34 glutamine codons in said coding region are CAG, the 25 asparagine codons in said coding region are AAC, the 29 lysine codons in said coding region are AAG, the 22 aspartic acid codons in said coding region

are GAC, the 14 glutamic acid codons in said coding region are GAG, the 5 tryptophan codons in said coding region are TGG, the 31 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 45 glycine codons in said coding region are GGC.; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

283. The polynucleotide of claim 282, wherein said codon optimized coding region comprises SEQ ID NO:37.

284. The polynucleotide of any one of claims 280 to 283, wherein said nucleic acid fragment encodes at least 50 contiguous amino acids of SEQ ID NO:14.

285. The polynucleotide of claim 284, wherein said nucleic acid fragment encodes at least 100 contiguous amino acids of SEQ ID NO:14.

286. The polynucleotide of claim 285, wherein said nucleic acid fragment encodes SEQ ID NO:14.

287. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO: 14, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:14;

wherein about 4 of the 13 phenylalanine codons in said coding region are TTT, and about 9 of the phenylalanine codons are TTC;

wherein about 1 of the 26 leucine codons in said coding region are TTA, about 6 of the leucine codons are TTG, about 7 of the leucine codons are CTT, about 3 of the leucine codons are CTC, about 5 of the leucine codons are CTA, and about 4 of the leucine codons are CTG;

wherein about 7 of the 11 isoleucine codons in said coding region are ATT, about 3 of the isoleucine codons are ATC, and about 1 of the isoleucine codons are ATA;

wherein the 7 methionine codons in said coding region are ATG;

wherein about 4 of the 11 valine codons in said coding region are GTT, about 4 of the valine codons are GTC, about 1 of the valine codons is GTA, and about 2 of the valine codons are GTG;

wherein about 10 of the 35 serine codons in said coding region are TCT, about 3 of the serine codons are TCC, about 9 of the serine codons are TCA, about 1 of the serine codons is TCG, about 7 of the serine codons are AGT, and about 5 of the serine codons are AGC;

wherein about 10 of the 31 proline codons in said coding region are CCT, about 9 of the proline codons are CCC, about 10 of the proline codons are CCA, and about 2 of the proline codons are CCG;

wherein about 17 of the 33 threonine codons in said coding region are ACT, about 5 of the threonine codons are ACC, about 11 of the threonine codons are ACA, and about 0 of the threonine codons is ACG;

wherein about 14 of the 34 alanine codons in said coding region are GCT, about 8 of the alanine codons are GCC, about 9 of the alanine codons are GCA, and about 3 of the alanine codons are GCG;

wherein about 2 of the 11 tyrosine codons in said coding region are TAT and about 9 of the tyrosine codons are TAC;

wherein about 3 of the 5 histidine codons in said coding region are CAT and about 2 of the histidine codons are CAC;

wherein about 24 of the 34 glutamine codons in said coding region are CAA and about 10 of the glutamine codons are CAG;

wherein about 16 of the 25 asparagine codons in said coding region are AAT and about 9 of the asparagine codons are AAC;

wherein about 20 of the 29 lysine codons in said coding region are AAA and about 9 of the lysine codons are AAG;

wherein about 10 of the 22 aspartic acid codons in said coding region are GAT and about 12 of the aspartic acid codons are GAC;

wherein about 7 of the 14 glutamic acid codons in said coding region are GAA and about 7 of the glutamic acid codons are GAG;

wherein about 5 tryptophan codons in said coding region are TGG;

wherein about 5 of the 31 arginine codons in said coding region are CGT, about 8 of the arginine codons are CGC, about 6 of the arginine codons are CGA, about 0 of the arginine codons are CGG, about 10 of the arginine codons are AGA, and about 2 of the arginine codons are AGG;

wherein and about 10 of the 45 glycine codons in said coding region are GGT, about 16 of the glycine codons are GGC, about 16 of the glycine codons are GGA, and about 3 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

288. The polynucleotide of claim 287, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO:14.

289. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO:14, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:14;

wherein the 13 phenylalanine codons in said coding region are TTC, the 26 leucine codons in said coding region are CTG, the 11 isoleucine codons in said coding region are ATC, the 7 methionine codons in said coding region are ATG, the 11 valine codons in said coding region are GTG, the 35 serine codons in said coding region are AGC, the 31 proline codons in said coding region are CCC, the 33 threonine codons in said coding region are ACC, the 34 alanine codons in said coding region are GCC, the 11 tyrosine

codons in said coding region are TAC, the 5 histidine codons in said coding region are CAC, the 34 glutamine codons in said coding region are CAG, the 25 asparagine codons in said coding region are AAC, the 29 lysine codons in said coding region are AAG, the 22 aspartic acid codons in said coding region are GAC, the 14 glutamic acid codons in said coding region are GAG, the 5 tryptophan codons in said coding region are TGG, the 31 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 45 glycine codons in said coding region are GGC.; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

290. The polynucleotide of claim 289, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO: 12.

291. The polynucleotide of claim 290, wherein said nucleic acid fragment encodes SEQ ID NO: 14.

292. The polynucleotide of any one of claims 280 to 291, wherein said nucleic acid fragment is ligated to a heterologous nucleic acid.

293. The polynucleotide of claim 292, wherein said heterologous nucleic acid encodes a heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment.

294. The polynucleotide of claim 293, wherein said heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment comprises a small self-assembly polypeptide, wherein said heterologous polypeptide self-assemble into multimers.

295. The polynucleotide of any one of claim 280 to 292, wherein said nucleic acid fragment is ligated to a nucleic acid operatively encoding for the secretion of said polypeptide.

296. The polynucleotide of any one of claims 280 to 295, which is DNA, and wherein said nucleic acid fragment is operably associated with a promoter.

297. The polynucleotide of any one of claims 280 to 295, which is RNA.

298. The polynucleotide of claim 297, which is messenger RNA (mRNA).

299. A vector comprising the polynucleotide of any one of claims 280 to 297.

300. The vector of claim 299, which is a plasmid.

301. A composition comprising the polynucleotide of any one of claims 280 to 298, and a carrier.

302. The composition of claim 301, further comprising a component selected from the group consisting of an adjuvant, and a transfection facilitating compound.

303. The composition of claim 302, wherein said adjuvant is selected from the group consisting of:

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) and a neutral lipid;

a cytokine;

mono-phosphoryl lipid A and trehalosedicorynomycolateAF (MPL + TDM);

a solubilized mono-phosphoryl lipid A formulation; and

CRL1005/BAK.

304. The composition of claim 303, wherein said adjuvant comprises(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE), and wherein said neutral lipid is selected from the group consisting of:

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE),

1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and

1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE).

305. The composition of claim 304, wherein said neutral lipid is DPyPE.

306. The composition of claim 302, comprising the transfection facilitating compound (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) (DMRIE).

307. The composition of claim 302, wherein said adjuvant or said transfection facilitating compound comprises CRL1005 and benzalkonium chloride (BAK).

308. The composition of claim 307, wherein said CRL1005 and BAK are present at concentrations selected from the group consisting of:

0.3 mM BAK and 7.5 mg/ml CRL 1005,

0.3 mM BAK and 34 mg/ml CRL 1005, and

0.3 mM BAK and 50 mg/ml CRL 1005.

309. The composition of claim 307 or claim 308, which is formulated by combining together the CRL1005, BAK, and polynucleotide at a temperature below the cloud point, without thermal cycling to a temperature above the cloud point..

310. A method to treat or prevent SARS-CoV infection in a vertebrate comprising: administering to a vertebrate in need thereof the polynucleotide of any one of claims 280 to 298 or the composition of any one of claims 301to 309.

311. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:16, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:16;

wherein about 5 of the 12 phenylalanine codons in said coding region are TTT, and about 7 of the phenylalanine codons are TTC;

wherein about 3 of the 26 leucine codons in said coding region are TTA, about 3 of the leucine codons are TTG, about 3 of the leucine codons are CTT, about 5 of the leucine codons are CTC, about 2 of the leucine codons are CTA, and about 10 of the leucine codons are CTG;

wherein about 4 of the 11 isoleucine codons in said coding region are ATT, about 5 of the isoleucine codons are ATC, and about 2 of the isoleucine codons are ATA;

wherein about 7 methionine codons in said coding region are ATG;

wherein about 2 of the 11 valine codons in said coding region are GTT, about 3 of the valine codons are GTC, about 1 of the valine codons is GTA, and about 5 of the valine codons are GTG;

wherein about 6 of the 35 serine codons in said coding region are TCT, about 8 of the serine codons are TCC, about 5 of the serine codons

are TCA, about 2 of the serine codons are TCG, about 6 of the serine codons are AGT, and about 8 of the serine codons are AGC;

wherein about 8 of the 28 proline codons in said coding region are CCT, about 9 of the proline codons are CCC, about 8 of the proline codons are CCA, and about 3 of the proline codons are CCG;

wherein about 7 of the 30 threonine codons in said coding region are ACT, about 11 of the threonine codons are ACC, about 9 of the threonine codons are ACA, and about 3 of the threonine codons are ACG;

wherein about 9 of the 33 alanine codons in said coding region are GCT, about 13 of the alanine codons are GCC, about 7 of the alanine codons are GCA, and about 4 of the alanine codons are GCG;

wherein about 5 of the 11 tyrosine codons in said coding region are TAT and about 6 of the tyrosine codons are TAC;

wherein about 2 of the 5 histidine codons in said coding region are CAT and about 3 of the histidine codons are CAC;

wherein about 9 of the 33 glutamine codons in said coding region are CAA and about 24 of the glutamine codons are CAG;

wherein about 12 of the 25 asparagine codons in said coding region are AAT and about 13 of the asparagine codons are AAC;

wherein about 9 of the 22 lysine codons in said coding region are AAA and about 13 of the lysine codons are AAG;

wherein about 9 of the 20 aspartic acid codons in said coding region are GAT and about 11 of the aspartic acid codons are GAC;

wherein about 5 of the 12 glutamic acid codons in said coding region are GAA and about 7 of the glutamic acid codons are GAG;

wherein about 5 tryptophan codons in said coding region are TGG;

wherein about 3 of the 31 arginine codons in said coding region are CGT, about 6 of the arginine codons are CGC, about 3 of the arginine codons are CGA, about 6 of the arginine codons are CGG, about 7 of the arginine codons are AGA, and about 6 of the arginine codons are AGG;

wherein about 7 of the 45 glycine codons in said coding region are GGT, about 15 of the glycine codons are GGC, about 12 of the glycine codons are GGA, and about 11 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

312. The polynucleotide of claim 311, wherein said codon optimized coding region comprises SEQ ID NO:38.

313. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:16, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:16;

wherein the 12 phenylalanine codons in said coding region are TTC, the 26 leucine codons in said coding region are CTG, the 11 isoleucine codons in said coding region are ATC, the 7 methionine codons in said coding region are ATG, the 11 valine codons in said coding region are GTG, the 35 serine codons in said coding region are AGC, the 28 proline codons in said coding region are CCC, the 30 threonine codons in said coding region are ACC, the 33 alanine codons in said coding region are GCC, the 11 tyrosine codons in said coding region are TAC, the 5 histidine codons in said coding region are CAC, the 33 glutamine codons in said coding region are CAG, the 25 asparagine codons in said coding region are AAC, the 22 lysine codons in said coding region are AAG, the 20 aspartic acid codons in said coding region are GAC, the 12 glutamic acid codons in said coding region are GAG, the 5 tryptophan codons in said coding region are TGG, the 31 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 45 glycine codons in said coding region are GGC.; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

314. The polynucleotide of claim 313, wherein said codon optimized coding region comprises SEQ ID NO:39.

315. The polynucleotide of any one of claims 311 to 314, wherein said nucleic acid fragment encodes at least 50 contiguous amino acids of SEQ ID NO:16.

316. The polynucleotide of claim 315, wherein said nucleic acid fragment encodes at least 100 contiguous amino acids of SEQ ID NO:16.

