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(54) Title: VECTORS FOR PRODUCING VIRUS-LIKE PARTICLES AND USES THEREOF

(57) Abstract: The present disclosure provides expression vectors and bacterial sequence-free vectors, such as ministring DNA (ms-DNA), for producing virus-like particles (VLPs) as well as compositions and methods thereof. In some aspects, the methods include treating viral infections in subjects with the vectors, compositions, and VLPs.

VECTORS FOR PRODUCING VIRUS-LIKE PARTICLES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This PCT application claims the priority benefit of U.S. Provisional Application Nos. 63/003,281, filed March 31, 2020, and 63/124,397, filed December 11, 2020, which are incorporated herein by reference in their entireties.

FIELD OF DISCLOSURE

[0002] The present disclosure provides vectors for producing virus-like particles (VLPs) and methods of treating subjects with the same.

BACKGROUND

[0003] Despite numerous advances in vaccine technologies, viral infections remain a prevalent health concern that are often under limited control. For example, the COVID-19 coronavirus pandemic became unlike anything the world had seen in over a century, both in terms of global spread and economic impact. It resulted in repeated shutdowns in much of the developed world, with continuously increasing death tolls and new infections.

[0004] COVID-19 causes a respiratory infection, along with acute respiratory distress syndrome in severe cases. Pre/asymptomatic airborne transmission and high viral titre early in the course of the disease significantly increase the infectiousness of COVID-19 compared to other coronaviruses such as SARS-CoV, making the development of vaccines critical for management of the pandemic.

[0005] VLPs represent potent vaccine candidates that mimic viral physicochemical properties and structure without potentiating viral growth (Cimica, V., & Galarza, J. M., *Clin. Immunol. 183*: 99–108 (2017)). As such, they confer strong humoral responses, but often limited cell-mediated responses against the 'whole virus' as they remain exogenously administered antigens. Furthermore, their production, purification, and storage are costly.

[0006] Existing vaccines have often shown limited cross-protection among different viral strains, complicated by the fact that viruses continue to mutate their genomes in response to evolutionary pressures.

[0007] There is a need for improved VLPs and methods of treating viral infections.

BRIEF SUMMARY

[0008] The present disclosure is directed to an expression vector comprising: an expression cassette that comprises a nucleic acid sequence encoding a recombinant protein comprising a conserved amino acid sequence from a virus fused to an immunogenic amino acid sequence, a target sequence for a first recombinase flanking each side of the expression cassette, and one or more additional target sequences for one or more additional recombinases integrated within non-binding regions of the target sequence for the first recombinase, wherein protein expressed intracellularly from the expression cassette is capable of forming a virus-like particle (VLP).

[0009] In some aspects, the immunogenic amino acid sequence is from the same virus as the conserved amino acid sequence. In some aspects, the conserved amino acid sequence is from a viral glycoprotein. In some aspects, the immunogenic amino acid sequence is from the same viral glycoprotein.

[0010] In some aspects, the expression cassette further comprises a nucleic acid sequence encoding a viral envelope protein and/or a nucleic acid sequence encoding a viral matrix protein. In some aspects, the viral envelope protein and/or the viral matrix protein are from the same virus as the conserved amino acid sequence.

[0011] In some aspects, the conserved amino acid sequence, the immunogenic amino acid sequence, the viral envelope protein, and/or the viral matrix protein is a consensus sequence.

[0012] In some aspects, the recombinant protein is capable of stimulating an immune response against the virus comprising neutralizing antibodies.

[0013] In some aspects, the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against the virus.

[0014] In some aspects, the immune response is cross-reactive to a related virus or strain.

[0015] In some aspects, the recombinant protein excludes amino acid sequences from the virus that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

[0016] In some aspects, the expression cassette comprises a single open reading frame comprising a nucleic acid sequence encoding a self-cleaving peptide between each nucleic acid sequence encoding a protein.

[0017] In some aspects, the virus is a coronavirus, an influenza virus, a human immunodeficiency virus, a human papillomavirus, a hepatitis virus, or an oncolytic virus.

[0018] In some aspects, the virus is a coronavirus. In some aspects, the coronavirus is COVID-19.

[0019] In some aspects, the expression cassette comprises nucleic acid sequences encoding a coronavirus Membrane (M) protein, a coronavirus Envelope (E) protein, and a recombinant protein comprising a conserved amino acid sequence and an immunogenic amino acid sequence from a coronavirus Spike (S) protein. In some aspects, the conserved amino acid sequence is from the S protein S2' cleavage site and internal fusion peptide (IFP).

[0020] In some aspects, the conserved amino acid sequence comprises SEQ ID NO:12.

[0021] In some aspects, the immunogenic amino acid sequence is from the S protein receptor-binding domain (RBD).

[0022] In some aspects, the immunogenic amino acid sequence is at least about 90% identical to SEQ ID NO:11.

[0023] In some aspects, the recombinant protein further comprises a transmembrane (TM) domain sequence from the S protein.

[0024] In some aspects, the recombinant protein excludes amino acid sequences from the S protein that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

[0025] In some aspects, the amino acid sequence of the recombinant protein is at least about 90% identical to SEQ ID NO:55.

[0026] In some aspects, the expression cassette comprises a single open reading frame translated as an amino acid sequence at least about 90% identical to SEQ ID NO:57.

[0027] In some aspects, the recombinant protein is capable of stimulating an immune response against COVID-19.

[0028] In some aspects, the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against COVID-19.

[0029] In some aspects, the immune response is cross-reactive to other coronaviruses. In some aspects, the immune response is cross-reactive to other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses.

[0030] In some aspects, the target sequence for the first recombinase and the one or more additional target sequences for the one or more additional recombinases are selected from the group consisting of the PY54 pal site, the N15 telRL site, the loxP site, φK02 telRL site, the FRT site, the phiC31 attP site, and the λ attP site. In some aspects, the expression vector comprises each of the target sequences. In some aspects, the expression vector comprises the Tel recombinase pal site and the telRL, loxP, and FRT recombinase target binding sequences integrated within the pal site.

[0031] In some aspects, the expression vector is for producing a bacterial sequence-free vector. In some aspects, the bacterial sequence-free vector has circular covalently closed ends. In some aspects, the bacterial sequence-free vector has linear covalently closed ends.

[0032] In some aspects, the expression vector further comprises at least one enhancer sequence flanking each side of the target sequence for the first recombinase. In some aspects, the at least one enhancer sequence is at least two enhancer sequences. In some aspects, the at least one enhancer sequence is a SV40 enhancer sequence.

[0033] The present disclosure is directed to a vector production system comprising recombinant cells designed to encode at least a first recombinase under the control of an inducible promoter, wherein the cells comprise any of the above expression vectors. In some aspects, the inducible promoter is thermally-regulated, chemically-regulated, IPTG regulated, glucose-regulated, arabinose inducible, T7 polymerase regulated, cold-shock inducible, pH inducible, or combinations thereof. In some aspects, the first recombinase is selected from telN and tel, and the expression vector incorporates the target sequence for at least the first recombinase. In some aspects, the recombinant cells have been further designed to encode a nuclease genome editing system, and wherein the expression vector further comprises a backbone sequence containing a cleavage site for the nuclease genome editing system. In some aspects, the nuclease genome editing system is a

CRISPR nuclease system comprising a Cas nuclease and gRNA, and the expression vector comprises a target sequence for the gRNA within the backbone sequence.

[0034] The present disclosure is directed to a method of producing a bacterial sequence-free vector comprising incubating any of the above vector production systems under suitable conditions for expression of the first recombinase.

[0035] The present disclosure is directed to a method of producing a bacterial sequence-free vector comprising incubating any of the above vector production systems that comprise recombinant cells designed to encode a nuclease genome editing system under suitable conditions for expression of the first recombinase and the nuclease genome editing system.

[0036] In some aspects, any of the above methods of producing a bacterial sequence-free vector further comprise harvesting the bacterial sequence-free vector.

[0037] The present disclosure is directed to a bacterial sequence-free vector produced by any of the above methods of producing a bacterial sequence-free vector.

[0038] The present disclosure is directed to a bacterial sequence-free vector comprising an expression cassette that comprises a nucleic acid sequence encoding a recombinant protein comprising a conserved amino acid sequence from a virus fused to an immunogenic amino acid sequence, wherein protein expressed intracellularly from the expression cassette is capable of forming a VLP.

[0039] In some aspects, the immunogenic amino acid sequence is from the same virus as the conserved amino acid sequence. In some aspects, the conserved amino acid sequence is from a viral glycoprotein. In some aspects, the immunogenic amino acid sequence is from the same viral glycoprotein.

[0040] In some aspects, the expression cassette further comprises a nucleic acid sequence encoding a viral envelope protein and/or a nucleic acid sequence encoding a viral matrix protein. In some aspects, the viral envelope protein and/or the viral matrix protein are from the same virus as the conserved amino acid sequence.

[0041] In some aspects, the conserved amino acid sequence, the immunogenic amino acid sequence, the viral envelope protein, and/or the viral matrix protein is a consensus sequence.

[0042] In some aspects, the recombinant protein is capable of stimulating an immune response against the virus comprising neutralizing antibodies.

[0043] In some aspects, the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against the virus.

[0044] In some aspects, the immune response is cross-reactive to a related virus or strain.

[0045] In some aspects, the recombinant protein excludes amino acid sequences from the virus that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

[0046] In some aspects, the expression cassette comprises a single open reading frame comprising a nucleic acid sequence encoding a self-cleaving peptide between each nucleic acid sequence encoding a protein.

[0047] In some aspects, the virus is a coronavirus, an influenza virus, a human immunodeficiency virus, a human papillomavirus, a hepatitis virus, or an oncolytic virus.

[0048] In some aspects, the virus is a coronavirus. In some aspects, the coronavirus is COVID-19.

[0049] In some aspects, the expression cassette comprises nucleic acid sequences encoding a coronavirus M protein, a coronavirus E protein, and a recombinant protein comprising a conserved amino acid sequence and an immunogenic amino acid sequence from a coronavirus S protein. In some aspects, the conserved amino acid sequence is from the S protein S2' cleavage site and IFP.

[0050] In some aspects, the conserved amino acid sequence comprises SEQ ID NO:12.

[0051] In some aspects, the immunogenic amino acid sequence is from the S protein RBD.

[0052] In some aspects, the immunogenic amino acid sequence is at least about 90% identical to SEQ ID NO:11.

[0053] In some aspects, the recombinant protein further comprises a TM domain sequence from the S protein.

[0054] In some aspects, the recombinant protein excludes amino acid sequences from the S protein that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

[0055] In some aspects, the amino acid sequence of the recombinant protein is SEQ ID NO:55.

[0056] In some aspects, the expression cassette comprises a single open reading frame translated as an amino acid sequence at least about 90% identical to SEQ ID NO:57.

[0057] In some aspects, the recombinant protein is capable of stimulating an immune response against COVID-19.

[0058] In some aspects, the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against COVID-19.

[0059] In some aspects, the immune response is cross-reactive to other coronaviruses. In some aspects, the immune response is cross-reactive to other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses.

[0060] In some aspects, the bacterial sequence-free vector further comprises at least one enhancer sequence flanking each side of the expression cassette. In some aspects, the at least one enhancer sequence is at least two enhancer sequences. In some aspects, the at least one enhancer sequence is a SV40 enhancer sequence.

[0061] In some aspects, the bacterial sequence-free vector comprises circular covalently closed ends.

[0062] In some aspects, the bacterial sequence-free vector comprises linear covalently closed ends.

[0063] The present disclosure is directed to a polynucleotide encoding an amino acid sequence at least about 90% identical to SEQ ID NO:57.

[0064] The present disclosure is directed to a recombinant cell comprising any of the above expression vectors or any of the above bacterial sequence-free vectors.

[0065] In some aspects, the present disclosure is directed to a method of producing a VLP, comprising culturing the recombinant cell under suitable conditions for production of the VLP from the expression vector or the bacterial sequence-free vector.

[0066] In some aspects, the method of producing a VLP further comprises isolating the VLP. In some aspects, the isolating is by affinity purification. In some aspects, the VLP is produced by any of the above expression vectors or any of the above bacterial sequence-free vectors wherein the virus is a coronavirus. In some aspects, the affinity purification comprises an angiotensin-converting enzyme 2 (ACE2) receptor peptide or an anti-S protein monoclonal antibody. In some aspects, the ACE2 receptor peptide comprises an amino acid sequence that is at least about 90% identical to the amino acid sequence of SEQ ID NO:70. In some aspects, the ACE2 receptor peptide comprises a biotin acceptor peptide (BAP) tag at the C-terminus or N-terminus of the peptide. In some aspects, the BAP tag comprises an amino acid sequence at least about 90% identical to the amino acid

sequence of SEQ ID NO:71. In some aspects, the ACE2 receptor peptide or anti-S protein monoclonal antibody is biotinylated and immobilized on a streptavidin-coated bead. In some aspects, the affinity purification comprises microfluidics and/or chromatography. In some aspects, the present disclosure is directed to a VLP produced by any of the methods of producing a VLP.

[0067] The present disclosure is directed to a VLP comprising a recombinant protein comprising a conserved amino acid sequence from a virus fused to an immunogenic amino acid sequence. In some aspects, the immunogenic amino acid sequence is from the same virus as the conserved amino acid sequence. In some aspects, the conserved amino acid sequence is from a viral glycoprotein. In some aspects, the immunogenic amino acid sequence is from the same viral glycoprotein.

[0068] In some aspects, the VLP further comprises a viral envelope protein and/or a viral matrix protein. In some aspects, the viral envelope protein and/or the viral matrix protein are from the same virus as the conserved amino acid sequence.

[0069] In some aspects, the conserved amino acid sequence, the immunogenic amino acid sequence, the viral envelope protein, and/or the viral matrix protein is a consensus sequence.

[0070] In some aspects, the recombinant protein is capable of stimulating an immune response against the virus comprising neutralizing antibodies.

[0071] In some aspects, the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against the virus.

[0072] In some aspects, the immune response is cross-reactive to a related virus or strain.

[0073] In some aspects, the recombinant protein excludes amino acid sequences from the virus that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

[0074] In some aspects, the virus is a coronavirus, an influenza virus, a human immunodeficiency virus, a human papillomavirus, a hepatitis virus, or an oncolytic virus. In some aspects, the virus is a coronavirus.

[0075] In some aspects, the coronavirus is COVID-19.

[0076] In some aspects, the VLP comprises a coronavirus Membrane (M) protein, a coronavirus Envelope (E) protein, and a recombinant protein comprising a conserved

amino acid sequence and an immunogenic amino acid sequence from a coronavirus Spike (S) protein.

[0077] In some aspects, the conserved amino acid sequence is from the S protein S2' cleavage site and internal fusion peptide (IFP).

[0078] In some aspects, the conserved amino acid sequence comprises SEQ ID NO:12.

[0079] In some aspects, the immunogenic amino acid sequence is from the S protein receptor-binding domain (RBD).

[0080] In some aspects, the immunogenic amino acid sequence is at least about 90% identical to SEQ ID NO:11.

[0081] In some aspects, the recombinant protein further comprises a transmembrane (TM) domain sequence from the S protein.

[0082] In some aspects, the recombinant protein excludes amino acid sequences from the S protein that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

[0083] In some aspects, the amino acid sequence of the recombinant protein is at least about 90% identical to SEQ ID NO:55.

[0084] The present disclosure is directed to a VLP comprising a recombinant protein at least about 90% identical to SEQ ID NO:55, an M protein at least about 90% identical to SEQ ID NO:1, and an E protein at least about 90% identical to SEQ ID NO:3.

[0085] In some aspects, the recombinant protein is capable of stimulating an immune response against COVID-19.

[0086] In some aspects, the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against COVID-19.

[0087] In some aspects, the immune response is cross-reactive to other coronaviruses.

[0088] In some aspects, the immune response is cross-reactive to other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses.

[0089] The present disclosure is directed to a composition comprising any of the above expression vectors, any of the above bacterial sequence-free vectors, or any of the above virus-like particles. In some aspects, the composition further comprises a delivery agent. In some aspects, the delivery agent is a nanoparticle. In some aspects, the delivery agent comprises a targeting ligand. In some aspects, the targeting ligand comprises a S protein

peptide. In some aspects, the S protein peptide comprises an amino acid sequence at least about 90% identical to any one of SEQ ID NOs:76-99.

[0090] The present disclosure is directed to a method of treating a viral infection in a subject, comprising administering to the subject any of the above expression vectors, any of the above bacterial sequence-free vectors, any of the above VLPs, or any of the above compositions, wherein intracellular expression of the expression vector or the bacterial sequence-free vector produces a VLP.

[0091] In some aspects, the administering is by parenteral or non-parenteral administration. In some aspects, the administering is by oral, pulmonary, intranasal, intravenous, epidermal, transdermal, subcutaneous, intramuscular, or intraperitoneal administration, or by inhalation.

[0092] In some aspects, the VLP stimulates an immune response in the subject comprising neutralizing antibodies against the viral infection.

[0093] In some aspects, the VLP stimulates a Th1 cell-mediated immune response in the subject against the viral infection.

[0094] In some aspects, the immune response is cross-reactive to a related virus or strain.

[0095] In some aspects, the VLP does not stimulate an immune response comprising non-neutralizing antibodies in the subject and/or does not stimulate a Th2 cell-mediated immune response in the subject.

[0096] In some aspects, the VLP cross-competes with the infecting virus for binding to a viral receptor.

[0097] In some aspects, the VLP cross-competes with a related virus or strain for binding to the viral receptor.

[0098] In some aspects, the viral infection is a coronavirus, an influenza virus, a human immunodeficiency virus, a human papillomavirus, a hepatitis virus, or an oncolytic virus.

[0099] In some aspects, the viral infection is a coronavirus. In some aspects, the viral infection is COVID-19.

[0100] In some aspects, the VLP stimulates an immune response in the subject comprising neutralizing antibodies against COVID-19.

[0101] In some aspects, the VLP stimulates a Th1 cell-mediated immune response in the subject against COVID-19.

[0102] In some aspects, the immune response is cross-reactive to other coronaviruses.

[0103] In some aspects, the immune response is cross-reactive to other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses.

[0104] In some aspects, the VLP does not stimulate an immune response comprising non-neutralizing antibodies in the subject and/or does not stimulate a Th2 cell-mediated immune response in the subject.

[0105] In some aspects, the administering is by inhalation.

[0106] In some aspects, the VLP cross-competes with COVID-19 for binding to ACE2 receptor, neuropilin-1, or other receptors.

[0107] In some aspects, the VLP cross-competes with other coronaviruses for binding to ACE2 receptor, neuropilin-1, and/or other receptors.

[0108] In some aspects, the VLP cross-competes with other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses for binding to ACE2 receptor, neuropilin-1, and/or other receptors.

BRIEF DESCRIPTION OF THE DRAWINGS

[0109] **Figure 1** shows a schematic representation of an exemplary expression cassette for producing a coronavirus VLP containing simian virus 40 enhancers (SV40E); a cytomegalovirus promoter (P_{CMV}); a sequence encoding a coronavirus Envelope (E) protein; a sequence encoding a coronavirus Membrane (M) protein; a sequence encoding a recombinant protein containing sequences from the receptor-binding domain (RBD), the second subunit cleavage domain and internal fusion peptide (S2'IFP), and transmembrane (TM) domain of a coronavirus S protein (referred to herein as a recombinant Spike (S) protein, RBD::S2'IFP::TM); sequences encoding 2A self-cleaving peptides from porcine teschovirus-1 (P2A) to separate the protein-encoding sequences of the expression cassette; and a polyadenylation (pA) signal.

[0110] **Figure 2** shows a vector map of an exemplary expression vector (pGL2-SS-CMV-VLP-BGH-SS) containing an expression cassette as described in Figure 1, in which the pA signal is from bovine growth hormone.

[0111] **Figure 3** shows in vitro expression of genes and protein from the expression vector of Figure 2. **(A)** shows a bar graph depicting relative expression of genes encoding the E protein, M protein, and recombinant S protein (RBD::S2'IFP::TM) as described in Figure 1 from cells containing the expression vector of Figure 2 (VLP) as well as control

cells without the expression vector (CTL). *** = $p<0.001$ and **** = $p<0.0001$. **(B)** shows a representative Western blot depicting expression of the recombinant S protein using an antibody that binds to the RBD (α-Spike (RBD)). Detection of beta-actin with the α-beta-actin antibody served as a loading control. Control = protein from cells without the expression vector. VLP = protein from cells containing the expression vector of Figure 2. **(C)** shows the relative mean intensity of recombinant S protein expression from Western blots (n=3) as described in (B).

[0112] **Figure 4** shows an exemplary msDNA-VLP (msDNA VLP Cov 19-BGH poly) as described herein that is encoded by the expression vector of Figure 2.

[0113] **Figure 5** shows the concentration (ng/mL) of antibodies that bind to the S1 subunit of the COVID-19 Spike protein (Spike AB) in serum from C57 mice at days 0, 7, 14, 21, 28, 35, 42, and 49 following intramuscular injection with the expression vector of Figure 2 at day 0 and day 14 (booster). **(A)** and **(B)** show a line graph and a bar graph of the antibody concentration, respectively.

[0114] **Figure 6** shows a sequence conservation analysis of representative COVID-19 genomes. **(A)** shows a bar plot in which the horizontal bars indicate the genomic positions on the x-axis of each of the COVID-19 genes listed on the y-axis as per the Wuhan reference genome (NC_045512.2). **(B)** shows a histogram in which bar heights correspond to the percentage of 3928 representative COVID-19 genomes that differed from the Wuhan reference genome at each genomic position.

[0115] **Figure 7** and **Figure 8** show histograms in which bar heights correspond to the percentage of analyzed genomes that differed from the Wuhan reference genome at each genomic position, with the analyzed genomes being: **(7)** 3928 representative COVID-19 genomes, 120 severe acute respiratory syndrome coronaviruses (SARS-CoV) genomes, and 257 Middle East respiratory syndrome coronaviruses (MERS-CoV) genomes, **(8A)** 233 COVID-19 genomes of variant strain B.1.1.7, **(8B)** 104 COVID-19 genomes of variant strain B.1.351, **(8C)** 39 COVID-19 genomes of variant strain P.1, and **(8D)** 62 COVID-19 genomes of variant strain B.1.427/429.

[0116] **Figure 9** shows an exemplary eukaryotic expression vector (pFastBac™ Dual-VLP) for VLP production in eukaryotic cells as described herein, containing the E, M, and recombinant S proteins as described in Figure 1.

DETAILED DESCRIPTION

[0117] The present disclosure provides expression vectors and bacterial sequence-free vectors (*e.g.*, ministring DNA (msDNA)) for producing virus-like particles (VLPs), vector production systems, and VLPs, as well as compositions and methods thereof. Some aspects of the present disclosure are directed to treating viral infections in a subject (*e.g.*, coronavirus infections in a human subject, such as COVID-19).

[0118] All publications cited herein are hereby incorporated by reference in their entireties, including without limitation all journal articles, books, manuals, patent applications, and patents cited herein, to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

I. Terms

[0119] In order that the present disclosure can be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

[0120] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0121] The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0122] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0123] The terms "about" or "comprising essentially of" refer to a value or composition that is within an acceptable error range for the particular value or composition as

determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" or "comprising essentially of" can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 10%. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value or composition.

[0124] As described herein, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Numeric ranges are inclusive of the numbers defining the range.

[0125] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 5th ed., 2013, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, 2006, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0126] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form.

[0127] Unless otherwise indicated, nucleotide sequences are written left to right in 5' to 3' orientation. Amino acid sequences are written left to right in amino to carboxy orientation.

[0128] The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0129] "Amino acid" is a molecule having the structure wherein a central carbon atom (the alpha-carbon atom) is linked to a hydrogen atom, a carboxylic acid group (the carbon atom of which is referred to herein as a "carboxyl carbon atom"), an amino group (the nitrogen atom of which is referred to herein as an "amino nitrogen atom"), and a side chain group, R. When incorporated into a peptide, polypeptide, or protein, an amino acid loses one or more atoms of its amino acid carboxylic groups in the dehydration reaction that links one amino acid to another. As a result, when incorporated into a protein, an amino acid is referred to as an "amino acid residue."

[0130] "Protein" or "polypeptide" refers to any polymer of two or more individual amino acids (whether or not naturally occurring) linked via a peptide bond, and occurs when the carboxyl carbon atom of the carboxylic acid group bonded to the alpha-carbon of one amino acid (or amino acid residue) becomes covalently bound to the amino nitrogen atom of amino group bonded to the non alpha-carbon of an adjacent amino acid. The term "protein" is understood to include the terms "polypeptide" and "peptide" (which, at times may be used interchangeably herein) within its meaning. In addition, proteins comprising multiple polypeptide subunits will also be understood to be included within the meaning of "protein" as used herein. Similarly, fragments of proteins and polypeptides are also within the scope of the disclosure and may be referred to herein as "proteins." In one aspect of the disclosure, a polypeptide comprises a chimera of two or more parental peptide segments. The term "polypeptide" is also intended to refer to and encompass the products of post-translation modification ("PTM") of the polypeptide, including without limitation disulfide bond formation, glycosylation, carbamylation, lipidation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, modification by non-naturally occurring amino acids, or any other manipulation or modification, such as conjugation with a labeling component. A polypeptide can be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis. An "isolated" polypeptide or a fragment, variant, or derivative thereof refers to a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can simply be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered

isolated for the purpose of the disclosure, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0131] "Domain" as used herein can be used interchangeably with the term "peptide segment" and refers to a portion or fragment of a larger polypeptide or protein. A domain need not on its own have functional activity, although in some instances, a domain can have its own biological activity.

[0132] "Fused," "operably linked," and "operably associated" are used interchangeably herein when referring to two or more domains to broadly refer to any chemical or physical coupling of the two or more domains in the formation of a recombinant polypeptide as disclosed herein. In one embodiment, a recombinant polypeptide as disclosed herein is a chimeric polypeptide comprising a plurality of domains from two or more different polypeptides.

[0133] Recombinant polypeptides (*i.e.*, recombinant proteins) comprising two or more domains and/or proteins as disclosed herein can be encoded by a single coding sequence that comprises polynucleotide sequences encoding each domain and/or protein. Unless stated otherwise, the polynucleotide sequences encoding each domain and/or protein are "in frame" such that translation of a single mRNA comprising the polynucleotide sequences results in a single polypeptide comprising each domain and/or protein. Typically, the domains and/or proteins in a recombinant polypeptide as described herein will be fused directly to one another or will be separated by a peptide linker. Various polynucleotide sequences encoding peptide linkers are known in the art and include, for example, self-cleaving peptides.

[0134] "Polynucleotide" or "nucleic acid" as used herein refers to a polymeric form of nucleotides. In some instances, a polynucleotide comprises a sequence that is either not immediately contiguous with the coding sequences or is immediately contiguous (on the 5′ end or on the 3′ end) with the coding sequences in the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, a cDNA) independent of other sequences. The nucleotides of the disclosure can be ribonucleotides, deoxyribonucleotides, or modified forms of either

nucleotide. A polynucleotide as used herein refers to, among others, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. The term polynucleotide encompasses genomic DNA or RNA (depending upon the organism, *i.e.*, RNA genome of viruses), as well as mRNA encoded by the genomic DNA, and cDNA. In certain embodiments, a polynucleotide comprises a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). By "isolated" nucleic acid or polynucleotide is intended a nucleic acid molecule, e.g., DNA or RNA, which has been removed from its native environment. For example, a nucleic acid molecule comprising a polynucleotide encoding a recombinant polypeptide contained in a vector is considered "isolated" for the purposes of the present disclosure. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) from other polynucleotides in a solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides of the present disclosure. Isolated polynucleotides or nucleic acids according to the present disclosure further include polynucleotides and nucleic acids (*e.g.*, nucleic acid molecules) produced synthetically.

[0135] As used herein, a "coding region" or "coding sequence" is a portion of a polynucleotide, which consists of codons translatable into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is typically 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, introns, and the like, are not part of a coding region. The boundaries of a coding region are typically determined by a start codon at the 5' terminus, encoding the amino-terminus of the resultant polypeptide, and a translation stop codon at the 3' terminus, encoding the carboxyl-terminus of the resulting polypeptide.