317. The polynucleotide of claim 316, wherein said nucleic acid fragment encodes SEQ ID NO:16.

318. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO: 16, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:16;

wherein about 5 of the 12 phenylalanine codons in said coding region are TTT, and about 7 of the phenylalanine codons are TTC;

wherein about 3 of the 26 leucine codons in said coding region are TTA, about 3 of the leucine codons are TTG, about 3 of the leucine codons are CTT, about 5 of the leucine codons are CTC, about 2 of the leucine codons are CTA, and about 10 of the leucine codons are CTG;

wherein about 4 of the 11 isoleucine codons in said coding region are ATT, about 5 of the isoleucine codons are ATC, and about 2 of the isoleucine codons are ATA;

wherein about 7 methionine codons in said coding region are ATG;

wherein about 2 of the 11 valine codons in said coding region are GTT, about 3 of the valine codons are GTC, about 1 of the valine codons is GTA, and about 5 of the valine codons are GTG;

wherein about 6 of the 35 serine codons in said coding region are TCT, about 8 of the serine codons are TCC, about 5 of the serine codons are TCA, about 2 of the serine codons are TCG, about 6 of the serine codons are AGT, and about 8 of the serine codons are AGC;

wherein about 8 of the 28 proline codons in said coding region are CCT, about 9 of the proline codons are CCC, about 8 of the proline codons are CCA, and about 3 of the proline codons are CCG;

wherein about 7 of the 30 threonine codons in said coding region are ACT, about 11 of the threonine codons are ACC, about 9 of the threonine codons are ACA, and about 3 of the threonine codons are ACG;

wherein about 9 of the 33 alanine codons in said coding region are GCT, about 13 of the alanine codons are GCC, about 7 of the alanine codons are GCA, and about 4 of the alanine codons are GCG;

wherein about 5 of the 11 tyrosine codons in said coding region are TAT and about 6 of the tyrosine codons are TAC;

wherein about 2 of the 5 histidine codons in said coding region are CAT and about 3 of the histidine codons are CAC;

wherein about 9 of the 33 glutamine codons in said coding region are CAA and about 24 of the glutamine codons are CAG;

wherein about 12 of the 25 asparagine codons in said coding region are AAT and about 13 of the asparagine codons are AAC;

wherein about 9 of the 22 lysine codons in said coding region are AAA and about 13 of the lysine codons are AAG;

wherein about 9 of the 20 aspartic acid codons in said coding region are GAT and about 11 of the aspartic acid codons are GAC;

wherein about 5 of the 12 glutamic acid codons in said coding region are GAA and about 7 of the glutamic acid codons are GAG;

wherein about 5 tryptophan codons in said coding region are TGG;

wherein about 3 of the 31 arginine codons in said coding region are CGT, about 6 of the arginine codons are CGC, about 3 of the arginine codons are CGA, about 6 of the arginine codons are CGG, about 7 of the arginine codons are AGA, and about 6 of the arginine codons are AGG;

wherein about 7 of the 45 glycine codons in said coding region are GGT, about 15 of the glycine codons are GGC, about 12 of the glycine codons are GGA, and about 11 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

319. The polynucleotide of claim 318, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO:16.

320. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO:16, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:16;

wherein the 12 phenylalanine codons in said coding region are TTC, the 26 leucine codons in said coding region are CTG, the 11 isoleucine codons in said coding region are ATC, the 7 methionine codons in said coding region are ATG, the 11 valine codons in said coding region are GTG, the 35 serine codons in said coding region are AGC, the 28 proline codons in said coding region are CCC, the 30 threonine codons in said coding region are ACC, the 33 alanine codons in said coding region are GCC, the 11 tyrosine codons in said coding region are TAC, the 5 histidine codons in said coding region are CAC, the 33 glutamine codons in said coding region are CAG, the

25 asparagine codons in said coding region are AAC, the 22 lysine codons in said coding region are AAG, the 20 aspartic acid codons in said coding region are GAC, the 12 glutamic acid codons in said coding region are GAG, the 5 tryptophan codons in said coding region are TGG, the 31 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 45 glycine codons in said coding region are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

321. The polynucleotide of claim 320, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO: 16.

322. The polynucleotide of claim 321, wherein said nucleic acid fragment encodes SEQ ID NO: 16.

323. The polynucleotide of any one of claims 311 to 322, wherein said nucleic acid fragment is ligated to a heterologous nucleic acid.

324. The polynucleotide of claim 323, wherein said heterologous nucleic acid encodes a heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment.

325. The polynucleotide of claim 324, wherein said heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment comprises a small self-assembly polypeptide, wherein said heterologous polypeptide self-assemble into multimers.

326. The polynucleotide of any one of claim 311 to 323, wherein said nucleic acid fragment is ligated to a nucleic acid operatively encoding for the secretion of said polypeptide.

327. The polynucleotide of any one of claims 311 to 326, which is DNA, and wherein said nucleic acid fragment is operably associated with a promoter.

328. The polynucleotide of any one of claims 311 to 326, which is RNA.

329. The polynucleotide of claim 328, which is messenger RNA (mRNA).

330. A vector comprising the polynucleotide of any one of claims 311 to 328.

331. The vector of claim 330, which is a plasmid.

332. A composition comprising the polynucleotide of any one of claims 311 to 329, and a carrier.

333. The composition of claim 332, further comprising a component selected from the group consisting of an adjuvant, and a transfection facilitating compound.

334. The composition of claim 333, wherein said adjuvant is selected from the group consisting of:

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) and a neutral lipid;

a cytokine;

mono-phosphoryl lipid A and trehalosedicorynomycolateAF (MPL + TDM);

a solubilized mono-phosphoryl lipid A formulation; and

CRL1005/BAK.

335. The composition of claim 334, wherein said adjuvant comprises(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE), and wherein said neutral lipid is selected from the group consisting of:

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE),

1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and

1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE).

336. The composition of claim 335, wherein said neutral lipid is DPyPE.

337. The composition of claim 332, comprising the transfection facilitating compound (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) (DMRIE).

338. The composition of claim 332, wherein said adjuvant or said transfection facilitating compound comprises CRL1005 and benzalkonium chloride (BAK).

339. The composition of claim 338, wherein said CRL1005 and BAK are present at concentrations selected from the group consisting of:

0.3 mM BAK and 7.5 mg/ml CRL 1005,

0.3 mM BAK and 34 mg/ml CRL 1005, and

0.3 mM BAK and 50 mg/ml CRL 1005.

340. The composition of claim 338 or claim 339, which is formulated by combining together the CRL1005, BAK, and polynucleotide at

a temperature below the cloud point, without thermal cycling to a temperature above the cloud point..

341. A method to treat or prevent SARS-CoV infection in a vertebrate comprising: administering to a vertebrate in need thereof the polynucleotide of any one of claims 311 to 329 or the composition of any one of claims 332 to 341.

342. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:19, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:19;

wherein about 5 of the 11 phenylalanine codons in said coding region are TTT, and about 6 of the phenylalanine codons are TTC;

wherein about 3 of the 31 leucine codons in said coding region are TTA, about 4 of the leucine codons are TTG, about 4 of the leucine codons are CTT, about 6 of the leucine codons are CTC, about 2 of the leucine codons are CTA, and about 12 of the leucine codons are CTG;

wherein about 6 of the 18 isoleucine codons in said coding region are ATT, about 9 of the isoleucine codons are ATC, and about 3 of the isoleucine codons are ATA;

wherein about 7 methionine codons in said coding region are ATG; wherein about 3 of the 16 valine codons in said coding region are GTT, about 4 of the valine codons are GTC, about 2 of the valine codons are GTA, and about 7 of the valine codons are GTG;

wherein about 2 of the 11 serine codons in said coding region are TCT, about 2 of the serine codons are TCC, about 2 of the serine codons are TCA, about 1 of the serine codons is TCG, about 1 of the serine codons is AGT, and about 3 of the serine codons are AGC;

wherein about 2 of the 6 proline codons in said coding region are CCT, about 2 of the proline codons are CCC, about 1 of the proline codons is CCA, and about 1 of the proline codons is CCG;

wherein about 3 of the 13 threonine codons a in said coding region e ACT, about 5 of the threonine codons are ACC, about 4 of the threonine codons are ACA, and about 1 of the threonine codons is ACG;

wherein about 5 of the 19 alanine codons in said coding region are GCT, about 8 of the alanine codons are GCC, about 4 of the alanine codons are GCA, and about 2 of the alanine codons are GCG;

wherein about 4 of the 9 tyrosine codons in said coding region are TAT and about 5 of the tyrosine codons are TAC;

wherein about 1 of the 3 histidine codons in said coding region is CAT and about 2 of the histidine codons are CAC;

wherein about 1 of the 5 glutamine codons in said coding region is CAA and about 4 of the glutamine codons are CAG;

wherein about 6 of the 13 asparagine codons in said coding region are AAT and about 7 of the asparagine codons are AAC;

wherein about 3 of the 6 lysine codons in said coding region are AAA and about 3 of the lysine codons are AAG;

wherein about 3 of the 6 aspartic acid codons in said coding region are GAT and about 3 of the aspartic acid codons are GAC;

wherein about 3 of the 7 glutamic acid codons in said coding region are GAA and about 4 of the glutamic acid codons are GAG;

wherein about 1 of the 3 cysteine codons in said coding region is TGT and about 2 of the cysteine codons are TGC;

wherein about 7 tryptophan codons in said coding region are TGG;

wherein about 1 of the 15 arginine codons in said coding region is CGT, about 3 of the arginine codons are CGC, about 2 of the arginine codons are CGA, about 3 of the arginine codons are CGG, about 3 of the arginine codons are AGA, and about 3 of the arginine codons are AGG;

wherein and about 2 of the 15 glycine codons in said coding region are GGT, about 5 of the glycine codons are GGC, about 4 of the glycine codons are GGA, and about 4 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

343. The polynucleotide of claim 342, wherein said codon optimized coding region comprises SEQ ID NO:40.

344. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:19, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:19;

wherein the 11 phenylalanine codons in said coding region are TTC, the 31 leucine codons in said coding region are CTG, the 18 isoleucine codons in said coding region are ATC, the 7 methionine codons in said coding region are ATG, the 16 valine codons in said coding region are GTG, the 11 serine codons in said coding region are AGC, the 6 proline codons in said coding region are CCC, the 13 threonine codons in said coding region are ACC, the 19 alanine codons in said coding region are GCC, the 19 tyrosine codons in said coding region are TAC, the 3 histidine codons in said coding region are CAC, the 5 glutamine codons in said coding region are CAG, the 13 asparagine codons in said coding region are AAC, the 6 lysine codons in said coding region are AAG, the 6 aspartic acid codons in said coding region are GAC, the 7 glutamic acid codons in said coding region are GAG, the 3 cysteine codons in said coding region are TGC, the 7 tryptophan codons in said coding region are TGG, the 15 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 43 glycine codons are GGC.; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

345. The polynucleotide of claim 344, wherein said codon optimized coding region comprises SEQ ID NO:41.

346. The polynucleotide of any one of claims 342 to 345, wherein said nucleic acid fragment encodes at least 50 contiguous amino acids of SEQ ID NO:19.

347. The polynucleotide of claim 346, wherein said nucleic acid fragment encodes at least 100 contiguous amino acids of SEQ ID NO:19

348. The polynucleotide of claim 347, wherein said nucleic acid fragment encodes SEQ ID NO:19.

349. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO: 19, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:19;

wherein about 5 of the 11 phenylalanine codons in said coding region are TTT, and about 6 of the phenylalanine codons are TTC;

wherein about 3 of the 31 leucine codons in said coding region are TTA, about 4 of the leucine codons are TTG, about 4 of the leucine codons are CTT, about 6 of the leucine codons are CTC, about 2 of the leucine codons are CTA, and about 12 of the leucine codons are CTG;

wherein about 6 of the 18 isoleucine codons in said coding region are ATT, about 9 of the isoleucine codons are ATC, and about 3 of the isoleucine codons are ATA;

wherein about 7 methionine codons in said coding region are ATG; wherein about 3 of the 16 valine codons in said coding region are GTT, about 4 of the valine codons are GTC, about 2 of the valine codons are GTA, and about 7 of the valine codons are GTG;

wherein about 2 of the 11 serine codons in said coding region are TCT, about 2 of the serine codons are TCC, about 2 of the serine codons are TCA, about 1 of the serine codons is TCG, about 1 of the serine codons is AGT, and about 3 of the serine codons are AGC;

wherein about 2 of the 6 proline codons in said coding region are CCT, about 2 of the proline codons are CCC, about 1 of the proline codons is CCA, and about 1 of the proline codons is CCG;

wherein about 3 of the 13 threonine codons a in said coding region e ACT, about 5 of the threonine codons are ACC, about 4 of the threonine codons are ACA, and about 1 of the threonine codons is ACG;

wherein about 5 of the 19 alanine codons in said coding region are GCT, about 8 of the alanine codons are GCC, about 4 of the alanine codons are GCA, and about 2 of the alanine codons are GCG;

wherein about 4 of the 9 tyrosine codons in said coding region are TAT and about 5 of the tyrosine codons are TAC;

wherein about 1 of the 3 histidine codons in said coding region is CAT and about 2 of the histidine codons are CAC;

wherein about 1 of the 5 glutamine codons in said coding region is CAA and about 4 of the glutamine codons are CAG;

wherein about 6 of the 13 asparagine codons in said coding region are AAT and about 7 of the asparagine codons are AAC;

wherein about 3 of the 6 lysine codons in said coding region are AAA and about 3 of the lysine codons are AAG;

wherein about 3 of the 6 aspartic acid codons in said coding region are GAT and about 3 of the aspartic acid codons are GAC;

wherein about 3 of the 7 glutamic acid codons in said coding region are GAA and about 4 of the glutamic acid codons are GAG;

wherein about 1 of the 3 cysteine codons in said coding region is TGT and about 2 of the cysteine codons are TGC;

wherein about 7 tryptophan codons in said coding region are TGG;

wherein about 1 of the 15 arginine codons in said coding region is CGT, about 3 of the arginine codons are CGC, about 2 of the arginine codons are CGA, about 3 of the arginine codons are CGG, about 3 of the arginine codons are AGA, and about 3 of the arginine codons are AGG;

wherein and about 2 of the 15 glycine codons in said coding region are GGT, about 5 of the glycine codons are GGC, about 4 of the glycine codons are GGA, and about 4 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

350. The polynucleotide of claim 349, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO:19.

351. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO:19, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:19;

wherein the 11 phenylalanine codons in said coding region are TTC, the 31 leucine codons in said coding region are CTG, the 18 isoleucine codons in said coding region are ATC, the 7 methionine codons in said coding region are ATG, the 16 valine codons in said coding region are GTG, the 11 serine codons in said coding region are AGC, the 6 proline codons in said coding region are CCC, the 13 threonine codons in said coding region are ACC, the 19 alanine codons in said coding region are GCC, the 19 tyrosine codons in said coding region are TAC, the 3 histidine codons in said coding region are CAC, the 5 glutamine codons in said coding region are CAG, the 13

asparagine codons in said coding region are AAC, the 6 lysine codons in said coding region are AAG, the 6 aspartic acid codons in said coding region are GAC, the 7 glutamic acid codons in said coding region are GAG, the 3 cysteine codons in said coding region are TGC, the 7 tryptophan codons in said coding region are TGG, the 15 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 43 glycine codons are GGC.; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

352. The polynucleotide of claim 351, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO: 19.

353. The polynucleotide of claim 352, wherein said nucleic acid fragment encodes SEQ ID NO: 19.

354. The polynucleotide of any one of claims 342 to 353, wherein said nucleic acid fragment is ligated to a heterologous nucleic acid.