[0136] As used herein, the term "expression control region" refers to a transcription control element that is operably associated with a coding region to direct or control expression of the product encoded by the coding region, including, for example, promoters, enhancers, operators, repressors, ribosome binding sites, translation leader

sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites, stem-loop structures, and transcription termination signals. For example, a coding region and a promoter are "operably associated" (*i.e.*, "operably linked") if induction of promoter function results in the transcription of mRNA comprising a coding region that encodes the product, and if the nature of the linkage between the promoter and the coding region does not interfere with the ability of the promoter to direct the expression of the product encoded by the coding region or interfere with the ability of the DNA template to be transcribed. Expression control regions include nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding region, and which influence the transcription, RNA processing, stability, or translation of the associated coding region. If a coding region is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0137] As used herein, the terms "host cell" and "cell" can be used interchangeably and can refer to any type of cell or a population of cells, *e.g.*, a primary cell, a cell in culture, or a cell from a cell line, that harbors or is capable of harboring a nucleic acid molecule (*e.g.*, a recombinant nucleic acid molecule). Host cells can be a prokaryotic cell, or alternatively, the host cells can be eukaryotic, for example, fungal cells, such as yeast cells, and various animal cells, such as insect cells or mammalian cells.

[0138] "Culture," "to culture" and "culturing," as used herein, means to incubate cells under *in vitro* conditions that allow for cell growth or division or to maintain cells in a living state. "Cultured cells," as used herein, means cells that are propagated *in vitro*.

[0139] A "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes, but is not limited to, vertebrates such as mammals, avians, pets, farm animals, nonhuman primates, sheep, cows, goats, pigs, chickens, dogs, cats, and rodents such as mice, rats, and guinea pigs. In preferred aspects, the subject is a human. The terms, "subject" and "patient" are used interchangeably herein.

[0140] "Administering" refers to the physical introduction of a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art.

[0141] The terms "treat," "treating," "treatment," or "therapy" of a subject as used herein, refer to any type of intervention or process performed on, or administering an active agent

to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, or slowing down or preventing the progression, development, severity or recurrence of a symptom, complication, condition or biochemical indicia associated with a disease or enhancing overall survival. Treatment can be of a subject having a disease or a subject who does not have a disease (*e.g.,* for prophylaxis, such as vaccination).

[0142] The term "effective dose" "effective dosage," or "effective amount" is defined as an amount sufficient to achieve or at least partially achieve a desired effect. A "therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent is any amount of the drug that, when used alone or in combination with another therapeutic agent, promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, an increase in overall survival (the length of time from either the date of diagnosis or the start of treatment for a disease that patients diagnosed with the disease are still alive), or a prevention of impairment or disability due to the disease affliction. A therapeutically effective amount or dosage of a drug includes a "prophylactically effective amount" or a "prophylactically effective dosage", which is any amount of the drug that, when administered alone or in combination with another therapeutic agent to a subject at risk of developing a disease or of suffering a recurrence of disease, inhibits the development or recurrence of the disease. The ability of a therapeutic agent to promote disease regression or inhibit the development or recurrence of the disease can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

[0143] Various aspects of the disclosure are described in further detail in the following subsections.

II. Vectors for producing VLPs

[0144] Bacterial sequence-free vectors and their production are described in U.S. Patent Nos. 9,290,778 and 9,862,954; Nafissi and Slavcev, *Microbial Cell Factories 11*:154 (2012); and Nafissi *et al.*, *Nucleic Acids 3*(6):e165 (2014), incorporated by reference herein in their entireties. These bacterial sequence-free vectors are produced from an expression vector (*e.g.*, a plasmid) that contains specialized "Super Sequence" ("SS") sites comprising target sequences for recombinases. The SS sites flank an expression

cassette containing a nucleic acid(s) of interest. When the expression vector is present in a recombinant cell that expresses an appropriate recombinase, bacterial sequence-free vector containing the expression cassette is separated from the backbone DNA of the expression vector. To produce a circular covalently closed (CCC) bacterial sequence-free vector, a production system is used in which the recombinant cell expresses a Cre or Flp recombinase, for example, and the expression vector contains corresponding target sequences for the recombinases. To produce a linear covalently closed (LCC) bacterial sequence-free vector, also referred to herein as a ministring DNA (msDNA), a production system is used in which the recombinant cell expresses a TelN or Tel recombinase, for example, and the expression vector contains corresponding target sequences for the recombinases The bacterial sequence-free vector can then be purified from the cells and used directly as a delivery vector. *See* U.S. Patent Nos. 9,290,778 and 9,862,954, Nafissi and Slavcev, and Nafissi *et al.*

[0145] msDNA vectors with LCC ends are torsion-free and not subject to gyrase-directed negative supercoiling during their production in *E. coli*. Exemplary msDNA vectors carry an expression cassette with a eukaryotic promoter, gene of interest (GOI), intron, and polyA sequence, and nuclear translocation enhancing sequences (Nafissi and Slavcev, and Nafissi *et al.*). Furthermore, due to its double stranded LCC topology, integration of msDNA into a cell's chromosome causes a chromosomal break, thereby eliminating the cell from the population. Thus, msDNA eliminates any risk of insertional mutagenesis, protecting patients who are administered the msDNA from potential genotoxicity and cancer (Nafissi *et al.*).

[0146] In some aspects, bacterial sequence-free vectors for producing VLPs as disclosed herein include CCC or LCC vectors produced according to any other method known in the art.

A. Expression vectors, expression cassettes, and vector production systems for producing bacterial sequence-free vectors and VLPs

[0147] Provided herein is an expression vector comprising: an expression cassette that comprises a nucleic acid sequence encoding a recombinant protein comprising a conserved amino acid sequence from a virus fused to an immunogenic amino acid sequence, wherein protein expressed intracellularly from the expression cassette is capable of forming a VLP.

[0148] Provided herein is an expression vector comprising: an expression cassette that comprises a nucleic acid sequence encoding a recombinant protein comprising a conserved amino acid sequence from a virus fused to an immunogenic amino acid sequence, a target sequence for a first recombinase flanking each side of the expression cassette, and one or more additional target sequences for one or more additional recombinases integrated within non-binding regions of the target sequence for the first recombinase, wherein protein expressed intracellularly from the expression cassette is capable of forming a VLP.

[0149] Conserved and immunogenic amino acid sequences include those known in the art as well as those determined through known techniques. For example, genome-based reverse vaccinology can be applied towards comparative genomics analysis, a field of biological research that can be used to compare genomic sequences between different pathogenic strains (*see*, *e.g.*, Sieb *et al.*, *Clin. Microbiol. Infect. 18*(Suppl. 5):109-116 (2012)). Other sequencing, structural, and computational approaches can also be used (*see*, *e.g.*, Liljeroos *et al.*, *J. Immunol. Res.* 2015: 156241; Sette and Rappuoli, *Immunity 33*(4):530-541 (2010)).

[0150] In some aspects, the immunogenic amino acid sequence is from the same virus as the conserved amino acid sequence. In some aspects, the conserved amino acid sequence is from a viral glycoprotein. In some aspects, the immunogenic amino acid sequence is from the same viral glycoprotein.

[0151] In some aspects, the expression cassette further comprises a nucleic acid sequence encoding a viral envelope protein and/or a nucleic acid sequence encoding a viral matrix protein. In some aspects, the viral envelope protein and/or the viral matrix protein are from the same virus as the conserved amino acid sequence.

[0152] In some aspects, the conserved amino acid sequence, the immunogenic amino acid sequence, the viral envelope protein, and/or the viral matrix protein is a consensus sequence.

[0153] In some aspects, the recombinant protein is capable of stimulating an immune response against the virus comprising neutralizing antibodies. Conserved sites, for example, are often recognized by broadly neutralizing antibodies and are susceptible to antibody inactivation (*see*, *e.g.*, Nabel, *N. Engl. J. Med. 368*(6): 551-560 (2013)).

[0154] In some aspects, the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against the virus. Cell-mediated immunity is the process by which cytotoxic T cells recognize antigen infected cells, to induce cell lysis.

[0155] In some aspects, the immune response is cross-reactive to a related virus or strain. For example, conserved sequences among different viral serotypes/strains can be utilized to provide protection against multiple serotypes/strains, including as a universal vaccine.

[0156] In some aspects, the recombinant protein excludes amino acid sequences from the virus that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

[0157] In some aspects, the expression cassette comprises a single open reading frame comprising a nucleic acid sequence encoding a self-cleaving peptide between each nucleic acid sequence encoding a protein such that the translation product of the expression cassette is cleaved intracellularly into two or more proteins. In some aspects, the self-cleaving peptide is a 2A self-cleaving peptide. In some aspects, the 2A self-cleaving peptide is P2A from porcine teschovirus-1. In some aspects, the 2A self-cleaving peptide is T2A from thosea asigna virus 2A.

[0158] In some aspects, the expression cassette comprises a nucleic acid sequence encoding a self-cleaving peptide between nucleic acid sequences encoding a viral matrix protein and a viral envelope protein, between nucleic acid sequences encoding a viral matrix protein and the recombinant protein, and/or between nucleic acid sequences encoding a viral envelope protein and the recombinant protein. In some aspects, the expression cassette comprises nucleic acid sequences from 5' to 3' encoding a viral matrix protein, a self-cleaving peptide, a viral envelope protein, a self-cleaving peptide, and the recombinant protein. In some aspects, the expression cassette comprises nucleic acid sequences from 5' to 3' encoding a viral envelope protein, a self-cleaving peptide, a viral matrix protein, a self-cleaving peptide, and the recombinant protein.

[0159] In some aspects, the expression cassette further comprises a nucleic acid sequence encoding a marker for gene expression. In some aspects, the marker for gene expression is a fluorescent reporter gene, such as green fluorescent protein (GFP), red fluorescent protein (RFP), yellow fluorescent protein (YFP), or near-infrared fluorescent protein (iRFP); a bioluminescent reporter genes such as luciferase; a selectable antibiotic marker; or LacZ. In some aspects, the expression cassette comprises a nucleic acid sequence

encoding a self-cleaving peptide between the nucleic acid sequence encoding a marker for gene expression and any other nucleic acid sequence encoding a protein.

[0160] The expression cassette can contain any expression control region known to those of skill in the art operably linked to the protein-encoding nucleic acid sequence(s). In some aspects, the expression control region is a promoter, enhancer, operator, repressor, ribosome binding site, translation leader sequence, intron, polyadenylation recognition sequence, RNA processing site, effector binding site, stem-loop structure, transcription termination signal, or combination thereof.

[0161] In some aspects, the target sequence for the first recombinase and the one or more additional target sequences for the one or more additional recombinases are selected from the group consisting of the PY54 pal site, the N15 telRL site, the loxP site, φK02 telRL site, the FRT site, the phiC31 attP site, and the λ attP site. In some aspects, the expression vector comprises each of the target sequences. In some aspects, the expression vector comprises the Tel recombinase pal site and the telRL, loxP, and FRT recombinase target binding sequences integrated within the pal site.

[0162] In some aspects, the expression vector is for producing a bacterial sequence-free vector. In some aspects, the bacterial sequence-free vector has circular covalently closed ends. In some aspects, the bacterial sequence-free vector has linear covalently closed ends.

[0163] In some aspects, the expression vector further comprises at least one enhancer sequence flanking each side of the target sequence for the first recombinase. In some aspects, the at least one enhancer sequence is at least two enhancer sequences. In some aspects, the at least one enhancer sequence is a SV40 enhancer sequence.

[0164] The source of the conserved amino acid sequence, the immunogenic amino acid sequence, and/or a viral protein as disclosed herein can be any virus associated with human or animal infection.

[0165] In some aspects, the virus is a coronavirus, an influenza virus, a human immunodeficiency virus, a human papillomavirus, a hepatitis virus, or an oncolytic virus.

[0166] In some aspects, the influenza virus is an influenza A virus. In some aspects, the influenza A virus is H1N1, H5N1, or H3N2.

[0167] In some aspects, the influenza virus is an influenza B virus.

[0168] In some aspects, the coronavirus is a human coronavirus such as, but not limited to, HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV-1, SARS-CoV-2 (*i.e.*, COVID-19)), and/or MERS-CoV.

[0169] In some aspects, the coronavirus is COVID-19 (*i.e.*, Wuhan-Hu-1 or a variant thereof such as, but not limited to, U.K. variant B.1.1.7, South African variant B.1.351, Brazilian variant P.1, or Californian variant B.1.427/429).

[0170] Provided herein is a vector production system comprising recombinant cells designed to encode at least a first recombinase under the control of an inducible promoter, wherein the cells comprise an expression vector as disclosed herein comprising a target for the at least first recombinase. In some aspects, the inducible promoter is thermally-regulated, chemically-regulated, IPTG regulated, glucose-regulated, arabinose inducible, T7 polymerase regulated, cold-shock inducible, pH inducible, or combinations thereof. In some aspects, the at least first recombinase is selected from telN and tel, and the expression vector incorporates the target sequence for the at least first recombinase. In some aspects, the at least first recombinase is selected from Cre or Flp, and the expression vector incorporates the target sequence for the at least first recombinase. In some aspects, the recombinant cells have been further designed to encode a nuclease genome editing system, and the expression vector further comprises a backbone sequence containing a cleavage site for the nuclease genome editing system. In some aspects, the nuclease genome editing system is a CRISPR nuclease system comprising a Cas nuclease and gRNA, and the expression vector comprises a target sequence for the gRNA within the backbone sequence.

[0171] Provided herein is a method of producing a bacterial sequence-free vector comprising incubating a vector production system as described herein under suitable conditions for expression of the at least first recombinase or the first recombinase and the nuclease genome editing system. In some aspects, the method further comprises harvesting the bacterial sequence-free vector. The present disclosure is also directed to a bacterial sequence-free vector produced by the method.

A.1 Expression cassettes comprising coronavirus sequences

[0172] Coronaviruses include any virus of the family *Coronaviridae*, including the subfamily *Coronovirinae*, and including the genuses *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. *See*, *e.g.*, Fung and Liu (2019).

Coronaviruses include human coronaviruses (HCoVs), such as HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronaviruses (SARS-CoV, *e.g.*, SARS-CoV-1 and SARS-CoV-2 (*i.e.*, COVID-19)), Middle East respiratory syndrome coronaviruses (MERS-CoV), zoonotic coronaviruses (*e.g.*, SARS-CoVs and MERS-CoVs), bat coronaviruses (BtCoVs), *Avian coronavirus*, *Murine coronavirus*, and bulbol coronavirus (BuCoV).