355. The polynucleotide of claim 354, wherein said heterologous nucleic acid encodes a heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment.

356. The polynucleotide of claim 355, wherein said heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment comprises a small self-assembly polypeptide, wherein said heterologous polypeptide self-assemble into multimers

357. The polynucleotide of any one of claim 342 to 357, wherein said nucleic acid fragment is ligated to a nucleic acid operatively encoding for the secretion of said polypeptide.

358. The polynucleotide of any one of claims 342 to 357, which is DNA, and wherein said nucleic acid fragment is operably associated with a promoter.

359. The polynucleotide of any one of claims 342 to 357, which is RNA.

360. The polynucleotide of claim 359, which is messenger RNA (mRNA).

361. A vector comprising the polynucleotide of any one of claims 342 to 359.

362. The vector of claim 361, which is a plasmid.

363. A composition comprising the polynucleotide of any one of claims 342 to 360, and a carrier.

364. The composition of claim 363, further comprising a component selected from the group consisting of an adjuvant, and a transfection facilitating compound.

365. The composition of claim 364, wherein said adjuvant is selected from the group consisting of:

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) and a neutral lipid;

a cytokine;

mono-phosphoryl lipid A and trehalosedicorynomycolateAF (MPL + TDM);

a solubilized mono-phosphoryl lipid A formulation; and

CRL1005/BAK.

366. The composition of claim 365, wherein said adjuvant comprises(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE), and wherein said neutral lipid is selected from the group consisting of:

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE),

1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and

1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE).

367. The composition of claim 366, wherein said neutral lipid is DPyPE.

368. The composition of claim 364, comprising the transfection facilitating compound (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) (DMRIE).

369. The composition of claim 364, wherein said adjuvant or said transfection facilitating compound comprises CRL1005 and benzalkonium chloride (BAK).

370. The composition of claim 369, wherein said CRL1005 and BAK are present at concentrations selected from the group consisting of:

0.3 mM BAK and 7.5 mg/ml CRL 1005,

0.3 mM BAK and 34 mg/ml CRL 1005, and

0.3 mM BAK and 50 mg/ml CRL 1005.

371. The composition of claim 369 or claim 370, which is formulated by combining together the CRL1005, BAK, and polynucleotide at a temperature below the cloud point, without thermal cycling to a temperature above the cloud point.

372. A method to treat or prevent SARS-CoV infection in a vertebrate comprising: administering to a vertebrate in need thereof the polynucleotide of any one of claims 342 to 360 or the composition of any one of claims 363 to 371.

373. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:21, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:21;

wherein about 1 of the 4 phenylalanine codons in said coding region are TTT, and about 3 of the phenylalanine codons are TTC;

wherein about 2 of the 14 leucine codons in said coding region are TTA, about 2 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 0 of the leucine codons are CTC, about 2 of the leucine codons are CTA, and about 2 of the leucine codons are CTG;

wherein about 1 of the 3 isoleucine codons in said coding region are ATT, about 1 of the isoleucine codons are ATC, and about 1 of the isoleucine codons are ATA;

wherein the 1 methionine codons in said coding region are ATG;

wherein about 6 of the 14 valine codons in said coding region are GTT, about 3 of the valine codons are GTC, about 3 of the valine codons are GTA, and about 2 of the valine codons are GTG;

wherein about 2 of the 7 serine codons in said coding region are TCT, about 0 of the serine codons are TCC, about 1 of the serine codons are

TCA, about 2 of the serine codons is TCG, about 1 of the serine codons is AGT, and about 1 of the serine codons are AGC;

wherein about 1 of the 2 proline codons in said coding region are CCT, about 0 of the proline codons are CCC, about 1 of the proline codons is CCA, and about 0 of the proline codons is CCG;

wherein about 1 of the 5 threonine codons in said coding region are ACT, about 0 of the threonine codons are ACC, about 2 of the threonine codons are ACA, and about 2 of the threonine codons is ACG;

wherein about 1 of the 4 alanine codons in said coding region are GCT, about 1 of the alanine codons are GCC, about 0 of the alanine codons are GCA, and about 2 of the alanine codons are GCG;

wherein about 0 of the 4 tyrosine codons in said coding region are TAT and about 4 of the tyrosine codons are TAC;

wherein about 3 of the 5 asparagine codons in said coding region are AAT and about 2 of the asparagine codons are AAC;

wherein about 2 of the 2 lysine codons in said coding region are AAA and about 0 of the lysine codons are AAG;

wherein about 1 of the 1 aspartic acid in said coding region codons are GAT and about 0 of the aspartic acid codons are GAC;

wherin about 3 of the 3 glutamic acid codons in said coding region are GAA and about 0 of the glutamic acid codons are GAG;

wherein about 1 of the 3 cysteine codons is in said coding region TGT and about 2 of the cysteine codons are TGC;

wherein about 1 of the 2 arginine codons in said coding region is CGT, about 0 of the arginine codons are CGC, about 1 of the arginine codons are CGA, about 0 of the arginine codons are CGG, about 0 of the arginine codons are AGA, and about 0 of the arginine codons are AGG;

wherein and about 1 of the 2 glycine codons in said coding region are GGT, about 0 of the glycine codons are GGC, about 1 of the glycine codons are GGA, and about 0 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

374. The polynucleotide of claim 373, wherein said codon optimized coding region comprises SEQ ID NO:42.

375. An isolated polynucleotide comprising a nucleic acid fragment which encodes at least 20 contiguous amino acids of SEQ ID NO:21, wherein said nucleic acid fragment is a fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:21;

wherein the 4 phenylalanine codons in said coding region are TTC, the 14 leucine codons in said coding region are CTG, the 18 isoleucine codons in said coding region are 3, the 1 methionine codon in said coding region is ATG, the 14 valine codons in said coding region are GTG, the 7 serine codons in said coding region are AGC, the 2 proline codons in said coding region are CCC, the 5 threonine codons in said coding region are ACC, the 4 alanine codons in said coding region are GCC, the 4 tyrosine codons in said coding region are TAC, the 5 asparagine codons in said coding region are AAC, the 2 lysine codons in said coding region are AAG, the 1 aspartic acid codon in said coding region is GAC, the 3 glutamic acid codons in said coding region are GAG, the 3 cysteine codons in said coding region are TGC, the 1 tryptophan codon in said coding region is TGG, the 2 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 2 glycine codons in said coding region are GGC.; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

376. The polynucleotide of claim 375, wherein said codon optimized coding region comprises SEQ ID NO:43.

377. The polynucleotide of any one of claims 373 to 376, wherein said nucleic acid fragment encodes at least 50 contiguous amino acids of SEQ ID NO:19.

378. The polynucleotide of claim 377, wherein said nucleic acid fragment encodes at least 100 contiguous amino acids of SEQ ID NO:19

379. The polynucleotide of claim 378, wherein said nucleic acid fragment encodes SEQ ID NO:19.

380. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO: 21, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:21;

wherein about 1 of the 4 phenylalanine codons in said coding region are TTT, and about 3 of the phenylalanine codons are TTC;

wherein about 2 of the 14 leucine codons in said coding region are TTA, about 2 of the leucine codons are TTG, about 6 of the leucine codons are CTT, about 0 of the leucine codons are CTC, about 2 of the leucine codons are CTA, and about 2 of the leucine codons are CTG;

wherein about 1 of the 3 isoleucine codons in said coding region are ATT, about 1 of the isoleucine codons are ATC, and about 1 of the isoleucine codons are ATA;

wherein the 1 methionine codons in said coding region are ATG;

wherein about 6 of the 14 valine codons in said coding region are GTT, about 3 of the valine codons are GTC, about 3 of the valine codons are GTA, and about 2 of the valine codons are GTG;

wherein about 2 of the 7 serine codons in said coding region are TCT, about 0 of the serine codons are TCC, about 1 of the serine codons are

TCA, about 2 of the serine codons is TCG, about 1 of the serine codons is AGT, and about 1 of the serine codons are AGC;

wherein about 1 of the 2 proline codons in said coding region are CCT, about 0 of the proline codons are CCC, about 1 of the proline codons is CCA, and about 0 of the proline codons is CCG;

wherein about 1 of the 5 threonine codons in said coding region are ACT, about 0 of the threonine codons are ACC, about 2 of the threonine codons are ACA, and about 2 of the threonine codons is ACG;

wherein about 1 of the 4 alanine codons in said coding region are GCT, about 1 of the alanine codons are GCC, about 0 of the alanine codons are GCA, and about 2 of the alanine codons are GCG;

wherein about 0 of the 4 tyrosine codons in said coding region are TAT and about 4 of the tyrosine codons are TAC;

wherein about 3 of the 5 asparagine codons in said coding region are AAT and about 2 of the asparagine codons are AAC;

wherein about 2 of the 2 lysine codons in said coding region are AAA and about 0 of the lysine codons are AAG;

wherein about 1 of the 1 aspartic acid in said coding region codons are GAT and about 0 of the aspartic acid codons are GAC;

wherin about 3 of the 3 glutamic acid codons in said coding region are GAA and about 0 of the glutamic acid codons are GAG;

wherein about 1 of the 3 cysteine codons is in said coding region TGT and about 2 of the cysteine codons are TGC;

wherein about 1 of the 2 arginine codons in said coding region is CGT, about 0 of the arginine codons are CGC, about 1 of the arginine codons are CGA, about 0 of the arginine codons are CGG, about 0 of the arginine codons are AGA, and about 0 of the arginine codons are AGG;

wherein and about 1 of the 2 glycine codons in said coding region are GGT, about 0 of the glycine codons are GGC, about 1 of the glycine codons are GGA, and about 0 of the glycine codons are GGG; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

381. The polynucleotide of claim 380, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO:21.

382. An isolated polynucleotide comprising a nucleic acid fragment which encodes a polypeptide at least 90% identical to SEQ ID NO:21, wherein said nucleic acid fragment is a variant fragment of a codon-optimized coding region for the polypeptide of SEQ ID NO:21;

wherein the 4 phenylalanine codons in said coding region are TTC, the 14 leucine codons in said coding region are CTG, the 18 isoleucine codons in said coding region are 3, the 1 methionine codon in said coding region is ATG, the 14 valine codons in said coding region are GTG, the 7 serine codons in said coding region are AGC, the 2 proline codons in said coding region are CCC, the 5 threonine codons in said coding region are ACC, the 4 alanine codons in said coding region are GCC, the 4 tyrosine codons in said coding region are TAC, the 5 asparagine codons in said coding region are AAC, the 2 lysine codons in said coding region are AAG, the 1 aspartic acid codon in said coding region is GAC, the 3 glutamic acid codons in said coding region are GAG, the 3 cysteine codons in said coding region are TGC, the 1 tryptophan codon in said coding region is TGG, the 2 arginine codons in said coding region are selected from the group consisting of CGG, AGA, or AGG, and the 2 glycine codons in said coding region are GGC; and

wherein said at least 20 contiguous amino acids, upon administration to a vertebrate, elicits a detectable immune response to the encoded polypeptide.

383. The polynucleotide of claim 382, wherein said nucleic acid fragment encodes a polypeptide at least 95% identical to SEQ ID NO: 19.

384. The polynucleotide of claim 383, wherein said nucleic acid fragment encodes SEQ ID NO: 19.

385. The polynucleotide of any one of claims 373 to 384, wherein said nucleic acid fragment is ligated to a heterologous nucleic acid.

386. The polynucleotide of claim 385, wherein said heterologous nucleic acid encodes a heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment.

387. The polynucleotide of claim 386, wherein said heterologous polypeptide fused to the polypeptide encoded by said nucleic acid fragment comprises a small self-assembly polypeptide, wherein said heterologous polypeptide self-assemble into multimers

388. The polynucleotide of any one of claim 373 to 385, wherein said nucleic acid fragment is ligated to a nucleic acid operatively encoding for the secretion of said polypeptide.

389. The polynucleotide of any one of claims 373 to 388, which is DNA, and wherein said nucleic acid fragment is operably associated with a promoter.

390. The polynucleotide of any one of claims 373 to 388, which is RNA.

391. The polynucleotide of claim 390, which is messenger RNA (mRNA).

392. A vector comprising the polynucleotide of any one of claims 373 to 390.

393. The vector of claim 392, which is a plasmid.

394. A composition comprising the polynucleotide of any one of claims 373 to 391, and a carrier.

395. The composition of claim 394, further comprising a component selected from the group consisting of an adjuvant, and a transfection facilitating compound.

396. The composition of claim 395, wherein said adjuvant is selected from the group consisting of:

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) and a neutral lipid;

a cytokine;

mono-phosphoryl lipid A and trehalosedicorynomycolateAF (MPL + TDM);

a solubilized mono-phosphoryl lipid A formulation; and

CRL1005/BAK.

397. The composition of claim 396, wherein said adjuvant comprises(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE), and wherein said neutral lipid is selected from the group consisting of:

1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE),

1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and

1,2-dimyristoyl-glycer-3-phosphoethanolamine (DMPE).

398. The composition of claim 397, wherein said neutral lipid is DPyPE.

399. The composition of claim 395, comprising the transfection facilitating compound (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) (DMRIE).

400. The composition of claim 395, wherein said adjuvant or said transfection facilitating compound comprises CRL1005 and benzalkonium chloride (BAK).

401. The composition of claim 400, wherein said CRL1005 and BAK are present at concentrations selected from the group consisting of:

0.3 mM BAK and 7.5 mg/ml CRL 1005,

0.3 mM BAK and 34 mg/ml CRL 1005, and

0.3 mM BAK and 50 mg/ml CRL 1005.

402. The composition of claim 400 or claim 401, which is formulated by combining together the CRL1005, BAK, and polynucleotide at a temperature below the cloud point, without thermal cycling to a temperature above the cloud point.

403. A method to treat or prevent SARS-CoV infection in a vertebrate comprising: administering to a vertebrate in need thereof the polynucleotide of any one of claims 373 to 391 or the composition of any one of claims 394 to 402.

404. The composition of any one of claims 115-123, 146-154, 177-185, 208-216, 239-247, 270-278, 301-309, 332-340, 363-371, 394-402 further comprising a conventional vaccine component of SARS-CoV selected from

the group consisting of inactivated virus, attenuated virus, a viral vector expressing an isolated SARS-CoV virus polypeptide, and isolated polypeptide from an SARS-CoV virus protein, fragment, variant or derivative thereof and/or one or more polynucleotides comprising at least one coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof.

405. The composition of claim 404 wherein said isolated polypeptide is any isolated SARS-CoV virus polypeptide fragment, variant or derivative thereof, selected from the group consisting of S, N, E, or M.

406. The composition of claim 404, wherein said isolated polypeptide comprises at least one immunogenic epitope capable of eliciting an immune response in a vertebrate.

407. The composition of claim 404, wherein the codon-optimized nucleic acid composition, and/or said one or more polynucleotides comprising at least one coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof and said isolated polypeptide from an SARS-CoV virus protein, fragment, variant or derivative thereof are in the same formulation.

408. The composition of claim 404, wherein said composition is a combinatorial polynucleotide vaccine composition or single formulation heterologous prime-boost vaccine composition.

409. The composition of claim 404, wherein the codon-optimized nucleic acid composition, and/or said one or more polynucleotides comprising at least one coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof, and said isolated polypeptide from a SARS-CoV virus protein, fragment, variant or derivative thereof, are in different

formulations wherein said different formulations are administered sequentially or simultaneously.

410. The composition of claim 404, wherein the combination of the codon-optimized nucleic acid composition, and/or said one or more polynucleotides comprising at least one coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof, and said isolated polypeptide from an SARS-CoV virus protein, fragment, variant or derivative thereof, provides an equivalent or higher immunological response than the individual components administered alone.

411. The composition of claim 404, wherein the combination of the codon-optimized nucleic acid composition, and/or said one or more polynucleotides comprising at least one coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof and said isolated polypeptide from an SARS-CoV virus protein, fragment, variant or derivative thereof, provides a broadened immune response.

412. The composition of claim 404, wherein the combination of codon-optimized nucleic acid composition, and/or said one or more polynucleotides comprising at least one coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof, and the isolated polypeptide, provides therapeutic benefit at lower doses than a therapeutically beneficial amount of either component administered alone.

413. The composition of claim 412, wherein the combination of codon-optimized nucleic acid composition, and/or one or more polynucleotides comprising at least one coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof, and the isolated polypeptide, provides therapeutic benefit at a dose of isolated polypeptide that is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least

50%, at least 60% or at least 70% less than administration of the polypeptide alone.

414. The composition of claim 404, wherein the combination of codon-optimized nucleic acid composition, and/or one or more polynucleotides comprising at least one coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof, and the isolated polypeptide provides, a broadened immune response at lower doses than a therapeutically beneficial amount of either component administered alone.

415. A method of eliciting an immune response by administration of the composition of claim 404, wherein said immune response is an equivalent or higher response than provided by administration of the composition in any one of claims 115-123, 146-154, 177-185, 208-216, 239-247, 270-278, 301-309, 332-340, 363-371, 394-402 or said isolated polypeptide from an SARS-CoV virus protein, fragment, variant or derivative thereof, or said one or more polynucleotides comprising at least one coding region encoding a SARS-CoV polypeptide, or a fragment, variant, or derivative thereof.

416. The method of claim 415, wherein said immune response is a broadened immune response.

417. The method of claim 415, wherein said immune response is achieved by administration of dose sparing amounts of said isolated polypeptide from an SARS-CoV virus protein, fragment, variant or derivative thereof, wherein said dose sparing amount is a concentration that is lower than conventionally administered.

418. The method of claim 417, wherein said immune response is be achieved by administration of dose sparing amounts of said isolated

polypeptide from an SARS-CoV virus protein, fragment, variant or derivative thereof, and wherein said immune response is a broadened immune response.

419. The method of claim 417, wherein said dose sparing amounts of isolated polypeptide from an SARS-CoV virus protein, fragment, variant or derivative thereof, are at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60% or at least 70% less than administration of polypeptide alone.

420. The method of claim 415, wherein said immune response is an enhanced cellular immune response.

421. The method of claim 415, wherein said immune response is an enhanced humoral immune response.

422. A method to treat or prevent SARS-CoV infection in a vertebrate comprising administering to a vertebrate in need thereof the composition of any one of claims 404-414.

423. An isolated antibody or fragment thereof that is produced from the administration to a vertebrate the polynucleotides selected from the group consisting of any one of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 18, or 20.

424. An isolated antibody or fragment thereof that is produced from the administration to a vertebrate the codon-optimized polynucleotides selected from the group consisting of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 18, or 20.

425. An isolated antibody or fragment thereof that is produced from the administration to a vertebrate the polypeptides selected from the group consisting of any one of SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 19, or 21.

426. The antibody or fragment thereof of claims 423 to 425, wherein said antibody is a human antibody.

427. The antibody or fragment thereof of claims 423 to 425, wherein said antibody is a polyclonal antibody.

428. A method of treating a SARS-CoV infection comprising administering to a patient in need, at least one isolated antibody of any one of claims 423 to 427.

429. The method of claim 428 wherein said method further comprises administering said antibody with at least one polynucleotide selected from the group consisting of SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 18, or 20.

430. The method of claim 428 wherein said method further comprises administering said antibody with at least one codon-optimized polynucleotide of any one of claims SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 18, or 20.

431. The method of claim 428 wherein said method further comprises administering said antibody with at least one polypeptide selected from the group consisting of SEQ ID Nos: 2, 4, 6, ,8, 10, 12, 14, 16, 19 or 21.

432. A method for raising a detectable immune response to multiple SARS-CoV polypeptides, comprising administering to a vertebrate a virus like particle comprising the SARS CoV polypeptides S, M, and E, wherein said virus like particle is administered in an amount sufficient to elicit a detectable immune response to one or more of said SARS-CoV polypeptides.

433. The method of claim 432, wherein said virus like particle is produced from polynucleotides encoding the polypeptides of SED ID NOs: 22, 18 and 20.

434. The method of claim 433, wherein said polynucleotides are codon optimized.

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ttttcacaaa tattacctga ccctctaaag ccaactaaga ggtcttttat tgaggacttg    360

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<212> PRT
<213> SARS-CoV Urbani strain

<400> 6

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Phe Ser Ile Ser Ile Thr Thr Glu Val Met Pro Val Ser Met Ala Lys
20 25 30

Thr Ser Val Asp Cys Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys
35 40 45

Ala Asn Leu Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg
50 55 60

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Ala Leu Ser Gly Ile Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val
65                  70                  75                  80

Phe Ala Gln Val Lys Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe
                85                  90                  95

Gly Gly Phe Asn Phe Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr
            100                 105                 110

Lys Arg Ser Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala
        115                 120                 125

Asp Ala Gly Phe Met Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn
    130                 135                 140

Ala Arg Asp Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu
145                 150                 155                 160

Pro Pro Leu Leu Thr Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu
                165                 170                 175

Val Ser Gly Thr Ala Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala
            180                 185                 190

Leu Gln Ile Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile
        195                 200                 205

Gly Val Thr Gln Asn Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn
    210                 215                 220

Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr
225                 230                 235                 240

Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln
                245                 250                 255

Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile
            260                 265                 270

Ser Ser Val Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala
        275                 280                 285

Glu Val Gln Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln
    290                 295                 300
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Thr Tyr Val Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser
305                 310                 315                 320

Ala Asn Leu Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser
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Lys Arg Val Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro
            340                 345                 350

Gln Ala Ala Pro His Gly Val Val Phe Leu His Val Thr Tyr Val Pro
        355                 360                 365

Ser Gln Glu Arg Asn Phe Thr Thr Ala Pro Ala Ile Cys His Glu Gly
    370                 375                 380

Lys Ala Tyr Phe Pro Arg Glu Gly Val Phe Val Phe Asn Gly Thr Ser
385                 390                 395                 400

Trp Phe Ile Thr Gln Arg Asn Phe Phe Ser Pro Gln Ile Ile Thr Thr
                405                 410                 415

Asp Asn Thr Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly Ile Ile
            420                 425                 430

Asn Asn Thr Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys
        435                 440                 445

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

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

Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu
                485                 490                 495

Ile Asp Leu Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro
            500                 505                 510

Trp
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<211> 3633
<212> DNA
<213> SARS-CoV Urbani strain

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ggtatttatt ttgctgccac agagaaatca aatgttgtcc gtggttgggt ttttggttct 360

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cgagcatgta actttgaatt gtgtgacaac cctttctttg ctgtttctaa acccatgggt 480

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gttcgtgatc taccttctgg ttttaacact ttgaaaccta tttttaagtt gcctcttggt 720

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ggaaaatatg agcaatatat taaatggcct tgg 3633

<210> 8
<211> 1211
<212> PRT
<213> SARS-CoV Urbani strain

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Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln His
35 40 45

Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg Ser
50 55 60

Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser Asn
65 70 75 80

Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val Ile
85 90 95

Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn Val
100 105 110

Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln Ser
115 120 125

Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys Asn
130 135 140

Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met Gly
145 150 155 160

Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr Phe
165 170 175

Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser Gly
180 185 190

Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly Phe
195 200 205

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Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp Leu
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Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu Gly
225                 230                 235                 240

Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro Ala
                245                 250                 255

Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr Leu
            260                 265                 270

Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile Thr
        275                 280                 285

Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys Ser
    290                 295                 300

Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn Phe
305                 310                 315                 320

Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr Asn
                325                 330                 335

Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser Val
            340                 345                 350

Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr Ser
        355                 360                 365

Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly Val
    370                 375                 380

Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala Asp
385                 390                 395                 400

Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly Gln
                405                 410                 415

Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Met
            420                 425                 430

Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser Thr
        435                 440                 445
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Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu Arg
    450                 455                 460

Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly Lys
465                 470                 475                 480

Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp Tyr
                485                 490                 495

Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val Val
            500                 505                 510

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

Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn Phe
    530                 535                 540

Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg Phe
545                 550                 555                 560

Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp Ser
                565                 570                 575

Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys Ser
            580                 585                 590

Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser Glu
        595                 600                 605

Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr Ala
    610                 615                 620

Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr Gly
625                 630                 635                 640

Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu His
                645                 650                 655

Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile Cys
            660                 665                 670

Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys Ser
        675                 680                 685

Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala Tyr
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690 695 700

Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile Thr
705 710 715 720

Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys Asn
725 730 735

Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu Gln
740 745 750

Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile Ala
755 760 765

Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys Gln
770 775 780

Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe Ser
785 790 795 800

Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile Glu
805 810 815

Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met Lys
820 825 830

Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile Cys
835 840 845

Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr Asp
850 855 860

Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala Thr
865 870 875 880

Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro Phe Ala
885 890 895

Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn Val
900 905 910

Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala Ile
915 920 925

Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly Lys
930 935 940

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Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu Val
945                 950                 955                 960

Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn Asp
                965                 970                 975

Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp Arg
            980                 985                 990

Leu Ile Thr Gly Arg Leu Gln Ser  Leu Gln Thr Tyr Val  Thr Gln Gln
        995                 1000                1005

Leu Ile  Arg Ala Ala Glu Ile  Arg Ala Ser Ala Asn  Leu Ala Ala
    1010                1015                1020

Thr Lys  Met Ser Glu Cys Val  Leu Gly Gln Ser Lys  Arg Val Asp
    1025                1030                1035