[0173] Coronavirus genomes are positive-sense, nonsegmented, single-stranded RNA ranging from about 27 to 32 kilobases (*see*, *e.g.*, Fung and Liu, *Annu. Rev. Microbiol. 73*:529-557 (2019)). For example, the complete genome of COVID-19 (also termed Wuhan-Hu-1 coronavirus (WHCV), SARS-CoV-2, and 2019-nCoV) has a size of 29.9 kb, compared to SARS-CoV and MERS-CoV with genomes of 27.9 kb and 30.1 kb, respectively (Zhou *et al.*, *Nature 579*: 270–273 (2020)). The COVID-19 genome has been found to be 96.2% identical to the Bat CoV RaTG13 genome, which is a type of SARS-CoV-2 found in bats and is likely the source of the virus transmitted to humans via unknown intermediate hosts.

[0174] Coronaviruses have a membrane (M) protein, which is the most abundant structural protein that supports the viral envelope and embeds in the envelope with three transmembrane domains. The M protein is essential for virus assembly and budding.

[0175] Envelope (E) protein is a small transmembrane protein in coronaviruses that is also present in the envelope at a lower amount than M protein. E protein is also engaged in virus assembly and egress.

[0176] The nucleocapsid (N) protein in coronaviruses binds to the RNA genome like beads-on-a-string, forming the helically symmetric nucleocapsid.

[0177] The virion surface of coronaviruses is decorated with the trimeric Spike (S) protein. Some betacoronaviruses also have dimeric hemagglutinin-esterase (HE) protein that make up shorter projections on the virion surface. S and HE protein each are type I transmembrane proteins with a large ectodomain and a short endodomain.

[0178] The S protein contains two subunits, S1 and S2, and is anchored in the viral envelope at its C-terminus. The S1 subunit of COVID-19, for example, contains the N-terminal domain (NTD) and receptor-binding domain (RBD), while the S2 subunit contains the fusion peptide (FP), internal fusion peptide (IFP), heptad repeat 1/2 (HR1/2), and the transmembrane domain (TM). The S protein's large ectodomain trimerizes and

forms the characteristic coronavirus spikes at the virion's surface. The S protein is responsible for receptor binding and virion entry to host cells (Fehr and Perlman, Coronaviruses: An Overview of Their Replication and Pathogenesis. In: Maier H., Bickerton E., Britton P. (eds) Coronaviruses. Methods in Molecular Biology, vol 1282. Humana Press, New York, NY; Wall *et al.*, *Cell* 180: 1–12 (2020)).

[0179] Fusion proteins from many viruses require a proteolytic event near a fusion peptide to enable the pathogen's entry into the target cell. For example, the S protein from COVID-19 possesses two cleavage sites, the first of which sits at the S1/S2 boundary but is not closely linked to the fusion peptide. A second cleavage site (S2') exposes the internal fusion peptide (IFP), a motif just downstream of S2' that is highly conserved across all sequenced coronaviruses. The sequence of IFP is SFIED**LLF**NKVTLADAGF (SEQ ID NO:7), within which the bolded LLF residues are critical for membrane fusion and infectivity (Madu *et al.*, *J. Virol. 83*(15): 7411-7421 (2009)). COVID-19 demonstrates the presence of a canonical furin-like cleavage motif at the S1/S2 site not found in other coronaviruses in the same clade, but similarly found in particularly virulent forms of influenza (H5N1). Cleavage via other proteases such as furin at the S1/S2 interface likely widens the tropism of the virus, making animal to human transmission more likely (Coutard *et al.*, *Antiviral Res. 176*:104742 (2020)).

[0180] In some aspects, the expression cassette comprises nucleic acid sequences encoding a coronavirus Membrane (M) protein, a coronavirus Envelope (E) protein, and a recombinant protein comprising a conserved amino acid sequence and an immunogenic amino acid sequence from a coronavirus Spike (S) protein. The M, E, and S proteins can be interchangeably referred to herein as M, E, and S glycoproteins.

[0181] In some aspects, the M protein comprises an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:1. In some aspects, the M protein comprises SEQ ID NO:1. In some aspects, the M protein is SEQ ID NO:1.

[0182] In some aspects, the nucleic acid sequence encoding the M protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:2. In some aspects, the nucleic acid sequence

encoding the M protein comprises SEQ ID NO:2. In some aspects, the nucleic acid sequence encoding the M protein is SEQ ID NO:2.

[0183] In some aspects, the E protein comprises an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:3. In some aspects, the E protein comprises SEQ ID NO:3. In some aspects, the E protein is SEQ ID NO:3. In some aspects, the E protein comprises a replacement of the proline located at amino acid number 71 in SEQ ID NO:3 (*i.e.*, at P71 in SEQ ID NO:3) with another amino acid. In some aspects, the replacement at P71 in SEQ ID NO:3 is a change from proline to leucine (*i.e.*, P71L).

[0184] In some aspects, the nucleic acid sequence encoding the E protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:4. In some aspects, the nucleic acid sequence encoding the E protein comprises SEQ ID NO:4. In some aspects, the nucleic acid sequence encoding the E protein is SEQ ID NO:4. In some aspects, the nucleic acid sequence encoding the E protein comprises a replacement of the codon for proline at nucleotide numbers 211-213 in SEQ ID NO:4 with a codon for another amino acid. In some aspects, the codon for proline at nucleotide numbers 211-213 in SEQ ID NO:4 is replaced with a codon for leucine.

[0185] In some aspects, the conserved amino acid sequence is from the S1 subunit or the S2 subunit of the S protein, the RBD of the S protein, the S protein S2' cleavage site and internal fusion peptide (IFP) of the S protein (referred to herein as S2'IFP), the M protein, or the E protein.

[0186] In some aspects, the conserved amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to any one of SEQ ID NOs:12-54. In some aspects, the conserved amino acid sequence comprises any one of SEQ ID NOs:12-54. In some aspects, the conserved amino acid sequence is any one of SEQ ID NOs:12-54.

[0187] In some aspects, the conserved amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%,

at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:7. In some aspects, the conserved amino acid sequence comprises SEQ ID NO:7. In some aspects, the conserved amino acid sequence is SEQ ID NO:7.

[0188] In some aspects, the nucleic acid sequence encoding the conserved amino acid sequence of the recombinant protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:8. In some aspects, the nucleic acid sequence encoding the conserved amino acid sequence of the recombinant protein comprises SEQ ID NO:8. In some aspects, the nucleic acid sequence encoding the conserved amino acid sequence of the recombinant protein is SEQ ID NO:8.

[0189] In some aspects, the immunogenic amino acid sequence is from the S protein receptor-binding domain (RBD).

[0190] In some aspects, the immunogenic amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:11. In some aspects, the immunogenic amino acid sequence comprises SEQ ID NO:11. In some aspects, the immunogenic amino acid sequence is SEQ ID NO:11. In some aspects, the immunogenic protein comprises a replacement of one or more of: lysine located at amino acid number 88 (*i.e.*, K88), leucine located at amino acid number 123 (*i.e.*, L123), glutamate located at amino acid number 155 (*i.e.*, E155), or asparagine located at amino acid number 172 (*i.e.*, N172) in SEQ ID NO:11 (corresponding to K417, L452, E484, and N501 in SEQ ID NO:5, respectively) with another amino acid. In some aspects, the replacement at K88 is K88N (*i.e.*, a change from lysine to asparagine). In some aspects, the replacement at K88 is K88T (*i.e.*, a change from lysine to threonine). In some aspects, the replacement at L123 is L123R (*i.e.*, a change from leucine to arginine). In some aspects, the replacement at E155 is E155K (*i.e.*, a change from glutamate to lysine). In some aspects, the replacement at N172 is N172Y (*i.e.*, a change from asparagine to tyrosine).

[0191] In some aspects, the nucleic acid sequence encoding the immunogenic amino acid sequence of the recombinant protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%,

at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:101. In some aspects, the nucleic acid sequence encoding the immunogenic amino acid sequence of the recombinant protein comprises SEQ ID NO:101. In some aspects, the nucleic acid sequence encoding the immunogenic amino acid sequence of the recombinant protein is SEQ ID NO:101. In some aspects, the nucleic acid sequence encoding the immunogenic protein comprises a replacement of one or more of: the codon for lysine at nucleotide numbers 262-264 of SEQ ID NO:101 with a codon for another amino acid, the codon for leucine at nucleotide numbers 367-369 of SEQ ID NO:101 with a codon for another amino acid, the codon for glutamate at nucleotide numbers 463-465 of SEQ ID NO:101 with a codon for another amino acid, or the codon for asparagine at nucleotide numbers 514-516 of SEQ ID NO:101 with a codon for another amino acid. In some aspects, the codon for lysine at nucleotide numbers 262-264 is replaced with a codon for asparagine or threonine. In some aspects, the codon for leucine at nucleotide numbers 367-369 is replaced with a codon for arginine. In some aspects, the codon for glutamate at nucleotide numbers 463-465 is replaced with a codon for lysine. In some aspects, the codon for asparagine at nucleotide numbers 514-516 is replaced with a codon for tyrosine.

[0192] In some aspects, the recombinant protein further comprises a transmembrane (TM) domain sequence from the S protein.

[0193] In some aspects, the TM domain sequence comprises an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:102. In some aspects, the TM domain sequence comprises SEQ ID NO:102. In some aspects, the TM domain sequence is SEQ ID NO:102.

[0194] In some aspects, the nucleic acid sequence encoding the TM domain sequence of the recombinant protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:103. In some aspects, the nucleic acid sequence encoding the TM domain sequence of the recombinant protein comprises SEQ ID NO:103. In some aspects, the nucleic acid sequence encoding the TM domain sequence of the recombinant protein is SEQ ID NO:103.

[0195] In some aspects, the recombinant protein comprises a conserved amino acid sequence from S2'IFP, an immunogenic amino acid sequence from the RBD, and a TM domain sequence of the S protein.

[0196] In some aspects, the recombinant protein excludes amino acid sequences from the S protein that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

[0197] In some aspects, the amino acid sequence of the recombinant protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:55. In some aspects, the amino acid sequence of the recombinant protein comprises SEQ ID NO:55. In some aspects, the amino acid sequence of the recombinant protein is SEQ ID NO:55. In some aspects, the recombinant protein comprises a replacement of one or more of K88, L123, E155, or N172 in SEQ ID NO:55 with another amino acid. In some aspects, the replacement at K88 is K88N. In some aspects, the replacement at K88 is K88T. In some aspects, the replacement at L123 is L123R. In some aspects, the replacement at E155 is E155K. In some aspects, the replacement at N172 is N172Y.

[0198] In some aspects, the nucleic acid sequence encoding the recombinant protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:56. In some aspects, the nucleic acid sequence encoding the recombinant protein comprises SEQ ID NO:56. In some aspects, the nucleic acid sequence encoding the recombinant protein is SEQ ID NO:56. In some aspects, the nucleic acid sequence encoding the recombinant protein comprises a replacement of one or more of: the codon for lysine at nucleotide numbers 262-264 of SEQ ID NO:56 with a codon for another amino acid, the codon for leucine at nucleotide numbers 367-369 of SEQ ID NO:56 with a codon for another amino acid, the codon for glutamate at nucleotide numbers 463-465 of SEQ ID NO:56 with a codon for another amino acid, or the codon for asparagine at nucleotide numbers 514-516 of SEQ ID NO:56 with a codon for another amino acid. In some aspects, the codon for lysine at nucleotide numbers 262-264 is replaced with a codon for asparagine or threonine. In some aspects, the codon for leucine at nucleotide numbers 367-369 is replaced with a codon for arginine. In some

aspects, the codon for glutamate at nucleotide numbers 463-465 is replaced with a codon for lysine. In some aspects, the codon for asparagine at nucleotide numbers 514-516 is replaced with a codon for tyrosine.

[0199] In some aspects, the expression cassette comprises a single open reading frame translated as an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:57. In some aspects, the expression cassette comprises a single open reading frame translated as an amino acid sequence comprising SEQ ID NO:57. In some aspects, the expression cassette comprises a single open reading frame translated as an amino acid sequence that is SEQ ID NO:57. In some aspects, the expression cassette comprises a single open reading frame translated as an amino acid sequence that comprises a replacement of one or more of P71, K423, L458, E490, or N507 in SEQ ID NO:57 with another amino acid. In some aspects, the replacement at P71 is P71L. In some aspects, the replacement at K423 is K423N. In some aspects, the replacement at K423 is K423T. In some aspects, the replacement at L458 is L458R. In some aspects, the replacement at E490 is E490K. In some aspects, the replacement at N507 is N507Y.

[0200] In some aspects, the expression cassette comprises a single open reading frame that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:58. In some aspects, the expression cassette comprises a single open reading frame that comprises SEQ ID NO:58. In some aspects, the expression cassette comprises a single open reading frame that is SEQ ID NO:58. In some aspects, the expression cassette comprises a single open reading frame that comprises a replacement of one or more of: the codon for proline at nucleotide numbers 211-213 in SEQ ID NO:58 with a codon for another amino acid, the codon for lysine at nucleotide numbers 1267-1269 of SEQ ID NO:58 with a codon for another amino acid, the codon for leucine at nucleotide numbers 1372-1374 of SEQ ID NO:58 with a codon for another amino acid, the codon for glutamate at nucleotide numbers 1468-1470 of SEQ ID NO:58 with a codon for another amino acid, or the codon for asparagine at nucleotide numbers 1519-1521 of SEQ ID NO:58 with a codon for another amino acid. In some aspects, the codon for proline at nucleotide numbers 211-213 in SEQ

ID NO:58 is replaced with a codon for leucine. In some aspects, the codon for lysine at nucleotide numbers 1267-1269 is replaced with a codon for asparagine or threonine. In some aspects, the codon for leucine at nucleotide numbers 1372-1374 is replaced with a codon for arginine. In some aspects, the codon for glutamate at nucleotide numbers 1468-1470 is replaced with a codon for lysine. In some aspects, the codon for asparagine at nucleotide numbers 1519-1521 is replaced with a codon for tyrosine.

[0201] In some aspects, the expression cassette is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to the nucleic acid sequence of any one of SEQ ID NOs:59-62. In some aspects, the expression cassette comprises the nucleic acid sequence of any one of SEQ ID NOs:59-62. In some aspects, the expression cassette is the nucleic acid sequence of any one of SEQ ID NOs:59-62.

[0202] In some aspects, the recombinant protein is capable of stimulating an immune response against COVID-19.

[0203] In some aspects, the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against COVID-19.

[0204] In some aspects, the immune response against COVID-19 is against Wuhan-Hu-1 and/or one or more variants such as, but not limited to, the U.K. variant B.1.1.7, the South African variant B.1.351, the Brazilian variant P.1, or the Californian variant B.1.427/429.

[0205] In some aspects, the immune response is cross-reactive to other coronaviruses. In some aspects, the immune response is cross-reactive to other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses.

[0206] Provided herein is a polynucleotide encoding an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:57. In some aspects, the polynucleotide encodes an amino acid sequence comprising SEQ ID NO:57. In some aspects, the polynucleotide encodes an amino acid sequence that is SEQ ID NO:57. In some aspects, the polynucleotide encodes an amino acid sequence that comprises a replacement of one or more of P71, K423, L458, E490, or N507 in SEQ ID NO:57 with another amino acid. In some aspects, the replacement at P71 is P71L. In some aspects, the replacement at K423

is K423N. In some aspects, the replacement at K423 is K423T. In some aspects, the replacement at L458 is L458R. In some aspects, the replacement at E490 is E490K. In some aspects, the replacement at N507 is N507Y.

[0207] Provided herein is a polynucleotide comprising a nucleic acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:58. In some aspects, the polynucleotide comprises SEQ ID NO:58. In some aspects, the polynucleotide is SEQ ID NO:58. In some aspects, the polynucleotide comprising a nucleic acid sequence that comprises a replacement of one or more of: the codon for proline at nucleotide numbers 211-213 in SEQ ID NO:58 with a codon for another amino acid, the codon for lysine at nucleotide numbers 1267-1269 of SEQ ID NO:58 with a codon for another amino acid, the codon for leucine at nucleotide numbers 1372-1374 of SEQ ID NO:58 with a codon for another amino acid, the codon for glutamate at nucleotide numbers 1468-1470 of SEQ ID NO:58 with a codon for another amino acid, or the codon for asparagine at nucleotide numbers 1519-1521 of SEQ ID NO:58 with a codon for another amino acid. In some aspects, the codon for proline at nucleotide numbers 211-213 in SEQ ID NO:58 is replaced with a codon for leucine. In some aspects, the codon for lysine at nucleotide numbers 1267-1269 is replaced with a codon for asparagine or threonine. In some aspects, the codon for leucine at nucleotide numbers 1372-1374 is replaced with a codon for arginine. In some aspects, the codon for glutamate at nucleotide numbers 1468-1470 is replaced with a codon for lysine. In some aspects, the codon for asparagine at nucleotide numbers 1519-1521 is replaced with a codon for tyrosine.

B. Bacterial sequence-free vectors

[0208] A bacterial sequence-free vector of the present disclosure can include any expression cassette of the present disclosure.

[0209] Provided herein is a bacterial sequence-free vector comprising an expression cassette that comprises a nucleic acid sequence encoding a recombinant protein comprising a conserved amino acid sequence from a virus fused to an immunogenic amino acid sequence, wherein protein expressed intracellularly from the expression cassette is capable of forming a VLP.

[0210] In some aspects, the immunogenic amino acid sequence is from the same virus as the conserved amino acid sequence. In some aspects, the conserved amino acid sequence is from a viral glycoprotein. In some aspects, the immunogenic amino acid sequence is from the same viral glycoprotein.

[0211] In some aspects, the expression cassette further comprises a nucleic acid sequence encoding a viral envelope protein and/or a nucleic acid sequence encoding a viral matrix protein. In some aspects, the viral envelope protein and/or the viral matrix protein are from the same virus as the conserved amino acid sequence.

[0212] In some aspects, the conserved amino acid sequence, the immunogenic amino acid sequence, the viral envelope protein, and/or the viral matrix protein is a consensus sequence.

[0213] In some aspects, the recombinant protein is capable of stimulating an immune response against the virus comprising neutralizing antibodies.

[0214] In some aspects, the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against the virus.

[0215] In some aspects, the immune response is cross-reactive to a related virus or strain.

[0216] In some aspects, the recombinant protein excludes amino acid sequences from the virus that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

[0217] In some aspects, the expression cassette comprises a single open reading frame comprising a nucleic acid sequence encoding a self-cleaving peptide between each nucleic acid sequence encoding a protein. Expression cassettes and self-cleaving peptides include those discussed above with respect to expression vectors.

[0218] In some aspects, the virus is a coronavirus, an influenza virus, a human immunodeficiency virus, a human papillomavirus, a hepatitis virus, or an oncolytic virus.

[0219] In some aspects, the influenza virus is an influenza A virus. In some aspects, the influenza A virus is H1N1, H5N1, or H3N2.

[0220] In some aspects, the influenza virus is an influenza B virus.

[0221] In some aspects, the coronavirus is a human coronavirus such as, but not limited to, HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV-1, SARS-CoV-2 (*i.e.*, COVID-19)), and/or MERS-CoV.

[0222] In some aspects, the coronavirus is COVID-19 (*i.e.*, Wuhan-Hu-1 or a variant thereof such as, but not limited to, U.K. variant B.1.1.7, South African variant B.1.351, Brazilian variant P.1, or Californian variant B.1.427/429).

[0223] In some aspects, the expression cassette comprises nucleic acid sequences encoding a coronavirus M protein, a coronavirus E protein, and a recombinant protein comprising a conserved amino acid sequence and an immunogenic amino acid sequence from a coronavirus S protein.

[0224] In some aspects, the M protein comprises an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:1. In some aspects, the M protein comprises SEQ ID NO:1. In some aspects, the M protein is SEQ ID NO:1.

[0225] In some aspects, the nucleic acid sequence encoding the M protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:2. In some aspects, the nucleic acid sequence encoding the M protein comprises SEQ ID NO:2. In some aspects, the nucleic acid sequence encoding the M protein is SEQ ID NO:2.

[0226] In some aspects, the E protein comprises an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:3. In some aspects, the E protein comprises SEQ ID NO:3. In some aspects, the E protein is SEQ ID NO:3. In some aspects, the E protein comprises a replacement of P71 in SEQ ID NO:3 with another amino acid. In some aspects, the replacement at P71 in SEQ ID NO:3 is P71L.

[0227] In some aspects, the nucleic acid sequence encoding the E protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:4. In some aspects, the nucleic acid sequence encoding the E protein comprises SEQ ID NO:4. In some aspects, the nucleic acid sequence encoding the E protein is SEQ ID NO:4. In some aspects, the nucleic acid sequence encoding the E protein comprises a replacement of the codon for proline at

nucleotide numbers 211-213 in SEQ ID NO:4 with a codon for another amino acid. In some aspects, the codon for proline at nucleotide numbers 211-213 in SEQ ID NO:4 is replaced with a codon for leucine.

[0228] In some aspects, the conserved amino acid sequence is from the S1 subunit or the S2 subunit of the S protein, the RBD of the S protein, the S protein S2' cleavage site and internal fusion peptide (IFP) of the S protein (referred to herein as S2'IFP), the M protein, or the E protein.

[0229] In some aspects, the conserved amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to any one of SEQ ID NOs:12-54. In some aspects, the conserved amino acid sequence comprises any one of SEQ ID NOs:12-54. In some aspects, the conserved amino acid sequence is any one of SEQ ID NOs:12-54.

[0230] In some aspects, the conserved amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:7. In some aspects, the conserved amino acid sequence comprises SEQ ID NO:7. In some aspects, the conserved amino acid sequence is SEQ ID NO:7.

[0231] In some aspects, the nucleic acid sequence encoding the conserved amino acid sequence of the recombinant protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:8. In some aspects, the nucleic acid sequence encoding the conserved amino acid sequence of the recombinant protein comprises SEQ ID NO:8. In some aspects, the nucleic acid sequence encoding the conserved amino acid sequence of the recombinant protein is SEQ ID NO:8.

[0232] In some aspects, the immunogenic amino acid sequence is from the S protein receptor-binding domain (RBD).

[0233] In some aspects, the immunogenic amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:11. In some aspects, the immunogenic amino acid sequence

comprises SEQ ID NO:11. In some aspects, the immunogenic amino acid sequence is SEQ ID NO:11. In some aspects, the immunogenic amino acid sequence comprises a replacement of one or more of: K88, L123, E155, or N172 in SEQ ID NO:11 with another amino acid. In some aspects, the replacement at K88 is K88N . In some aspects, the replacement at K88 is K88T. In some aspects, the replacement at L123 is L123R. In some aspects, the replacement at E155 is E155K. In some aspects, the replacement at N172 is N172Y.

[0234] In some aspects, the nucleic acid sequence encoding the immunogenic amino acid sequence of the recombinant protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:101. In some aspects, the nucleic acid sequence encoding the immunogenic amino acid sequence of the recombinant protein comprises SEQ ID NO:101. In some aspects, the nucleic acid sequence encoding the immunogenic amino acid sequence of the recombinant protein is SEQ ID NO:101. In some aspects, the nucleic acid sequence encoding the immunogenic amino acid sequence comprises a replacement of one or more of: the codon for lysine at nucleotide numbers 262-264 of SEQ ID NO:101 with a codon for another amino acid, the codon for leucine at nucleotide numbers 367-369 of SEQ ID NO:101 with a codon for another amino acid, the codon for glutamate at nucleotide numbers 463-465 of SEQ ID NO:101 with a codon for another amino acid, or the codon for asparagine at nucleotide numbers 514-516 of SEQ ID NO:101 with a codon for another amino acid. In some aspects, the codon for lysine at nucleotide numbers 262-264 is replaced with a codon for asparagine or threonine. In some aspects, the codon for leucine at nucleotide numbers 367-369 is replaced with a codon for arginine. In some aspects, the codon for glutamate at nucleotide numbers 463-465 is replaced with a codon for lysine. In some aspects, the codon for asparagine at nucleotide numbers 514-516 is replaced with a codon for tyrosine.

[0235] In some aspects, the recombinant protein further comprises a transmembrane (TM) domain sequence from the S protein.

[0236] In some aspects, the TM domain sequence comprises an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at

least about 99% identical to SEQ ID NO:102. In some aspects, the TM domain sequence comprises SEQ ID NO:102. In some aspects, the TM domain sequence is SEQ ID NO:102.

[0237] In some aspects, the nucleic acid sequence encoding the TM domain sequence of the recombinant protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:103. In some aspects, the nucleic acid sequence encoding the TM domain sequence of the recombinant protein comprises SEQ ID NO:103. In some aspects, the nucleic acid sequence encoding the TM domain sequence of the recombinant protein is SEQ ID NO:103.

[0238] In some aspects, the recombinant protein comprises a conserved amino acid sequence from S2'IFP, an immunogenic amino acid sequence from the RBD, and a TM domain sequence of the S protein.

[0239] In some aspects, the recombinant protein excludes amino acid sequences from the S protein that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

[0240] In some aspects, the amino acid sequence of the recombinant protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:55. In some aspects, the amino acid sequence of the recombinant protein comprises SEQ ID NO:55. In some aspects, the amino acid sequence of the recombinant protein is SEQ ID NO:55. In some aspects, the amino acid sequence of the recombinant protein comprises a replacement of one or more of K88, L123, E155, or N172 in SEQ ID NO:55 with another amino acid. In some aspects, the replacement at K88 is K88N. In some aspects, the replacement at K88 is K88T. In some aspects, the replacement at L123 is L123R. In some aspects, the replacement at E155 is E155K. In some aspects, the replacement at N172 is N172Y.

[0241] In some aspects, the nucleic acid sequence encoding the recombinant protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:56. In some aspects, the nucleic acid sequence encoding the recombinant protein comprises SEQ ID NO:56. In some aspects, the nucleic

acid sequence encoding the recombinant protein is SEQ ID NO:56. In some aspects, the nucleic acid sequence encoding the recombinant protein comprises a replacement of one or more of: the codon for lysine at nucleotide numbers 262-264 of SEQ ID NO:56 with a codon for another amino acid, the codon for leucine at nucleotide numbers 367-369 of SEQ ID NO:56 with a codon for another amino acid, the codon for glutamate at nucleotide numbers 463-465 of SEQ ID NO:56 with a codon for another amino acid, or the codon for asparagine at nucleotide numbers 514-516 of SEQ ID NO:56 with a codon for another amino acid. In some aspects, the codon for lysine at nucleotide numbers 262-264 is replaced with a codon for asparagine or threonine. In some aspects, the codon for leucine at nucleotide numbers 367-369 is replaced with a codon for arginine. In some aspects, the codon for glutamate at nucleotide numbers 463-465 is replaced with a codon for lysine. In some aspects, the codon for asparagine at nucleotide numbers 514-516 is replaced with a codon for tyrosine.

[0242] In some aspects, the expression cassette comprises a single open reading frame translated as an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:57. In some aspects, the expression cassette comprises a single open reading frame translated as an amino acid sequence comprising SEQ ID NO:57. In some aspects, the expression cassette comprises a single open reading frame translated as an amino acid sequence that is SEQ ID NO:57. In some aspects, the expression cassette comprises a single open reading frame translated as an amino acid sequence that comprises a replacement of one or more of P71, K423, L458, E490, or N507 in SEQ ID NO:57 with another amino acid. In some aspects, the replacement at P71 is P71L. In some aspects, the replacement at K423 is K423N. In some aspects, the replacement at K423 is K423T. In some aspects, the replacement at L458 is L458R. In some aspects, the replacement at E490 is E490K. In some aspects, the replacement at N507 is N507Y.