Phe Cys  Gly Lys Gly Tyr His  Leu Met Ser Phe Pro  Gln Ala Ala
    1040                1045                1050

Pro His  Gly Val Val Phe Leu  His Val Thr Tyr Val  Pro Ser Gln
    1055                1060                1065

Glu Arg  Asn Phe Thr Thr Ala  Pro Ala Ile Cys His  Glu Gly Lys
    1070                1075                1080

Ala Tyr  Phe Pro Arg Glu Gly  Val Phe Val Phe Asn  Gly Thr Ser
    1085                1090                1095

Trp Phe  Ile Thr Gln Arg Asn  Phe Phe Ser Pro Gln  Ile Ile Thr
    1100                1105                1110

Thr Asp  Asn Thr Phe Val Ser  Gly Asn Cys Asp Val  Val Ile Gly
    1115                1120                1125

Ile Ile  Asn Asn Thr Val Tyr  Asp Pro Leu Gln Pro  Glu Leu Asp
    1130                1135                1140

Ser Phe  Lys Glu Glu Leu Asp  Lys Tyr Phe Lys Asn  His Thr Ser
    1145                1150                1155

Pro Asp  Val Asp Leu Gly Asp  Ile Ser Gly Ile Asn  Ala Ser Val
    1160                1165                1170
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Val Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys
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Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Lys Tyr
1190 1195 1200

Glu Gln Tyr Ile Lys Trp Pro Trp
1205 1210

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<211> 2093
<212> DNA
<213> SARS-CoV Urbani strain

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caagctccta attacactca acatacttca tctatgaggg gggtttacta tcctgatgaa 180

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<212> PRT
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Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln His
35 40 45

Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg Ser
50 55 60

Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser Asn
65 70 75 80

Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val Ile
85 90 95

Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn Val

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            100                 105                 110

Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln Ser
        115                 120                 125

Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys Asn
    130                 135                 140

Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met Gly
145                 150                 155                 160

Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr Phe
                165                 170                 175

Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser Gly
            180                 185                 190

Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly Phe
        195                 200                 205

Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp Leu
    210                 215                 220

Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu Gly
225                 230                 235                 240

Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro Ala
                245                 250                 255

Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr Leu
            260                 265                 270

Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile Thr
        275                 280                 285

Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys Ser
    290                 295                 300

Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn Phe
305                 310                 315                 320

Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr Asn
                325                 330                 335

Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser Val
            340                 345                 350
```

```
Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr Ser
        355                 360                 365

Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly Val
    370                 375                 380

Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala Asp
385                 390                 395                 400

Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly Gln
                405                 410                 415

Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Met
            420                 425                 430

Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser Thr
        435                 440                 445

Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu Arg
    450                 455                 460

Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly Lys
465                 470                 475                 480

Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp Tyr
                485                 490                 495

Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val Val
            500                 505                 510

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

Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn Phe
    530                 535                 540

Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg Phe
545                 550                 555                 560

Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp Ser
                565                 570                 575

Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys Ser
            580                 585                 590
```

```
Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser Glu
        595                 600                 605

Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr Ala
    610                 615                 620

Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr Gly
625                 630                 635                 640

Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu His
                645                 650                 655

Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile Cys
            660                 665                 670

Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys Ser
        675                 680                 685

Ile Val Ala Tyr Thr Met Ser Leu Gly Ala
    690                 695
```

<210> 11
<211> 1623
<212> DNA
<213> SARS-CoV Urbani strain

<400> 11

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atggatgcaa tgaagagagg gctctgctgt gtgctgctgc tgtgtggagc agtcttcgtt     60

tcgcccagcg ctagaggatc gggagatagt tcaattgctt actctaataa caccattgct    120

atacctacta acttttcaat tagcattact acagaagtaa tgcctgtttc tatggctaaa    180

acctccgtag attgtaatat gtacatctgc ggagattcta ctgaatgtgc taatttgctt    240

ctccaatatg gtagcttttg cacacaacta aatcgtgcac tctcaggtat tgctgctgaa    300

caggatcgca acacacgtga agtgttcgct caagtcaaac aaatgtacaa aaccccaact    360

ttgaaatatt ttggtggttt taatttttca caaatattac ctgaccctct aaagccaact    420

aagaggtctt ttattgagga cttgctcttt aataaggtga cactcgctga tgctggcttc    480

atgaagcaat atggcgaatg cctaggtgat attaatgcta gagatctcat ttgtgcgcag    540

aagttcaatg gacttacagt gttgccacct ctgctcactg atgatatgat tgctgcctac    600

actgctgctc tagttagtgg tactgccact gctggatgga catttggtgc tggcgctgct    660

cttcaaatac cttttgctat gcaaatggca tataggttca atggcattgg agttacccaa    720

aatgttctct atgagaacca aaaacaaatc gccaaccaat ttaacaaggc gattagtcaa    780
```

attcaagaat cacttacaac aacatcaact gcattgggca agctgcaaga cgttgttaac 840

cagaatgctc aagcattaaa cacacttgtt aaacaactta gctctaattt tggtgcaatt 900

tcaagtgtgc taaatgatat cctttcgcga cttgataaag tcgaggcgga ggtacaaatt 960

gacaggttaa ttacaggcag acttcaaagc cttcaaacct atgtaacaca acaactaatc 1020

agggctgctg aaatcagggc ttctgctaat cttgctgcta ctaaaatgtc tgagtgtgtt 1080

cttggacaat caaaaagagt tgacttttgt ggaaagggct accaccttat gtccttccca 1140

caagcagccc cgcatggtgt tgtcttccta catgtcacgt atgtgccatc ccaggagagg 1200

aacttcacca cagcgccagc aatttgtcat gaaggcaaag catacttccc tcgtgaaggt 1260

gtttttgtgt ttaatggcac ttcttggttt attacacaga ggaacttctt ttctccacaa 1320

ataattacta cagacaatac atttgtctca ggaaattgtg atgtcgttat tggcatcatt 1380

aacaacacag tttatgatcc tctgcaacct gagctcgact cattcaaaga agagctggac 1440

aagtacttca aaaatcatac atcaccagat gttgatcttg gcgacatttc aggcattaac 1500

gcttctgtcg tcaacattca aaaagaaatt gaccgcctca atgaggtcgc taaaaattta 1560

aatgaatcac tcattgacct tcaagaattg ggaaaatatg agcaatatat taaatggcct 1620

tgg 1623

<210> 12
<211> 541
<212> PRT
<213> SARS-CoV Urbani strain

<400> 12

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro Ser Ala Arg Gly Ser Gly Asp Ser Ser Ile
20 25 30

Ala Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser
35 40 45

Ile Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp
50 55 60

Cys Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu
65 70 75 80

Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly
85 90 95

```
Ile Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val
            100                 105                 110

Lys Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn
        115                 120                 125

Phe Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe
    130                 135                 140

Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe
145                 150                 155                 160

Met Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu
                165                 170                 175

Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu
            180                 185                 190

Thr Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr
        195                 200                 205

Ala Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro
    210                 215                 220

Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln
225                 230                 235                 240

Asn Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys
                245                 250                 255

Ala Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu
            260                 265                 270

Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr
        275                 280                 285

Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu
    290                 295                 300

Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile
305                 310                 315                 320

Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr
                325                 330                 335
```

```
Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu Ala
            340                 345                 350

Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val Asp
        355                 360                 365

Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ala Ala Pro
    370                 375                 380

His Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ser Gln Glu Arg
385                 390                 395                 400

Asn Phe Thr Thr Ala Pro Ala Ile Cys His Glu Gly Lys Ala Tyr Phe
                405                 410                 415

Pro Arg Glu Gly Val Phe Val Phe Asn Gly Thr Ser Trp Phe Ile Thr
            420                 425                 430

Gln Arg Asn Phe Phe Ser Pro Gln Ile Ile Thr Thr Asp Asn Thr Phe
        435                 440                 445

Val Ser Gly Asn Cys Asp Val Val Ile Gly Ile Ile Asn Asn Thr Val
    450                 455                 460

Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Glu Leu Asp
465                 470                 475                 480

Lys Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp Ile
                485                 490                 495

Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg
            500                 505                 510

Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln
        515                 520                 525

Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp
    530                 535                 540
```

<210> 13
<211> 1269
<212> DNA
<213> SARS-CoV Urbani strain

<400> 13
atgtctgata atggacccca atcaaaccaa cgtagtgccc cccgcattac atttggtgga 60

cccacagatt caactgacaa taaccagaat ggaggacgca atggggcaag gccaaaacag 120

```
cgccgacccc aaggtttacc caataatact gcgtcttggt tcacagctct cactcagcat    180

ggcaaggagg aacttagatt ccctcgaggc cagggcgttc caatcaacac caatagtggt    240

ccagatgacc aaattggcta ctaccgaaga gctacccgac gagttcgtgg tggtgacggc    300

aaaatgaaag agctcagccc cagatggtac ttctattacc taggaactgg cccagaagct    360

tcacttccct acggcgctaa caaagaaggc atcgtatggg ttgcaactga gggagccttg    420

aatacaccca aagaccacat tggcacccgc aatcctaata acaatgctgc caccgtgcta    480

caacttcctc aaggaacaac attgccaaaa ggcttctacg cagagggaag cagaggcggc    540

agtcaagcct cttctcgctc ctcatcacgt agtcgcggta attcaagaaa ttcaactcct    600

ggcagcagta ggggaaattc tcctgctcga atggctagcg gaggtggtga aactgccctc    660

gcgctattgc tgctagacag attgaaccag cttgagagca aagtttctgg taaaggccaa    720

caacaacaag gccaaactgt cactaagaaa tctgctgctg aggcatctaa aaagcctcgc    780

caaaaacgta ctgccacaaa acagtacaac gtcactcaag catttgggag acgtggtcca    840

gaacaaaccc aaggaaattt cggggaccaa gacctaatca gacaaggaac tgattacaaa    900

cattggccgc aaattgcaca atttgctcca agtgcctctg cattctttgg aatgtcacgc    960

attggcatgg aagtcacacc ttcgggaaca tggctgactt atcatggagc cattaaattg   1020

gatgacaaag atccacaatt caaagacaac gtcatactgc tgaacaagca cattgacgca   1080

tacaaaacat tcccaccaac agagcctaaa aaggacaaaa agaaaaagac tgatgaagct   1140

cagcctttgc cgcagagaca aaagaagcag cccactgtga ctcttcttcc tgcggctgac   1200

atggatgatt tctccagaca acttcaaaat tccatgagtg gagcttctgc tgattcaact   1260

caggcataa                                                           1269
```

<210> 14
<211> 422
<212> PRT
<213> SARS-CoV Urbani strain

<400> 14

```
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

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> 15
<211> 1209
<212> DNA
<213> SARS-CoV Urbani strain

<400> 15
atgtctgata atggacccca atcaaaccaa cgtagtgccc cccgcattac atttggtgga 60

cccacagatt caactgacaa taaccagaat ggaggacgca atggggcaag gccaaaacag 120

cgccgacccc aaggtttacc caataatact gcgtcttggt tcacagctct cactcagcat 180

ggcaaggagg aacttagatt ccctcgaggc agggcgttc caatcaacac caatagtggt 240

ccagatgacc aaattggcta ctaccgaaga gctacccgac gagttcgtgg tggtgacggc 300

aaaatgaaag agctcagccc cagatggtac ttctattacc taggaactgg cccagaagct 360

tcacttccct acggcgctaa caaagaaggc atcgtatggg ttgcaactga gggagccttg 420

aatacaccca aagaccacat tggcacccgc aatcctaata acaatgctgc caccgtgcta 480

caacttcctc aaggaacaac attgccaaaa ggcttctacg cagagggaag cagaggcggc 540

agtcaagcct cttctcgctc ctcatcacgt agtcgcggta attcaagaaa ttcaactcct 600

ggcagcagta ggggaaattc tcctgctcga atggctagcg gaggtggtga aactgccctc 660

gcgctattgc tgctagacag attgaaccag cttgagagca aagtttctgg taaaggccaa 720

caacaacaag gccaaactgt cactaagaaa tctgctgctg aggcatctaa aaagcctcgc 780

caaaaacgta ctgccacaaa acagtacaac gtcactcaag catttgggag acgtggtcca 840

gaacaaaccc aaggaaattt cggggaccaa gacctaatca gacaaggaac tgattacaaa 900

cattggccgc aaattgcaca atttgctcca agtgcctctg cattctttgg aatgtcacgc 960

attggcatgg aagtcacacc ttcgggaaca tggctgactt atcatggagc cattaaattg 1020

gatgacaaag atccacaatt caaagacaac gtcatactgc tgaacaagca cattgacgca 1080

taccctttgc cgcagagaca aaagaagcag cccactgtga ctcttcttcc tgcggctgac 1140

atggatgatt tctccagaca acttcaaaat tccatgagtg gagcttctgc tgattcaact 1200

caggcataa 1209

<210> 16
<211> 402
<212> PRT
<213> SARS-CoV Urbani strain

<400> 16

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

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 Pro Leu Pro Gln Arg Gln Lys
        355                 360                 365
```

```
Lys Gln Pro Thr Val Thr Leu Leu Pro Ala Ala Asp Met Asp Asp Phe
    370                 375                 380

Ser Arg Gln Leu Gln Asn Ser Met Ser Gly Ala Ser Ala Asp Ser Thr
385                 390                 395                 400

Gln Ala
```