[0243] In some aspects, the expression cassette comprises a single open reading frame that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:58. In some aspects, the expression cassette comprises a single open reading frame that comprises SEQ ID NO:58. In some

aspects, the expression cassette comprises a single open reading frame that is SEQ ID NO:58. In some aspects, the expression cassette comprises a single open reading frame that comprises a replacement of one or more of: the codon for proline at nucleotide numbers 211-213 in SEQ ID NO:58 with a codon for another amino acid, the codon for lysine at nucleotide numbers 1267-1269 of SEQ ID NO:58 with a codon for another amino acid, the codon for leucine at nucleotide numbers 1372-1374 of SEQ ID NO:58 with a codon for another amino acid, the codon for glutamate at nucleotide numbers 1468-1470 of SEQ ID NO:58 with a codon for another amino acid, or the codon for asparagine at nucleotide numbers 1519-1521 of SEQ ID NO:58 with a codon for another amino acid. In some aspects, the codon for proline at nucleotide numbers 211-213 in SEQ ID NO:58 is replaced with a codon for leucine. In some aspects, the codon for lysine at nucleotide numbers 1267-1269 is replaced with a codon for asparagine or threonine. In some aspects, the codon for leucine at nucleotide numbers 1372-1374 is replaced with a codon for arginine. In some aspects, the codon for glutamate at nucleotide numbers 1468-1470 is replaced with a codon for lysine. In some aspects, the codon for asparagine at nucleotide numbers 1519-1521 is replaced with a codon for tyrosine.

[0244] In some aspects, the expression cassette is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to any one of SEQ ID NOs:59-62. In some aspects, the expression cassette comprises any one of SEQ ID NOs:59-62. In some aspects, the expression cassette is any one of SEQ ID NOs:59-62.

[0245] In some aspects, the recombinant protein is capable of stimulating an immune response against COVID-19.

[0246] In some aspects, the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against COVID-19.

[0247] In some aspects, the immune response against COVID-19 is against Wuhan-Hu-1 and/or one or more variants such as, but not limited to, the U.K. variant B.1.1.7, the South African variant B.1.351, the Brazilian variant P.1, or the Californian variant B.1.427/429.

[0248] In some aspects, the immune response is cross-reactive to other coronaviruses. In some aspects, the immune response is cross-reactive to other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses.

[0249] In some aspects, the bacterial sequence-free vector further comprises at least one enhancer sequence flanking each side of the expression cassette. In some aspects, the at least one enhancer sequence is at least two enhancer sequences. In some aspects, the at least one enhancer sequence is a SV40 enhancer sequence.

[0250] In some aspects, the bacterial sequence-free vector comprises circular covalently closed ends.

[0251] In some aspects, the bacterial sequence-free vector comprises linear covalently closed ends. In some aspects, the bacterial sequence-free vector is a msDNA as disclosed herein. A vector map for an exemplary msDNA is shown in Figure 4.

[0252] In some aspects, the bacterial sequence-free vector is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:104. In some aspects, the bacterial sequence-free vector comprises SEQ ID NO:104. In some aspects, the bacterial sequence-free vector is SEQ ID NO:104.

III. VLPs

[0253] In some aspects, a VLP as disclosed herein is produced from the expression cassette of an expression vector and/or the expression cassette of a bacterial sequence-free vector as described herein.

[0254] Provided herein is a recombinant cell comprising an expression vector or a bacterial sequence-free vector as described herein.

[0255] In some aspects, the recombinant cell is a yeast, bacteria, archaebacteria, fungi, insect, or animal cell, including a mammalian cell. In some aspects, recombinant cells include *Drosophila melanogaster* cells, *Saccharomyces cerevisiae* or other yeasts, *E. coli*, *Bacillus subtilis*, Sf9 cells, C129 cells, HEK293 cells, Neurospora, BHK, CHO, COS, HeLa cells, Hep G2 cells, and human cells and cell lines.

[0256] In some aspects, the expression vector is for expression in a human cell or cell line such as the exemplary vector shown in Figure 2.

[0257] In some aspects, the expression vector is a baculovirus vector such as the exemplary vector shown in Figure 5 and the cell type is an insect cell (*e.g.*, Sf9 cells).

[0258] In some aspects, the present disclosure is directed to a method of producing a VLP, comprising culturing the recombinant cell comprising the expression vector or the

bacterial sequence-free vector under suitable conditions for production of the VLP from the expression vector or the bacterial sequence-free vector.

[0259] In some aspects, the method of producing a VLP further comprises isolating the VLP. In some aspects, the VLP produced by any of the above expression vectors or any of the above bacterial sequence-free vectors wherein the virus is a coronavirus.

[0260] In some aspects, the VLP is isolated from a cell lysate.

[0261] In some aspects, the isolating is by affinity purification. In some aspects, the affinity purification comprises microfluidics and/or chromatography.

[0262] In some aspects, the affinity purification comprises an angiotensin-converting enzyme 2 (ACE2) receptor peptide or an anti-S protein monoclonal antibody.

[0263] In some aspects, the ACE2 receptor peptide comprises an amino acid sequence that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:70. In some aspects, the ACE2 receptor peptide comprises SEQ ID NO:70. In some aspects, the ACE2 receptor peptide is SEQ ID NO:70.

[0264] In some aspects, the ACE2 receptor peptide comprises a biotin acceptor peptide (BAP) tag at the C-terminus or N-terminus of the peptide. In some aspects, the BAP tag comprises an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:71. In some aspects, the BAP tag comprises SEQ ID NO:71. In some aspects, the BAP tag is SEQ ID NO:71.

[0265] In some aspects, the ACE2 receptor peptide or anti-S protein monoclonal antibody is biotinylated and immobilized on a streptavidin-coated bead. In some aspects, the affinity purification comprises microfluidics and/or chromatography.

[0266] In some aspects, the present disclosure is directed to a VLP produced by the method.

[0267] Provided herein is a VLP comprising a recombinant protein comprising a conserved amino acid sequence from a virus fused to an immunogenic amino acid sequence.

[0268] In some aspects, the immunogenic amino acid sequence is from the same virus as the conserved amino acid sequence.

[0269] In some aspects, the conserved amino acid sequence is from a viral glycoprotein. In some aspects, the immunogenic amino acid sequence is from the same viral glycoprotein.

[0270] In some aspects, the VLP further comprises a viral envelope protein and/or a viral matrix protein. In some aspects, the viral envelope protein and/or the viral matrix protein are from the same virus as the conserved amino acid sequence.

[0271] In some aspects, the conserved amino acid sequence, the immunogenic amino acid sequence, the viral envelope protein, and/or the viral matrix protein is a consensus sequence.

[0272] In some aspects, the recombinant protein is capable of stimulating an immune response against the virus comprising neutralizing antibodies.

[0273] In some aspects, the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against the virus.

[0274] In some aspects, the immune response is cross-reactive to a related virus or strain.

[0275] In some aspects, the recombinant protein excludes amino acid sequences from the virus that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

[0276] In some aspects, the virus is a coronavirus, an influenza virus, a human immunodeficiency virus, a human papillomavirus, a hepatitis virus, or an oncolytic virus.

[0277] In some aspects, the influenza virus is an influenza A virus. In some aspects, the influenza A virus is H1N1, H5N1, or H3N2.

[0278] In some aspects, the influenza virus is an influenza B virus.

[0279] In some aspects, the coronavirus is a human coronavirus such as, but not limited to, HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV-1, SARS-CoV-2 (*i.e.*, COVID-19)), and/or MERS-CoV.

[0280] In some aspects, the coronavirus is COVID-19 (*i.e.*, Wuhan-Hu-1 or a variant thereof such as, but not limited to, U.K. variant B.1.1.7, South African variant B.1.351, Brazilian variant P.1, or Californian variant B.1.427/429).

[0281] In some aspects, the VLP comprises a coronavirus M protein, a coronavirus E protein, and a recombinant protein comprising a conserved amino acid sequence and an immunogenic amino acid sequence from a coronavirus S protein.

[0282] In some aspects, the M protein comprises an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:1. In some aspects, the M protein comprises SEQ ID NO:1. In some aspects, the M protein is SEQ ID NO:1.

[0283] In some aspects, the E protein comprises an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:3. In some aspects, the E protein comprises SEQ ID NO:3. In some aspects, the E protein is SEQ ID NO:3. In some aspects, the E protein comprises a replacement of P71 in SEQ ID NO:3 with another amino acid. In some aspects, the replacement at P71 in SEQ ID NO:3 is P71L.

[0284] In some aspects, the conserved amino acid sequence is from the S1 subunit or the S2 subunit of the S protein, the RBD of the S protein, the S protein S2' cleavage site and internal fusion peptide (IFP) of the S protein, the M protein, or the E protein.

[0285] In some aspects, the conserved amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to any one of SEQ ID NOs:12-54. In some aspects, the conserved amino acid sequence comprises any one of SEQ ID NOs:12-54. In some aspects, the conserved amino acid sequence is any one of SEQ ID NOs:12-54.

[0286] In some aspects, the conserved amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:7. In some aspects, the conserved amino acid sequence comprises SEQ ID NO:7. In some aspects, the conserved amino acid sequence is SEQ ID NO:7.

[0287] In some aspects, the immunogenic amino acid sequence is from the S protein receptor-binding domain (RBD).

[0288] In some aspects, the immunogenic amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:11. In some aspects, the immunogenic amino acid sequence comprises SEQ ID NO:11. In some aspects, the immunogenic amino acid sequence is SEQ ID NO:11. In some aspects, the immunogenic amino acid sequence comprises a replacement of one or more of: K88, L123, E155, or N172 in SEQ ID NO:11 with another amino acid. In some aspects, the replacement at K88 is K88N . In some aspects, the replacement at K88 is K88T. In some aspects, the replacement at L123 is L123R. In some aspects, the replacement at E155 is E155K. In some aspects, the replacement at N172 is N172Y.

[0289] In some aspects, the recombinant protein further comprises a transmembrane (TM) domain sequence from the S protein.

[0290] In some aspects, the TM domain sequence comprises an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:102. In some aspects, the TM domain sequence comprises SEQ ID NO:102. In some aspects, the TM domain sequence is SEQ ID NO:102.

[0291] In some aspects, the recombinant protein comprises a conserved amino acid sequence from S2'IFP, an immunogenic amino acid sequence from the RBD, and a TM domain sequence of the S protein.

[0292] In some aspects, the recombinant protein excludes amino acid sequences from the S protein that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

[0293] In some aspects, the amino acid sequence of the recombinant protein is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:55. In some aspects, the amino acid sequence of the recombinant protein comprises SEQ ID NO:55. In some aspects, the amino acid sequence of the recombinant protein is SEQ ID NO:55. In some aspects, the amino acid sequence of the recombinant protein comprises a replacement of one or more of K88, L123, E155,

or N172 in SEQ ID NO:55 with another amino acid. In some aspects, the replacement at K88 is K88N. In some aspects, the replacement at K88 is K88T. In some aspects, the replacement at L123 is L123R. In some aspects, the replacement at E155 is E155K. In some aspects, the replacement at N172 is N172Y.

[0294] Provided herein is a VLP comprising a recombinant protein at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:55, an M protein at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:1, and an E protein at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to SEQ ID NO:3.

[0295] Provided herein is a VLP comprising a recombinant protein that comprises SEQ ID NO:55, an M protein that comprises SEQ ID NO:1, and an E protein that comprises SEQ ID NO:3.

[0296] Provided herein is a VLP comprising the recombinant protein of SEQ ID NO:55, the M protein of SEQ ID NO:1, and the E protein of SEQ ID NO:3.

[0297] In some aspects, the recombinant protein is capable of stimulating an immune response against COVID-19.

[0298] In some aspects, the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against COVID-19.

[0299] In some aspects, the immune response against COVID-19 is against Wuhan-Hu-1 and/or one or more variants such as, but not limited to, the U.K. variant B.1.1.7, the South African variant B.1.351, the Brazilian variant P.1, or the Californian variant B.1.427/429

[0300] In some aspects, the immune response is cross-reactive to other coronaviruses.

[0301] In some aspects, the immune response is cross-reactive to other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses.

IV. Compositions

[0302] Provided herein is a composition comprising any of the expression vectors, bacterial sequence-free vectors, or VLPs as described herein.

[0303] In some aspects, the composition further comprises a physiologically acceptable carrier, excipient, or stabilizer. *See*, *e.g.*, *Remington: The Science and Practice of Pharmacy*, 22nd ed. (2013). Acceptable carriers, excipients, or stabilizers can include those that are nontoxic to a subject. In some aspects, the composition or one or more components of the composition are sterile. A sterile component can be prepared, for example, by filtration (*e.g.*, by a sterile filtration membrane) or by irradiation (*e.g.*, by gamma irradiation).

[0304] An excipient of the present invention can be described as a "pharmaceutically acceptable" excipient when added to a pharmaceutical composition, meaning that the excipient is a compound, material, composition, salt, and/or dosage form which is, within the scope of sound medical judgment, suitable for contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problematic complications over the desired duration of contact commensurate with a reasonable benefit/risk ratio. In some aspects, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized international pharmacopeia for use in animals, and more particularly in humans. Various excipients can be used. In some aspects, the excipient can be, but is not limited to, an alkaline agent, a stabilizer, an antioxidant, an adhesion agent, a separating agent, a coating agent, an exterior phase component, a controlled-release component, a solvent, a surfactant, a humectant, a buffering agent, a filler, an emollient, or combinations thereof. Excipients in addition to those discussed herein can include excipients listed in, though not limited to, *Remington: The Science and Practice of Pharmacy*, 22nd ed. (2013). Inclusion of an excipient in a particular classification herein (e.g., "solvent") is intended to illustrate rather than limit the role of the excipient. A particular excipient can fall within multiple classifications.

[0305] A pharmaceutical composition of the disclosure is formulated to be compatible with its intended route of administration. Exemplary routes of administration include enteral, topical, parenteral, oral, pulmonary, intranasal, intravenous, epidermal, transdermal, subcutaneous, intramuscular, or intraperitoneal administration, or inhalation. "Parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection or infusion, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic,

intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intrapleural, and intrasternal injection and infusion, as well as in vivo electroporation. In some aspects, the formulation is administered via a non-parenteral route, in some aspects, orally. Other non-parenteral routes include a topical, epidermal, or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically.

[0306] In some aspects, the pharmaceutical composition is lyophilized.

[0307] A variety of methods are known in the art and are suitable for introduction of nucleic acids into a cell. Examples include, but are not limited to, electroporation, calcium phosphate mediated transfer, nucleofection, sonoporation, heat shock, magnetofection, liposome mediated transfer, microinjection, microprojectile mediated transfer (nanoparticles), cationic polymer mediated transfer (DEAE-dextran, polyethylenimine, polyethylene glycol (PEG), and the like), or cell fusion.

[0308] Nanoparticle carriers such as liposomes, micelles, and polymeric nanoparticles have been investigated for improving bioavailability and pharmacokinetic properties of therapeutics via various mechanisms, for example, the enhanced permeability and retention (EPR) effect.

[0309] Further improvement can be achieved by conjugation of targeting ligands onto nanoparticles to achieve selective delivery to a target cell. For example, receptor-targeted nanoparticle delivery has been shown to improve therapeutic responses both *in vitro* and *in vivo*. Targeting ligands that have been investigated include folate, transferrin, antibodies, peptides, and aptamers. Additionally, multiple functionalities can be incorporated into the design of nanoparticles, *e.g.*, to enable imaging and to trigger intracellular drug release.

[0310] In some aspects, the composition further comprises a delivery agent. In some aspects, the delivery agent is a nanoparticle. In some aspects, the delivery agent is selected from the group consisting of liposomes, non-lipid polymeric molecules, endosomes, and any combination thereof.

[0311] In some aspects, the delivery agent (*e.g.*, a nanoparticle) comprises a targeting ligand.

[0312] In some aspects, the targeting ligand comprises a S protein peptide with binding affinity to the ACE2 receptor (*e.g.*, for delivery of an expression vector, bacterial sequence-free vector, or VLP comprising coronavirus sequences).

[0313] In some aspects, the S protein peptide is from a conserved region of the S protein. In some aspects, the length of the S protein peptide is from 3 amino acids to 100 amino acids, including any length or range of lengths therein, such as 3 amino acids to 90, 80, 70, 60, 50, 40, 30, 20, or 10 amino acids.

[0314] In some aspects, the S protein peptide comprises an amino acid sequence at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to any one of SEQ ID NOs:76-99. In some aspects, the S protein peptide comprises any one of SEQ ID NOs:76-99. In some aspects, the S protein peptide is any one of SEQ ID NOs:76-99.

V. Therapeutic Uses and Methods

[0315] The expression vectors, bacterial sequence-free vectors (e.g., msDNA), VLPs, and compositions as described herein can be utilized for prophylactic or therapeutic treatment of a subject in need thereof, including as a vaccine against a viral infection (*e.g.*, a coronavirus infection such as COVID-19) infection or as a treatment for individuals infected with a virus.

[0316] Provided herein is a vaccine for a viral infection comprising an expression vector, bacterial sequence-free vector, VLP, or composition as described herein.

[0317] Provided herein is a method of treating a viral infection in a subject, comprising administering to the subject an expression vector, bacterial sequence-free vector, VLP, or composition as described herein, wherein intracellular expression of the expression vector or the bacterial sequence-free vector in the subject produces a VLP.

[0318] Provided herein is an expression vector, bacterial sequence-free vector, VLP, or composition as described herein for use in treating a viral infection in a subject, wherein intracellular expression of the expression vector or the bacterial sequence-free vector in the subject produces a VLP.

[0319] Provided herein is use of an expression vector, bacterial sequence-free vector, VLP, or composition for treating a viral infection in a subject, wherein intracellular

expression of the expression vector or the bacterial sequence-free vector in the subject produces a VLP.

[0320] Provided herein is use of an expression vector, bacterial sequence-free vector, VLP, or composition for the preparation of a medicament for treating a viral infection in a subject, wherein intracellular expression of the expression vector or the bacterial sequence-free vector in the subject produces a VLP.

[0321] The expression vector, bacterial sequence-free vector, or composition can be administered to a subject by any route of administration that is effective in treating the viral infection.

[0322] In some aspects, the administering is by enteral, topical, parenteral, oral, pulmonary, intranasal, intravenous, epidermal, transdermal, subcutaneous, intramuscular, or intraperitoneal administration, or inhalation.

[0323] In some aspects, the administering is by parenteral or non-parenteral administration.

[0324] In some aspects, the parenteral administration is by injection or infusion.

[0325] In some aspects, the parenteral administration is by intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intrapleural, or intrasternal injection or infusion, or by in vivo electroporation.

[0326] In some aspects, the non-parenteral administration is oral, topical, epidermal, mucosal, intranasal, vaginal, rectal, or sublingual.

[0327] In some aspects, the administering is by oral, pulmonary, intranasal, intravenous, epidermal, transdermal, subcutaneous, intramuscular, or intraperitoneal administration, or by inhalation.

[0328] In some aspects, the administering is by the route of viral infection and transmission.

[0329] In some aspects, the route of viral infection and transmission is mucosal.

[0330] In some aspects, the administering is by oral, nasal, or pulmonary administration for a respiratory tract infection. In some aspects, the administering is by nasal administration.

[0331] Applying the inhalation and intranasal routes of administration provide a powerful opportunity to generate supporting immune responses via lungs and nasopharyngeal-associated lymphoid tissues (NALT) in addition to efficient, targeted, and non-invasive delivery of a VLP as described herein to lower respiratory tract tissue.

[0332] In some aspects, the administering is vaginal administration for a sexually transmitted infection.

[0333] In some aspects, the administering is by intramuscular, subcutaneous, or intradermal administration where both the site and depth of injection effect the immune response. Intramuscular injection offers a powerful alternative and commonly used technique for vaccine administration, particularly as it is validated and readily re-administered.

[0334] Administering can be performed, for example, once, a plurality of times, and/or over one or more extended periods. In some aspects, the administering is one time, two times (*e.g.*, a first administration followed by a second administration about 1, about 2, about 3, about 4 or more weeks later), once about every week, once about every month, once about every 2 months, once about every 3 months, once about every 4 months, once about every 6 months, once about every year, or once about every decade.

[0335] The expression cassette as described herein provides a VLP conferring a robust humoral immune response with the benefits of a DNA vaccine for internal processing of intracellular pathogen epitopes for T-cell presentation and cell-mediated immunity. In some aspects, immunodominance is successfully conferred to the conserved amino acid sequence of the recombinant protein, and the vaccine generates universal coronavirus immunity.

[0336] In some aspects, VLPs that self-assemble intracellularly from translation products of the expression cassette (whether from the expression vector or a bacterial sequence-free vector as described herein) generate a Th1 cell-mediated response as presented in: 1) an MHC-I context to prime specific cytotoxic T-cell activity against virally infected cells; 2) an MHC-II context in phagocytic antigen presenting cells (APCs) for complementary humoral and cell-mediated support.

[0337] In some aspects, intracellular assembly of VLP from the expression cassettes as described herein eliminates potential vaccine-mediated TH2 immunopathology and any associated requirement for adjuvant therapy.

[0338] In some aspects, the VLP stimulates an immune response in the subject comprising neutralizing antibodies against the viral infection.

[0339] In some aspects, the VLP stimulates a Th1 cell-mediated immune response in the subject against the viral infection.

[0340] In some aspects, the immune response is cross-reactive to a related virus or strain.

[0341] In some aspects, the VLP does not stimulate an immune response comprising non-neutralizing antibodies in the subject and/or does not stimulate a Th2 cell-mediated immune response in the subject.

[0342] In some aspects, the VLP induces antibodies that block viral receptor binding, viral genome uncoating, and/or genome injection.

[0343] In some aspects, the VLP cross-competes with the infecting virus for binding to a viral receptor.

[0344] In some aspects, the VLP cross-competes with a related virus or strain for binding to the viral receptor.

[0345] In some aspects, the viral infection is a coronavirus, an influenza virus, a human immunodeficiency virus, a human papillomavirus, a hepatitis virus, or an oncolytic virus.

[0346] In some aspects, the influenza virus is an influenza A virus. In some aspects, the influenza A virus is H1N1, H5N1, or H3N2.

[0347] In some aspects, the influenza virus is an influenza B virus.

[0348] In some aspects, the coronavirus is a human coronavirus such as, but not limited to, HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV-1, SARS-CoV-2 (*i.e.*, COVID-19)), and/or MERS-CoV.

[0349] In some aspects, the coronavirus is COVID-19 (*i.e.*, Wuhan-Hu-1 or a variant thereof such as, but not limited to, U.K. variant B.1.1.7, South African variant B.1.351, Brazilian variant P.1, or Californian variant B.1.427/429).

[0350] In some aspects, the VLP stimulates an immune response in the subject comprising neutralizing antibodies against COVID-19.

[0351] In some aspects, the VLP stimulates a Th1 cell-mediated immune response in the subject against COVID-19.

[0352] In some aspects, the immune response against COVID-19 is against Wuhan-Hu-1 and/or one or more variants such as, but not limited to, the U.K. variant B.1.1.7, the South African variant B.1.351, the Brazilian variant P.1, or the Californian variant B.1.427/429.

[0353] In some aspects, the immune response is cross-reactive to other coronaviruses.

[0354] In some aspects, the immune response is cross-reactive to other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses.

[0355] In some aspects, the VLP does not stimulate an immune response comprising non-neutralizing antibodies in the subject and/or does not stimulate a Th2 cell-mediated immune response in the subject.

[0356] In some aspects, the administering is by inhalation.

[0357] The cellular ligand for COVID-19 and many other coronaviruses is the ACE2 receptor found in the lower respiratory tract of humans, which regulates both cross-species and human-to-human transmission. The ACE2 receptor is bound by the S glycoprotein on the surface of coronavirus that, upon fusion, forms a replication-transcription complex in a double membrane vesicle (Letko *et al.*, *Nat. Microbiol.* *5*(4): 562–569 (2020); Wan *et al.*, *J. Virol.* *4*(7) e00127-20 (2020)). The continuous replication and synthesis of nested sets of subgenomic RNAs encode accessory proteins and structural proteins for the viral particles to bud. This causes the virion-containing vesicles to fuse with plasma membrane ultimately releasing the virus into the host (Fehr and Perlman). Hypertensive patients on adrenergic blocking agents (beta-blockers) to control blood pressure are particularly susceptible to infection as beta blockers stimulate ACE2 receptor over-expression in the respiratory tract facilitating viral binding and infection. Susceptibility has also been noted in patients underlying medical conditions such as COPD, diabetes, and cardiovascular disease (Guan *et al.*, *Eur. Resp. Journal*, 2000547; DOI: 10.1183/13993003.00547-2020 (2020)).

[0358] In some aspects, a VLP against coronavirus (*e.g.*, COVID-19) as described herein not only delivers a therapeutic DNA vaccine, but also competes for available coronavirus receptor sites in respiratory tissue, attenuating further infection.

[0359] In some aspects, the extrusion of functional VLPs (expressing surface RBD) from cells further promotes competitive interference for available ACE2 receptors on target cells and promotes interaction with B-cells to ensure a robust neutralizing humoral response.

[0360] In some aspects, the S2'IFP domain for presentation exposes the highly conserved site and confers immuno-dominance to the determinant via hapten-carrier response.

[0361] In some aspects, the VLP cross-competes with COVID-19 for binding to ACE2 receptor, neuropilin-1, and/or other receptors.

[0362] In some aspects, the VLP cross-competes with other coronaviruses for binding to ACE2 receptor, neuropilin-1, and/or other receptors.

[0363] In some aspects, the VLP cross-competes with other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses for binding to ACE2 receptor, neuropilin-1, and/or other receptors.

[0364] The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

A. Generation of expression vectors for producing bacterial sequence-free vectors and VLPs

[0365] Four expression vectors were produced by cloning sequences derived from COVID-19 into the multicloning site between two specialized supersequence (SS) sites in a ministring expression vector (Mediphage Bioceuticals, Inc., Toronto, CA) as described in U.S. Patent Nos. 9,290,778 and 9,862,954, incorporated by reference herein in their entireties.

[0366] The sequences derived from COVID-19 included sequences encoding Envelope (E) protein (GenBank Accession No. QHD43418.1; SEQ ID NO:3) and Membrane (M) protein (GenBank Accession No. QHD43419.1; SEQ ID NO:1). Additionally, a sequence encoding a recombinant Spike (S) protein was produced that contained a fusion of sequences associated with the receptor-binding domain (RBD), the S2' cleavage site and internal fusion peptide (S2'IFP), and the transmembrane (TM) domain (RBD::S2'IFP::TM; SEQ ID NO:55) of the COVID-19 S protein (GenBank Accession No. QHD43416.1; SEQ ID NO:5). The recombinant S protein was engineered to exclude amino acid sequences from the S protein that stimulate an immune response comprising non-neutralizing antibodies and to exclude amino acid sequences that stimulate a Th2 cell-mediated immune response.

[0367] The expression cassettes of three of the expression vectors contained the E protein, the M protein, and the recombinant S protein fused into a single polynucleotide

(SEQ ID NO:58) via sequences encoding the self-cleaving peptide P2A from porcine teschovirus-1 2A under the control of a cytomegalovirus (CMV) promoter. **Figure 1** illustrates an exemplary expression cassette.

[0368] One of the three expression vectors contained the expression cassette "CMV-E-P2A-M-P2A-RBD::S2'IFP::TM-bGHpolyA" (SEQ ID NO:60), which contained a bovine growth hormone (bGH) polyadenylation (polyA) signal. A map of the expression vector containing the expression cassette is shown in **Figure 2** (pGL2-SS-CMV-VLP-BGH-SS, SEQ ID NO:63).

[0369] Another of the three expression vectors contained the expression cassette "CMV-E-P2A-M-P2A-RBD::S2'IFP::TM-SV40polyA" (SEQ ID NO:59), which contained a simian virus 40 (SV40) polyA.

[0370] Another of the three expression vectors contained the expression cassette "CMV-E-P2A-M-P2A-RBD::S2'IFP::TM-T2A-GFP-SV40polyA" (SEQ ID NO:61), which contained a green fluorescent protein (GFP) fused to the COVID-19 sequences via a sequence encoding the self-cleaving peptide T2A from thosea asigna virus 2A and a SV40 polyA.

[0371] A fourth expression vector contained the expression cassette "CMV-E-P2A-M-T2A-MCS-bGHpolyA" (SEQ ID NO:62), which contained a single polynucleotide having the E protein and the M protein fused to one another via a sequence encoding P2A in turn fused to a multiple cloning site (MCS) via a sequence encoding T2A. The expression cassette also contained a CMV promoter and a bGH polyA. The MCS is for insertion of additional sequences, such as recombinant proteins comprising conserved and immunogenic sequences as disclosed herein.