<210> 17
<211> 20
<212> PRT
<213> SARS-CoV Urbani strain

<400> 17

```
Lys Thr Phe Pro Pro Thr Glu Pro Lys Lys Asp Lys Lys Lys Lys Thr
1               5                   10                  15

Asp Glu Ala Gln
            20
```

<210> 18
<211> 666
<212> DNA
<213> SARS-CoV Urbani strain

<400> 18

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atggcagaca acggtactat taccgttgag gagcttaaac aactcctgga acaatggaac     60

ctagtaatag gtttcctatt cctagcctgg attatgttac tacaatttgc ctattctaat    120

cggaacaggt ttttgtacat aataaagctt gttttcctct ggctcttgtg gccagtaaca    180

cttgcttgtt ttgtgcttgc tgctgtctac agaattaatt gggtgactgg cgggattgcg    240

attgcaatgg cttgtattgt aggcttgatg tggcttagct acttcgttgc ttccttcagg    300

ctgtttgctc gtacccgctc aatgtggtca ttcaacccag aaacaaacat tcttctcaat    360

gtgcctctcc gggggacaat tgtgaccaga ccgctcatgg aaagtgaact tgtcattggt    420

gctgtgatca ttcgtggtca cttgcgaatg gccggacacc ccctagggcg ctgtgacatt    480

aaggacctgc caaaagagat cactgtggct acatcacgaa cgctttctta ttacaaatta    540

ggagcgtcgc agcgtgtagg cactgattca ggttttgctg catacaaccg ctaccgtatt    600

ggaaactata aattaaatac agaccacgcc ggtagcaacg acaatattgc tttgctagta    660

cagtaa                                                               666
```

<210> 19
<211> 221
<212> PRT

<213> SARS-CoV Urbani strain

<400> 19

```
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

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> 20

<211> 231
<212> DNA
<213> SARS-CoV Urbani strain

<400> 20
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cttgctttcg tggtattctt gctagtcaca ctagccatcc ttactgcgct tcgattgtgt 120

gcgtactgct gcaatattgt taacgtgagt ttagtaaaac caacggttta cgtctactcg 180

cgtgttaaaa atctgaactc ttctgaagga gttcctgatc ttctggtcta a 231

<210> 21
<211> 76
<212> PRT
<213> SARS-CoV Urbani strain

<400> 21

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> 22
<211> 3768
<212> DNA
<213> SARS-CoV Urbani strain

<400> 22
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acttttgatg atgttcaagc tcctaattac actcaacata cttcatctat gaggggggtt 120

tactatcctg atgaaatttt tagatcagac actctttatt taactcagga tttatttctt 180

ccattttatt ctaatgttac agggtttcat actattaatc atacgtttgg caaccctgtc 240

ataccttta aggatggtat ttattttgct gccacagaga aatcaaatgt tgtccgtggt 300

tgggtttttg gttctaccat gaacaacaag tcacagtcgg tgattattat taacaattct 360

actaatgttg ttatacgagc atgtaacttt gaattgtgtg acaaccctt ctttgctgtt 420

tctaaaccca tgggtacaca gacacatact atgatattcg ataatgcatt taattgcact 480

ttcgagtaca tatctgatgc cttttcgctt gatgtttcag aaaagtcagg taattttaaa 540

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Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
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Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
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Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
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Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
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Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
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Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
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Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
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Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
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Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
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Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
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Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
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Pro His  Gly Val Val Phe Leu  His Val Thr Tyr Val  Pro Ser Gln
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Glu Arg  Asn Phe Thr Thr Ala  Pro Ala Ile Cys His  Glu Gly Lys
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Ala Tyr  Phe Pro Arg Glu Gly  Val Phe Val Phe Asn  Gly Thr Ser
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Trp Phe  Ile Thr Gln Arg Asn  Phe Phe Ser Pro Gln  Ile Ile Thr
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Ile Ile  Asn Asn Thr Val Tyr  Asp Pro Leu Gln Pro  Glu Leu Asp
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Pro Asp  Val Asp Leu Gly Asp  Ile Ser Gly Ile Asn  Ala Ser Val
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<223> Fully Optimized soluble S1 protein

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<220>
<223> Fully optimized S2 protein

<400> 28
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atctgcggag attctacaga atgtgcaaac ttgctgctac agtatggatc gttctgtacc 180

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<210> 29
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<212> DNA
<213> Artificial Sequence

<220>
<223> Uniform Optimization of S2 protein

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<210> 30
<211> 3633
<212> DNA
<213> Artificial Sequence

<220>
<223> Fully optimized TPA-S protein

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<213> Artificial Sequence

<220>
<223> Uniform optimization of TPA-S protein

<400> 31
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ggcgaggtgt tcaacgccac caagttcccc agcgtgtacg cctgggagcg gaagaagatc 1080

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agcttcgtgg tgaagggcga cgacgtgcgg cagatcgccc ccggccagac cggcgtgatc 1260

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gccaaccagt tcaacaaggc catcagccag atccaggaga gcctgaccac caccagcacc 2820

gccctgggca agctgcagga cgtggtgaac cagaacgccc aggccctgaa caccctggtg 2880

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ggcaactgcg acgtggtgat cggcatcatc aacaacaccg tgtacgaccc cctgcagccc 3420

gagctggaca gcttcaagga ggagctggac aagtacttca agaaccacac cagccccgac 3480

gtggacctgg gcgacatcag cggcatcaac gccagcgtgg tgaacatcca gaaggagatc 3540

gaccggctga acgaggtggc caagaacctg aacgagagcc tgatcgacct gcaggagctg 3600

ggcaagtacg agcagtacat caagtggccc tgg 3633

<210> 32
<211> 2094
<212> DNA
<213> Artificial Sequence

<220>
<223> Fully optimized soluble TPA-S1 protein

<400> 32
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agtccatccg cccgggggtc gggatctgac ctagatagat gcacgacctt cgatgacgtg 120

caggcaccaa attacaccca acatacttca tccatgcgcg gcgtttacta tcccgacgaa 180

atcttccgga gtgacaccct gtatctgact caggacctgt ttctgccctt ctacagcaat 240

gtgacaggct ttcacaccat taaccatacc ttcgggaatc cagtaatccc ttttaaggat 300

gggatttact ttgctgctac tgagaaaagt aatgttgtca gggggtgggt ttttggctca 360

acaatgaaca ataagtctca gagtgtcatc atcattaaca attctaccaa tgtagtcatc 420

agagcatgca acttcgagct ctgtgataac cctttctttg ctgtgtctaa gcccatgggc 480

actcaaacac ataccatgat cttcgacaat gcgttcaatt gtacctttga gtatatatca 540

gacgccttca gcctagacgt ctcggaaaag tccggaaact ttaaacacct gcgggaattc 600

gtgtttaaga acaaagatgg atttttgtac gtatacaagg gttatcagcc tatcgatgtc 660

gtgcgtgatc tgccctccgg cttcaacacc ctgaagccta tattcaaact acccctaggg 720

atcaacatca ccaattttag ggcaatactt acggcatttt ccccagccca ggacatctgg 780

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ctgaaatgct ccgtaaagag cttcgaaatt gataaaggaa tctatcagac cagcaacttc 960

cgggtcgtgc cctctggcga cgttgtccgg ttccccaaca tcaccaacct ctgcccattc 1020

ggcgaggtgt tcaacgctac aaaattccca agtgtctacg cctgggagag gaaaaagatc 1080

tctaattgtg tggcagatta ttccgtgtta tacaacagca cattcttctc aacgttcaag 1140

tgttatggcg tgagcgccac caagcttaac gacctctgct tctccaatgt atacgctgac 1200

tcttttgtgg ttaagggaga cgatgtgcga cagatcgccc cggggcaaac cggagtgatt 1260

gcggactaca actataaact gcccgacgat ttcatgggtt gtgtgcttgc ttggaatacg 1320

aggaacattg acgcaacgag caccgggaac tataattaca aatatcgtta cctgcgccat 1380

gggaaactca gacctttga acgagatatt agcaacgtcc ctttctcacc ggatgggaag 1440

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aacaatgttt tccagactca ggccggttgt ctgattggcg ccgagcacgt cgacacatct 1980

tacgagtgcg atattcccat aggtgccggc atttgtgcga gctaccacac tgtatcactg 2040

ctgagaagca caagccagaa atcaattgtg gcatacacaa tgtccttggg agca 2094

<210> 33
<211> 2091
<212> DNA
<213> Artificial Sequence

<220>
<223> Uniform optimization of soluble TPA-S1 protein

<400> 33
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atcttccgga gcgacaccct gtacctgacc caggacctgt tcctgccctt ctacagcaac 240

gtgaccggct tccacaccat caaccacacc ttcggcaacc ccgtgatccc cttcaaggac 300

ggcatctact tcgccgccac cgagaagagc aacgtggtgc ggggctgggt gttcggcagc 360

accatgaaca acaagagcca gagcgtgatc atcatcaaca acagcaccaa cgtggtgatc 420

cgggcctgca acttcgagct gtgcgacaac cccttcttcg ccgtgagcaa gcccatgggc 480

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cgggtggtgc ccagcggcga cgtggtgcgg ttccccaaca tcaccaacct gtgccccttc 1020

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cggaacatcg acgccaccag caccggcaac tacaactaca agtaccggta cctgcggcac 1380

ggcaagctgc ggcccttcga gcgggacatc agcaacgtgc ccttcagccc cgacggcaag 1440

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gcccccgcca ccgtgtgcgg ccccaagctg agcaccgacc tgatcaagaa ccagtgcgtg 1620

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cagcccttcc agcagttcgg ccgggacgtg agcgacttca ccgacagcgt gcgggacccc 1740

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tacgagtgcg acatccccat cggcgccggc atctgcgcca gctaccacac cgtgagcctg 2040

ctgcggagca ccagccagaa gagcatcgtg gcctacacca tgagcctggg c 2091

<210> 34
<211> 1623
<212> DNA
<213> Artificial Sequence

<220>
<223> Fully optimized soluble TPA-S2

<400> 34
atggatgcaa tgaaaagagg cctgtgttgt gttctgctgc tgtgtggggc ggtatttgtg 60

agtccctctg ccaggggaag cggcgacagc agtatagcct actcaaacaa taccatcgcc 120

attcctacaa atttttccat ctcaatcacg acggaagtca tgccagttag catggccaaa 180

acctctgtcg actgcaacat gtacatctgc ggagactcta ctgagtgcgc aaacctgctc 240

ttgcagtatg gctcgttttg cacccagttg aatcgggccc tcagtggcat tgccgcagaa 300

caagatcgga ataccaggga ggtcttcgcg caagtcaagc agatgtacaa aacccctaca 360

ctcaaatact tcgggggggtt caactttagc caaatcctgc cagaccccct caagcctact 420

aagcgcagtt ttatcgaaga cttactcttt aataaggtga cattagctga tgccggattc 480

atgaagcagt acggagagtg cctgggggat atcaacgcgc gggacctaat ctgtgcccag 540

aagttcaacg gtctgacagt gcttccgcct ctcctgaccg atgatatgat cgcagcttac 600

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ctgcaaatcc cattcgcgat gcagatggca tacagattta acggcattgg agtcacccag 720

aatgtcctat acgagaacca gaagcaaatc gctaaccagt tcaacaaagc catatcccag 780

attcaggagt cccttactac aaccagtact gctttaggta aactgcaaga tgtagtgaac 840

cagaacgctc aggccttaaa taccccttgtt aaacagctat cctcaaactt tggggctatc 900

tcctccgtgc tcaacgatat cctgagccgc ctcgataagg tggaagcgga ggtccagatc 960

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aataatactg tgtatgaccc gctgcagccc gaactggaca gctttaaaga ggagctggac 1440

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gcctctgtgg taaacattca gaaggagatc gatcggctga acgaagtggc taagaatctg 1560

aatgaatcat tgattgacct tcaggagttg ggcaagtatg agcagtatat taaatggcca 1620

tgg 1623

<210> 35
<211> 1623
<212> DNA
<213> Artificial Sequence

<220>
<223> Uniform optimization of TPA-S2 protein

<400> 35
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accagcgtgg actgcaacat gtacatctgc ggcgacagca ccgagtgcgc caacctgctg 240

ctgcagtacg gcagcttctg cacccagctg aaccgggccc tgagcggcat cgccgccgag 300

caggaccgga acacccggga ggtgttcgcc caggtgaagc agatgtacaa gacccccacc 360

ctgaagtact tcggcggctt caacttcagc cagatcctgc ccgacccccct gaagcccacc 420

aagcggagct tcatcgagga cctgctgttc aacaaggtga ccctggccga cgccggcttc 480

atgaagcagt acggcgagtg cctgggcgac atcaacgccc gggacctgat ctgcgcccag 540

aagttcaacg gcctgaccgt gctgcccccc ctgctgaccg acgacatgat cgccgcctac 600

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cagaacgccc aggccctgaa caccctggtg aagcagctga gcagcaactt cggcgccatc 900

agcagcgtgc tgaacgacat cctgagccgg ctggacaagg tggaggccga ggtgcagatc 960

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gccagcgtgg tgaacatcca gaaggagatc gaccggctga acgaggtggc caagaacctg 1560

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tgg 1623

<210> 36
<211> 1269
<212> DNA
<213> Artificial Sequence

<220>
<223> Fully optimized N protein

<400> 36
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ccaacagaca gtaccgataa caaccagaac ggcggaagaa acggggccag gcccaagcag 120

cggagacctc agggattacc aaataatacc gcaagctggt tcacagccct gacccagcat 180

ggaaaagagg aactgagatt ccctagagga caaggggtgc ctattaatac taatagcggg 240

cctgacgatc aaattggcta ttatcgacgt gcgactcgcc gtgttagagg gggggacggg 300

aagatgaagg agcttagccc acgctggtac ttttactatc tgggaaccgg acctgaagct 360

agtctgccct acggcgctaa caaggaggga atagtatggg tcgccacgga aggtgcgttg 420

aatactccga aagatcacat cggcaccaga aatcctaaca ataacgccgc aaccgtgcta 480

caattacccc agggaactac tctgccgaag gggttctatg cggagggaag ccgcggcggc 540

tcacaagcca gttcacgctc cagctcccgg tcgaggggta attcccgaaa cagcaccccg 600

ggatcatcta ggggaaactc tcccgcccgg atggcctcag gcggcggcga aacagctctg 660

gctctgctat tgctggaccg gctcaaccag ctcgagtcca aagtctctgg taaaggtcag 720

cagcagcagg gtcaaacagt gaccaaaaaa agtgcagccg aggccagcaa gaaaccacgc 780

cagaaacgta cggccacaaa gcaatacaat gtgacccaag cctttggaag gcgggggccc 840

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cactggccgc agatcgctca gtttgcacct agcgcctccg ctttctttgg catgagtcgg 960

attggcatgg aggtgacacc atcaggtact tggttaacgt accacggggc aatcaaactt 1020

gatgataaag atccccagtt taaggacaac gttatcctcc tgaataagca tattgacgcc 1080

tataagacct tccccccaac cgaaccaaag aaggacaaga agaagaagac agacgaggca 1140

cagcctctcc cccagaggca gaaaaagcag cctactgtca cccttctgcc cgctgcagac 1200

atggatgact tttcccgcca actccagaac tctatgagtg gggcttccgc tgactctacg 1260

caggcctga 1269

<210> 37
<211> 1266
<212> DNA
<213> Artificial Sequence

<220>
<223> Uniform optimization of N protein

<400> 37
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cccaccgaca gcaccgacaa caaccagaac ggcggcagaa acggcgccag acccaagcag 120

agaagacccc agggcctgcc caacaacacc gccagctggt tcaccgccct gacccagcac 180

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cccgacgacc agatcggcta ctacagaaga gccaccagaa gagtgagagg cggcgacggc 300

aagatgaagg agctgagccc cagatggtac ttctactacc tgggcaccgg ccccgaggcc 360

agcctgccct acggcgccaa caaggagggc atcgtgtggg tggccaccga gggcgccctg 420

aacaccccca aggaccacat cggcaccaga aaccccaaca acaacgccgc caccgtgctg 480

cagctgcccc agggcaccac cctgcccaag ggcttctacg ccgagggcag cagaggcggc 540

agccaggcca gcagcagaag cagcagcaga agcagaggca acagcagaaa cagcaccccc 600

ggcagcagca gaggcaacag ccccgccaga atggccagcg gcggcggcga gaccgccctg 660

gccctgctgc tgctggacag actgaaccag ctggagagca aggtgagcgg caagggccag 720

cagcagcagg gccagaccgt gaccaagaag agcgccgccg aggccagcaa gaagcccaga 780

cagaagagaa ccgccaccaa gcagtacaac gtgacccagg ccttcggcag aagaggcccc 840

gagcagaccc agggcaactt cggcgaccag gacctgatca gacagggcac cgactacaag 900

cactggcccc agatcgccca gttcgccccc agcgccagcg ccttcttcgg catgagcaga 960

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gacgacaagg acccccagtt caaggacaac gtgatcctgc tgaacaagca catcgacgcc 1080

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cagcccctgc cccagagaca gaagaagcag cccaccgtga ccctgctgcc cgccgccgac 1200

atggacgact tcagcagaca gctgcagaac agcatgagcg gcgccagcgc cgacagcacc 1260

caggcc 1266

<210> 38
<211> 1209
<212> DNA
<213> Artificial Sequence

<220>
<223> Fully optimized N protein lacking NLS

<400> 38
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ccaaccgact caacagacaa taatcagaac ggaggacgca atggtgcacg tcctaagcag 120

agacgccccc aagggctgcc taataataca gcaagttggt ttaccgcact cacacaacat 180

ggaaaggaag agttgcggtt cccccgcggc cagggcgtgc ccatcaacac aaatagcgga 240

cccgacgatc agatcggata ttaccgaaga gctacaagga gagttcgcgg cggggatggc 300

aagatgaagg agctatcacc acgatggtac ttctattacc tcgggacagg cccagaggcc 360

tcgctaccat acggggccaa caaggagggt attgtctggg tcgctaccga aggggccctg 420

aatacaccta aagaccacat aggtaccaga aatcccaaca ataacgccgc gaccgtgtta 480

cagcttcctc agggaacgac ccttccaaaa gggttttacg ccgaaggatc tcggggaggg 540

tcacaggcta gctcccgtag ctcctcaagg tccaggggga attctagaaa cagtacaccc 600

ggctctagcc gtggtaactc cccagctcgc atggcatccg gcggagggga aaccgctctg 660

gctctgctcc tgttagatcg gttgaaccaa ctggaatcga aggtatccgg aaagggacag 720

cagcagcaag gccagactgt gactaagaag tccgcggccg aggccagtaa gaaaccccgc 780

cagaaacgaa ctgccaccaa acagtataat gtgacacagg ccttcggcag acggggtcca 840

gagcagaccc aaggcaactt cggggatcag gacctgattc ggcagggtac cgactataag 900

cactggccgc aaattgctca gtttgctccc agtgcgagtg ccttcttcgg catgtctagg 960

atcgggatgg aggttactcc tagcggcact tggcttactt atcacggagc catcaaactc 1020

gatgataagg acccacagtt taaggataac gtgattctgc tgaacaaaca tatagacgcg 1080

taccctctcc cgcaaaggca gaaaaaacag cctaccgtca cgttactgcc tgccgcagac 1140

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caagcttga 1209

<210> 39
<211> 1206
<212> DNA
<213> Artificial Sequence

<220>
<223> Uniform optimization of N protein lacking NLS

<400> 39
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agaagacccc agggcctgcc caacaacacc gccagctggt tcaccgccct gacccagcac 180

ggcaaggagg agctgagatt ccccagaggc cagggcgtgc ccatcaacac caacagcggc 240

cccgacgacc agatcggcta ctacagaaga gccaccagaa gagtgagagg cggcgacggc 300

aagatgaagg agctgagccc cagatggtac ttctactacc tgggcaccgg ccccgaggcc 360

agcctgccct acggcgccaa caaggagggc atcgtgtggg tggccaccga gggcgccctg 420

aacaccccca aggaccacat cggcaccaga aaccccaaca acaacgccgc caccgtgctg 480

cagctgcccc agggcaccac cctgcccaag ggcttctacg ccgagggcag cagaggcggc 540

agccaggcca gcagcagaag cagcagcaga agcagaggca acagcagaaa cagcaccccc 600

ggcagcagca gaggcaacag ccccgccaga atggccagcg gcggcggcga gaccgccctg 660

gccctgctgc tgctggacag actgaaccag ctggagagca aggtgagcgg caagggccag 720

cagcagcagg gccagaccgt gaccaagaag agcgccgccg aggccagcaa gaagcccaga 780

cagaagagaa ccgccaccaa gcagtacaac gtgacccagg ccttcggcag aagaggcccc 840

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cactggcccc agatcgccca gttcgccccc agcgccagcg ccttcttcgg catgagcaga 960

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gacgacaagg acccccagtt caaggacaac gtgatcctgc tgaacaagca catcgacgcc 1080

taccccctgc cccagagaca gaagaagcag cccaccgtga ccctgctgcc cgccgccgac 1140

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<210> 40
<211> 666
<212> DNA
<213> Artificial Sequence

<220>
<223> Fully optimized M protein

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cgcaataggt ttttgtacat tatcaagctg gtcttccttt ggctgctctg gcccgtaaca 180

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gtgcctttac gcggcactat cgtgacccgg cctctaatgg aatccgagct ggtaattggc 420

gcagtcatca taaggggggca cctcagaatg gccgggcacc cacttgggag atgcgacatc 480

aaggatctgc cgaaggaaat tactgttgca acttcacgaa cgctgagcta ttacaaactg 540

ggagctagcc agagagtggg taccgactcc ggcttcgctg cctacaaccg ctaccgtatc 600

ggaaattaca aactcaacac agatcatgca ggaagcaatg ataacatcgc cctcctggtc 660

cagtga 666

<210> 41
<211> 663
<212> DNA
<213> Artificial Sequence

<220>
<223> Uniform optimization of M protein

<400> 41
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gccgtgatca tcagaggcca cctgagaatg gccggccacc ccctgggcag atgcgacatc 480

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ggcgccagcc agagagtggg caccgacagc ggcttcgccg cctacaacag atacagaatc 600

ggcaactaca agctgaacac cgaccacgcc ggcagcaacg acaacatcgc cctgctggtg 660

cag 663

<210> 42
<211> 231
<212> DNA
<213> Artificial Sequence

<220>
<223> Fully optimized E protein

<400> 42
atgtacagct ttgtgtctga agaaacagga acgttgatag ttaatagtgt tttgcttttc 60

ttagcgttcg tagtcttcct tcttgtcaca cttgccattt taactgcgct tcgtctatgc 120

gcttactgtt gcaatatcgt aaacgtgtcg cttgttaaac caacggttta cgtatactcg 180

cgagttaaaa acctgaattc ttcagaaggt gttcctgatc tgctagtcta a 231

<210> 43
<211> 231
<212> DNA
<213> Artificial Sequence

<220>
<223> Uniform optimization of E protein

<400> 43
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ctggccttcg tggtgttcct gctggtgacc ctggccatcc tgaccgccct gcggctgtgc 120

gcctactgct gcaacatcgt gaacgtgagc ctggtgaagc ccaccgtgta cgtgtacagc 180

cgggtgaaga acctgaacag cagcgagggc gtgcccgacc tgctggtgtg a 231

<210> 44
<211> 3588
<212> DNA
<213> Artificial Sequence

<220>
<223> Minimal optimization of soluble S protein

<400> 44
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acgtttgacg acgtgcaggc ccccaactac acccagcata catccagcat gaggggcgtt 120

tactaccccg atgagatctt tagaagtgat actctgtatc tgactcagga cctgtttctg 180

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tgggtgttcg gcagtactat gaacaacaag tctcagtctg tgataataat caacaactcc 360

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tcgaagccca tgggcactca gacccataca atgatctttg ataacgcctt caactgcacc 480

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aacgtgtatg ccgatagctt cgtcgtcaag ggcgacgacg tcagacagat cgcccccggc 1200

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atacagaagg agatcgatag actgaacgag gtggccaaga acctgaacga gtctctgata 3540

gacctgcagg agctgggcaa gtacgagcag tacatcaagt ggccctgg 3588

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<211> 2049
<212> DNA
<213> Artificial Sequence

<220>
<223> Minimal optimization of soluble S1 protein

<400> 45
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acattcatgc tgaagtacga tgagaacggc acaattacgg atgccgtaga ttgcagtcag 840

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cataccgtgt ctctgctgag atctacctct cagaagagta tcgttgccta cactatgtcc 2040

ctgggcgcc 2049

<210> 46
<211> 1539
<212> DNA
<213> Artificial Sequence

<220>
<223> Minimal optimization of soluble S2 protein

<400> 46
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ataactaccg aggtgatgcc cgtgagcatg gccaagacat cggtagattg caacatgtac 120

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cccgatgtgg acctgggcga catttctggc ataaacgcct ccgtcgtcaa catccagaag 1440

gagatagata gactgaacga ggttgccaag aacctgaacg agtccctgat cgatctgcag 1500

gagctgggca agtacgagca gtatataaag tggccctgg 1539

<210> 47
<211> 1620
<212> DNA
<213> Artificial Sequence

<220>
<223> Minimal optimization of TPA-S protein

<400> 47
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agccccagcg cccggggcag tggcgacagc agcatcgcct attcgaacaa cactattgcc 120

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actagtgtag actgcaacat gtacatctgc ggcgactcta ctgagtgcgc caacctgctg 240

ctgcagtatg gctctttctg cacccagctg aacagagccc tgagtggcat cgccgccgag 300

caggaccgga acacaagaga ggttttcgcc caggtaaagc agatgtacaa gacccccact 360

ctgaagtatt ttggcggctt caacttctct cagatcctgc ccgatcccct gaagcccacc 420

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aagtttaacg gcctgacggt cctgcccccc ctgctgacag atgatatgat cgccgcctac 600

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aacgagtcac tgatcgatct gcaggagctg ggcaagtacg agcagtatat taagtggccc 1620

<210> 48
<211> 231
<212> DNA
<213> Artificial Sequence

<220>
<223> Minimal optimization of E protein

<400> 48
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<210> 49
<211> 1620
<212> DNA
<213> Artificial Sequence

<220>
<223> Minimal optimization of TPA-S2 protein

<400> 49
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ctgcagtatg gctctttctg cacccagctg aacagagccc tgagtggcat cgccgccgag 300

caggaccgga acacaagaga ggttttcgcc caggtaaagc agatgtacaa gacccccact 360

ctgaagtatt ttggcggctt caacttctct cagatcctgc ccgatccct gaagcccacc 420

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ctgcagatcc ccttcgccat gcagatggcc tatagattta acggcatagg cgtaactcag 720

aacgtcctgt acgagaacca gaagcagatc gccaaccagt ttaacaaggc catctcccag 780

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gccagtgtcg tgaacataca gaaggagatc gataggctga acgaggtggc caagaacctg 1560

aacgagtcac tgatcgatct gcaggagctg ggcaagtacg agcagtatat taagtggccc 1620

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<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence contain in VR9208

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tactaccccg acgagatttt cagaagcgac accctgtacc tcacccagga cctgttcctg 180

cccttctaca gcaacgtgac cggcttccac accatcaacc acaccttcgg caaccccgtg 240

atccctttca aggacggcat ctacttcgcc gccaccgaga agagcaatgt ggtgcggggc 300

tgggtgttcg gcagcaccat gaacaacaag agccagagcg tgatcatcat caacaacagc 360

accaacgtgg tgatccgggc ctgcaatttc gagctgtgcg acaacccttt cttcgccgtg 420

tccaaaccta tgggcaccca gacccacacc atgatcttcg acaacgcctt caactgcacc 480

ttcgagtaca tcagcgacgc cttcagcctg gatgtgagcg agaagagcgg caacttcaag 540

cacctgcggg agttcgtgtt caagaacaag gacggcttcc tgtacgtgta caagggctac 600

cagcccatcg acgtggtgag agacctgccc agcggcttca acaccctgaa gcccatcttc 660

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cacgtggaca ccagctacga gtgcgacatc cccatcggag ccggcatctg cgccagctac 1980

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<210> 51

<400> 51
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<210> 52

<400> 52
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<210> 53
<211> 231
<212> DNA
<213> Artificial Sequence

<220>
<223> Minimal optimization of E protein

<400> 53
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gcctactgct gcaacatcgt gaacgtctct ctggtaaagc ccacagttta cgtgtattct 180

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<210> 54
<211> 1542
<212> DNA
<213> Artificial Sequence

<220>
<223> Optimized soluble S2 protein with MET

<400> 54
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tacatctgcg gagattctac tgaatgtgct aatttgcttc tccaatatgg tagcttttgc 180

acacaactaa atcgtgcact ctcaggtatt gctgctgaac aggatcgcaa cacacgtgaa 240

gtgttcgctc aagtcaaaca aatgtacaaa accccaactt tgaaatattt tggtggtttt 300

aatttttcac aaatattacc tgaccctcta aagccaacta agaggtcttt tattgaggac 360

ttgctcttta ataaggtgac actcgctgat gctggcttca tgaagcaata tggcgaatgc 420

ctaggtgata ttaatgctag agatctcatt tgtgcgcaga agttcaatgg acttacagtg 480

ttgccacctc tgctcactga tgatatgatt gctgcctaca ctgctgctct agttagtggt 540

actgccactg ctggatggac atttggtgct ggcgctgctc ttcaaatacc ttttgctatg 600

caaatggcat ataggttcaa tggcattgga gttacccaaa atgttctcta tgagaaccaa 660

aaacaaatcg ccaaccaatt taacaaggcg attagtcaaa ttcaagaatc acttacaaca 720

acatcaactg cattgggcaa gctgcaagac gttgttaacc agaatgctca agcattaaac 780

acacttgtta aacaacttag ctctaatttt ggtgcaattt caagtgtgct aaatgatatc 840

ctttcgcgac ttgataaagt cgaggcggag gtacaaattg acaggttaat tacaggcaga 900

cttcaaagcc ttcaaaccta tgtaacacaa caactaatca gggctgctga aatcagggct 960

tctgctaatc ttgctgctac taaaatgtct gagtgtgttc ttggacaatc aaaaagagtt 1020

gacttttgtg gaaagggcta ccaccttatg tccttcccac aagcagcccc gcatggtgtt 1080

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tcttggttta ttacacagag gaacttcttt tctccacaaa taattactac agacaataca 1260

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tcaccagatg ttgatcttgg cgacatttca ggcattaacg cttctgtcgt caacattcaa 1440

aaagaaattg accgcctcaa tgaggtcgct aaaaatttaa atgaatcact cattgacctt 1500

caagaattgg gaaaatatga gcaatatatt aaatggcctt gg 1542

<210> 55
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> H-2Kd binding pepride

<400> 55

Thr Tyr Gln Arg Thr Arg Ala Leu Val
1 5

<210> 56
<211> 514
<212> PRT
<213> Artificial Sequence

<220>
<223> Optimized S2 protein with MET

<400> 56

Met Asp Ser Ser Ile Ala Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr
1 5 10 15

Asn Phe Ser Ile Ser Ile Thr Thr Glu Val Met Pro Val Ser Met Ala
20 25 30

Lys Thr Ser Val Asp Cys Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu
35 40 45

Cys Ala Asn Leu Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn
50 55 60

Arg Ala Leu Ser Gly Ile Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu
65 70 75 80

Val Phe Ala Gln Val Lys Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr
85 90 95

Phe Gly Gly Phe Asn Phe Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro
100 105 110

Thr Lys Arg Ser Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu
115 120 125

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Ala Asp Ala Gly Phe Met Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile
    130                 135                 140

Asn Ala Arg Asp Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val
145                 150                 155                 160

Leu Pro Pro Leu Leu Thr Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala
                165                 170                 175

Leu Val Ser Gly Thr Ala Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala
            180                 185                 190

Ala Leu Gln Ile Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly
        195                 200                 205

Ile Gly Val Thr Gln Asn Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala
    210                 215                 220

Asn Gln Phe Asn Lys Ala Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr
225                 230                 235                 240

Thr Ser Thr Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala
                245                 250                 255

Gln Ala Leu Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala
            260                 265                 270

Ile Ser Ser Val Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu
        275                 280                 285

Ala Glu Val Gln Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu
    290                 295                 300

Gln Thr Tyr Val Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala
305                 310                 315                 320

Ser Ala Asn Leu Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln
                325                 330                 335

Ser Lys Arg Val Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe
            340                 345                 350

Pro Gln Ala Ala Pro His Gly Val Val Phe Leu His Val Thr Tyr Val
        355                 360                 365
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Pro Ser Gln Glu Arg Asn Phe Thr Thr Ala Pro Ala Ile Cys His Glu
370 375 380

Gly Lys Ala Tyr Phe Pro Arg Glu Gly Val Phe Val Phe Asn Gly Thr
385 390 395 400

Ser Trp Phe Ile Thr Gln Arg Asn Phe Phe Ser Pro Gln Ile Ile Thr
405 410 415

Thr Asp Asn Thr Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly Ile
420 425 430

Ile Asn Asn Thr Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe
435 440 445

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

Asp Leu Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln
465 470 475 480

Lys Glu Ile Asp Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser
485 490 495

Leu Ile Asp Leu Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp
500 505 510

Pro Trp

<210> 57
<211> 1242
<212> DNA
<213> Artificial Sequence

<220>
<223> Fragment of S protein

<400> 57
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ggcgtgtact accccgacga gattttcaga agcgacaccc tgtacctcac ccaggacctg 180

ttcctgccct tctacagcaa cgtgaccggc ttccacacca tcaaccacac cttcggcaac 240

cccgtgatcc ctttcaagga cggcatctac ttcgccgcca ccgagaagag caatgtggtg 300

cggggctggg tgttcggcag caccatgaac aacaagagcc agagcgtgat catcatcaac 360

aacagcacca acgtggtgat ccgggcctgc aatttcgagc tgtgcgacaa ccctttcttc 420

gccgtgtcca aacctatggg cacccagacc cacaccatga tcttcgacaa cgccttcaac 480

tgcaccttcg agtacatcag cgacgccttc agcctggatg tgagcgagaa gagcggcaac 540

ttcaagcacc tgcgggagtt cgtgttcaag aacaaggacg gcttcctgta cgtgtacaag 600

ggctaccagc ccatcgacgt ggtgagagac ctgcccagcg gcttcaacac cctgaagccc 660

atcttcaagc tgcccctggg catcaacatc accaacttcc gggccatcct caccgccttt 720

agccctgccc aggatatctg gggcaccagc gccgctgcct acttcgtggg ctacctgaag 780

cctaccacct tcatgctgaa gtacgacgag aacggcacca tcaccgatgc cgtggactgc 840

agccagaacc ccctggccga gctgaagtgc agcgtgaaga gcttcgagat cgacaagggc 900

atctaccaga ccagcaactt cagagtggtg cctagcggcg atgtggtgag gttccccaat 960

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cctggccaga ccggcgtgat cgccgactac aactacaagc tt 1242

<210> 58
<211> 412
<212> PRT
<213> Artificial Sequence

<220>
<223> Fragment of S protein

<400> 58

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

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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
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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
405 410

<210> 59
<211> 1432
<212> DNA
<213> Artificial Sequence

<220>
<223> Fragment of S protein

<400> 59
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gccctgaact gctactggcc cctgaacgac tacggcttct acaccaccac cggcatcggc 240

tatcagccct acagagtggt ggtgctgagc ttcgagctgc tgaacgcccc tgccaccgtg 300

tgcggcccca agctgagcac cgacctcatc aagaaccagt gcgtgaactt caacttcaac 360

ggcctcaccg gcaccggcgt gctcaccccc agcagcaaga gattccagcc cttccagcag 420

ttcggcaggg acgtgagcga tttcaccgac agcgtgaggg atcctaagac cagcgagatc 480

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cacgccgacc agctcacccc cgcctggaga atctacagca ccggcaacaa cgtgttccag 660

acccaggccg gctgcctcat cggcgccgag cacgtggaca ccagctacga gtgcgacatc 720

cccatcggag ccggcatctg cgccagctac cacaccgtga gcctgctgag aagcaccagc 780

cagaagagca tcgtggccta caccatgagc ctgggcgccg acagcagcat cgcctacagc 840

aacaacacca tcgccatccc caccaacttc agcatctcca tcaccaccga ggtgatgccc 900

gtgagcatgg ccaagaccag cgtggattgc aacatgtaca tctgcggcga cagcaccgag 960

tgcgccaacc tgctgctgca gtacggcagc ttctgcaccc agctgaacag agccctgagc 1020

ggcattgccg ccgagcagga cagaaacacc agggaggtgt tcgcccaggt gaagcagatg 1080

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ggcgccggag ccgccctgca gatccccttc gccatgcaga tggcctaccg gt 1432

<210> 60
<211> 1118
<212> DNA
<213> Artificial Sequence

<220>
<223> Fragment of S protein

<400> 60
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ccctgggcaa gctgcaggac gtggtgaacc agaacgccca ggccctgaat accctggtga 180

agcagctgag cagcaacttc ggcgccatca gcagcgtgct gaacgacatc ctgagcaggc 240

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gaaagtacga gcagtacatc aagtggccct ggtacgtgtg gctgggcttc atcgccggcc 960

tcatcgccat cgtgatggtg accatcctgc tgtgctgcat gaccagctgc tgctcctgcc 1020

tgaagggcgc ctgcagctgt ggcagctgct gcaagttcga cgaggacgac tcagagcccg 1080

tgctgaaggg cgtgaagctg cactacacct gaagatct 1118

<210> 61
<211> 3780
<212> DNA
<213> Artificial Sequence

<220>
<223> Mutated S protein

<400> 61
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ggcgtgtact accccgacga gattttcaga agcgacaccc tgtacctcac ccaggacctg 180

ttcctgccct tctacagcaa cgtgaccggc ttccacacca tcaaccacac cttcggcaac 240

cccgtgatcc ctttcaagga cggcatctac ttcgccgcca ccgagaagag caatgtggtg 300

cggggctggg tgttcggcag caccatgaac aacaagagcc agagcgtgat catcatcaac 360

aacagcacca acgtggtgat ccgggcctgc aatttcgagc tgtgcgacaa ccctttcttc 420

gccgtgtcca aacctatggg cacccagacc cacaccatga tcttcgacaa cgccttcaac 480

tgcaccttcg agtacatcag cgacgccttc agcctggatg tgagcgagaa gagcggcaac 540

ttcaagcacc tgcgggagtt cgtgttcaag aacaaggacg gcttcctgta cgtgtacaag 600

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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

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Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
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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
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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

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
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Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
    610                 615                 620

Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
625                 630                 635                 640

His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
                645                 650                 655

Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
            660                 665                 670

Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
        675                 680                 685

Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
    690                 695                 700

Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
705                 710                 715                 720

Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
                725                 730                 735

Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
            740                 745                 750

Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
        755                 760                 765

Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe
    770                 775                 780

Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
785                 790                 795                 800

Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
                805                 810                 815

Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
            820                 825                 830

Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
        835                 840                 845

Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
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    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
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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
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Gly Val  Lys Leu His Tyr Thr
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aagcagagga gaccccaggg cctgcccaac aacaccgcca gctggttcac cgccctcacc    180
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<223> Uniform optimization of S2 protein with MET

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