[0372] The expression vectors containing the expression cassettes of SEQ ID NOs:59-62 are the same as the expression vector of **Figure 2** and SEQ ID NO:63 except for the different expression cassette.

B. Expression of COVID-19 genes

[0373] Human lung A549 cells (1×10^6) were electroporated with 1 µg of the expression vector shown in **Figure 2**, or no expression vector. Total RNA was extracted after 48 hours after electroporation and converted to cDNA libraries. 1 µL of cDNA was used as template for Real Time qRT-PCR for E, M, and RBD::S2'IFP::TM transgenes using the

gene-specific primers for E, M, and RBD, respectively, shown below in **Table 1**. Expression of the transgenes was normalized to β-actin expression.

Table 1. Primer Sequences

Gene	Forward Primer	Reverse Primer
E	ACTGCTGCAACATCGTGAAC (SEQ ID NO:64)	TGCTAGAATTCAGGTTCTTCACC (SEQ ID NO:65)
M	TTCCTGTGGCTGCTGTGG (SEQ ID NO:66)	ATGACCAGCTCGCTTTCCAG (SEQ ID NO:67)
RBD::S2'IFP::TM	ATCAGCACAGAGATCTACCAGG (SEQ ID NO:68)	AGCACCACCACTCTGTAAGG (SEQ ID NO:69)

[0374] As shown in **Figure 3(A)**, each of the transgenes was detected in cDNA libraries from cells electroporated with the expression vector ("VLP") but not in cDNA libraries from control cells ("CTL"). The relative gene expression shown in the figure was calculated by ΔΔCT method. Statistical analysis was performed using 1-way ANOVA (*** = $p<0.001$, **** = $p<0.0001$).

C. Expression of recombinant Spike protein

[0375] HEK 293 cells (1 x 10^6) were transfected with 2 µg of the expression vector of Figure 2 using Lipofectamine® 3000 Reagent (Invitrogen). Protein samples were collected 48 hours after transfection. Western blots were prepared by loading 50 µg of whole protein lysate from transfected cells as well as from control cells that were not transfected. A rabbit polyclonal anti-RBD antibody was used to in the detection of recombinant S protein, while a rabbit polyclonal anti-beta-actin antibody was used in the detection of beta-actin as a loading control. An anti-rabbit-horse radish peroxidase (HRP) antibody and chemiluminescence imaging was used for signal detection. A representative Western blot is shown in **Figure 3(B)**, showing that recombinant S protein was detected in protein isolated from cells transfected with the expression vector ("VLP") but not in protein isolated from control cells.

[0376] The relative mean protein intensity of recombinant S protein expression in transfected and control cells was determined by densitometry analysis of Western blot images (n=3). *See* **Figure 3(C)**.

Example 2

Stimulation of antibody production by VLP expression vectors

[0377] The expression vector of **Figure 2** was encapsulated in lipid nanoparticles (Entos Pharmaceuticals) and administered to C57 mice at a dose of 100 μg via intramuscular injection at day 0 followed by a booster dose of 100 μg via intramuscular injection at day 14. Serum was collected via tail vein every 7 days through day 49.

[0378] Antibody concentrations in mouse serum were assessed by indirect ELISA by binding to purified S1 protein (Abclonal, Inc.).

[0379] Serum was diluted to 1% in PBS and then added to ELISA plates containing the S1 protein. Mouse serum antibodies that bound to the S1 protein were detected by anti-mouse IgG SULFO-TAG™ conjugated antibody (Meso Scale Diagnostics, LLC).

[0380] Antibody concentrations are shown in **Figures 5A** and **5B**. Concentrations peaked at day 21 at about 5000 ng/mL, with consistent expression maintained at about 3000 ng/mL through day 49.

Example 3

A. Characterization of COVID-19 genomic sequence conservation

[0381] A total of 3928 representative complete COVID-19 genomes were downloaded from the GISAID database (https://www.gisaid.org). Collection dates for the genomes ranged from December 2019 to February 2021 and contained all major variant strains as well as the Wuhan reference genome (NC_045512.2). Genomes were aligned to the Wuhan reference genome using the MAFFT multiple sequence alignment program. Sequence conservation and nucleotide frequency analyses were performed.

[0382] **Figure 6** shows a sequence conservation analysis of the 3928 representative COVID-19 genomes. **(A)** Horizontal tracks indicate the genomic positions (indicated on the x-axis) of all COVID-19 genes (depicted on the y-axis) as per the Wuhan reference genome. **(B)** The bar heights in the histogram correspond to the percent of genomes that

differed from the Wuhan reference genome in each given genomic position. The bar plot and histogram were generated in R version 3.6.1 using the ggplot2 package.

[0383] As shown in **Figure 6**, the COVID-19 genome has a relatively high level of sequence conservation with few key genomic variants. Ignoring variable 5' and 3' end regions, only three genomic positions were found to differ from the reference genome in >50% of sequences. Two of these single nucleotide polymorphisms (SNPs) were found within ORF1ab (the first (C241T) in an intergenic region and the second (C14408T -> L4715)) within a coding region, and the third (D614G) within the Spike (S) protein.

B. Characterization of human beta coronavirus genomic sequence conservation

[0384] In addition to the 3928 representative complete COVID-19 genomes discussed in part A of this example, 120 SARS-CoV (the virus responsible for SARS) genomes and 257 MERS-CoV (the virus responsible for MERS) genomes were downloaded from the NCBI GenBank® database. Genomes were aligned to the COVID-19 Wuhan reference genome using the MAFFT multiple sequence alignment program. The comparison was possible due to similar genomic organization across these three viral genomes. Sequence conservation and nucleotide frequency analyses were performed.

[0385] **Figure 7** shows a histogram in which the bar heights correspond to the percent of genomes that differed from the Wuhan reference genome in each given genomic position. The histogram was generated in R version 3.6.1 using the ggplot2 package.

[0386] As shown in **Figure 7**, the genomes of other prominent human beta coronaviruses (SARS-CoV and MERS-CoV) also have relatively high levels of sequence conservation as compared to the COVID-19 genome.

C. Identification of functionally relevant mutations in prominent variant COVID-19 strains

[0387] The 3928 COVID-19 sequences discussed in part A were filtered for those belonging to key variant strains (U.K. variant B.1.1.7 (n=233), South African variant B.1.351 (n=104), Brazilian variant P.1 (n=39), and Californian variant B.1.427/429 (n=62)). Genomes of the four variant strains were independently aligned to the SARS-CoV-2 Wuhan reference genome (NC_045512.2) using the MAFFT multiple sequence alignment program. Sequence conservation and nucleotide frequency analyses were performed. Functional importance was determined via assessment of BLOSUM 62 matrix score, surface exposure analysis (via PyMol), and literature review.

[0388] **Figures 8A-D** shows histograms in which the bar heights correspond to the percent of the variant genomes (B.1.1.7 in **8A**, B.1.351 in **8B**, P.1 in naïve, and B.1.427/429 in **8D**) that differed from the Wuhan reference genome in each given genomic position. The histograms were generated in R version 3.6.1 using the ggplot2 package.

[0389] **Table 2** shows a summary of the identified SNPs from variant COVID-19 strains located in regions of the COVID-19 genome contained within the expression cassette shown in **Figure 1**.

Table 2. Summary of Identified SNPs

Expression Cassette Sequences	COVID-19 Variants			
	U.K. (B.1.1.7)	South Africa (B.1.357)	Brazil (P.1)	California (B.1.427/429)
RBD	AAT > TAT → N501Y	AAT > TAT → N501Y GAA > AAA → E484K AAG > AAT → K417N	AAT > TAT → N501Y GAA > AAA → E484K AAG > ACG → K417T	CTG > CGG → L452R
S2'IFP	-	-	-	-
E	-	CCT > CTT → P71L	-	-
M	-	-	-	TTC > TTT → F53F

[0390] SNPs identified in the receptor-binding domain (RBD) region of the Spike (S) protein of the variant COVID-19 strains were mapped onto a referenced Protein Data Bank (PDB) structure (PBD ID: 6VXX) to assess surface exposure. The N501, K417, and L452 residues were determined to be surface exposed and therefore of potentially greater consequence. The E484 residue was determined not to be surface exposed.

[0391] Surface exposure of SNPs identified in the Envelope (E) protein of the variant COVID-19 strains were assessed via structural information in Bianchi *et al.*, *BioMed*

Research International, https://doi.org/10.1155/2020/4389089 (2020). The P71 residue was determined to be surface exposed and therefore of potentially greater consequence.

[0392] The SNP identified in the membrane (M) protein results in a synonymous mutation and therefore functional analysis was not performed.

[0393] Overall, the analysis showed that sequences selected for the VLP expression cassette as shown in **Figure 1** are relatively robust against COVID-19 variants, especially the S2'IFP site which is completely conserved across all key variant strains as well as in other coronaviruses (SARS-CoV and MERS-CoV).

Example 4

A. Generation of bacterial sequence-free vectors for producing VLP

[0394] DNA ministrings for producing VLP (msDNA-VLP) are produced in inducible *E. coli* cells from the expression vectors described in Example 1 according to methods described in U.S. Patent Nos. 9,290,778 and 9,862,954.

[0395] msDNA-VLP is purified and concentrated, with quality control testing for purity and sequence.

B. Complexation of bacterial sequence-free vectors with nanoparticles

[0396] The purified msDNA-VLP and a control msDNA (msDNA-control) expressing a marker protein (*e.g.*, GFP) are complexed with nanoparticles (*e.g.*, lipid nanoparticles (LNPs)). In other studies, commercial LNPs have demonstrated strong transfection efficiency in lung in vivo with msDNA (unpublished data). Commercial LNPs are used as in vitro controls. Commercial JetPEI (https://www.polyplus-transfection.com/products/cgmp-grade-in-vivo-jetpei/) is used as an in vivo control.

[0397] The msDNA nanoparticles are lyophilized for in vitro and in vivo tests.

C. In vitro VLP formation and immune responses from bacterial sequence-free vectors

[0398] The msDNA nanoparticles (*i.e.*, as described in part B of this example) as well as naked msDNA (*i.e.*, msDNA-VLP as described in part A of this example and msDNA-control that are not complexed with nanoparticles) are delivered into a human cell line expressing ACE2 receptors (*e.g.*, A549 cells (ATCC CCL-185)), vascular endothelial cell, or alveolar epithelial cells (Yen, T.-T., *et al.*, *Journal of Virology 80*(6): 2684-2693

(2006); Qian, Z. *et al.*, *American Journal of Respiratory Cell and Molecular Biology* *48*(6): 742-748 (2013)). Efficiency of the delivery and mean fluorescence are assessed.

[0399] Intracellular VLP formation is assessed by transmission electron microscopy.

[0400] Cytokine storm and over-activity of inflammation response would be assessed in cell cultures using immune assay techniques.

D. Production of VLP in vitro in a eukaryotic expression system

[0401] A eukaryotic expression vector is produced comprising M-P2A-E and RBD::S2'::TM under control of a promoter for VLP production in eukaryotic cells. An exemplary baculoviral expression vector for VLP production in Sf9 cells is shown in **Figure 9**. VLP is produced in vitro and purified using standard techniques.

E. In vivo VLP production and immune responses from bacterial sequence-free vectors

[0402] The msDNA nanoparticles (*i.e.*, as described in part B of this example) are administered by inhalation, intranasal, or intramuscular routes in an animal model. Cytokine profiles, immunoglobulin profiles, and protective effects against COVID-19 are determined.

[0403] For inhalation and intranasal routes, the following administrations are performed: (1) lyophilized msDNA-VLP or msDNA-control nanoparticles are administered by inhalation in one or multiple doses (e.g., dosing at 1, 2, 3, and/or 4 weeks; dosing at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 months; and/or annual intervals); (2) lyophilized msDNA-VLP or msDNA-control nanoparticles are administered by inhalation in one or multiple doses (e.g., dosing at 1, 2, 3, and/or 4 weeks; dosing at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 months; and/or annual intervals), followed by intranasal administration of a booster of purified VLP (*i.e.*, as described in part D of this example) in one or multiple doses (e.g., dosing at 1, 2, 3, and/or 4 weeks; dosing at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 months; and/or annual intervals); or (3) intranasal administration of purified VLP in one or multiple doses (e.g., dosing at 1, 2, 3, and/or 4 weeks; dosing at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 months; and/or annual intervals).

[0404] For intramuscular routes, the following administrations are performed: (1) msDNA-VLP or msDNA-control nanoparticles are administered by injection in one or multiple doses (e.g., dosing at 1, 2, 3, and/or 4 weeks; dosing at 2, 3, 4, 5, 6, 7, 8, 9, 10,

11, and/or 12 months; and/or annual intervals); (2) msDNA-VLP or msDNA-control nanoparticles are administered by injection in one or multiple doses (e.g., dosing at 1, 2, 3, and/or 4 weeks; dosing at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 months; and/or annual intervals), followed by injection of a booster of purified VLP in one or multiple doses (e.g., dosing at 1, 2, 3, and/or 4 weeks; dosing at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 months; and/or annual intervals); or (3) injection of a booster of purified VLP in one or multiple doses (e.g., dosing at 1, 2, 3, and/or 4 weeks; dosing at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 months; and/or annual intervals).

Example 5

Affinity purification of VLPs

[0405] A 64-residue ACE2 receptor peptide ("ACE2-64") was identified as a sufficient interaction interface for binding coronavirus S protein following analysis of four co-crystal structures of S protein and ACE2 receptor as well as one co-crystal structure of lipoprotein E and ACE2 receptor. The amino acid sequence of ACE2-64 is: STIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAF LKEQSTLAQMY (SEQ ID NO:70).

[0406] The peptide is encoded on an expression plasmid encoding a biotin acceptor peptide (BAP) tag (*e.g.*, GLNDIFEAQKIEWHE (SEQ ID NO:71)) at the C-terminus or N-terminus of ACE2-64 (*i.e.*, SEQ ID NO:72, encoded by SEQ ID NO:73, or SEQ ID NO:74, encoded by SEQ ID NO:75, respectively). The expression plasmid is transformed into a *BirA* positive *E. coli* strain, which results in one-step *in vivo* biotinylation of ACE2-64. The cells are lysed, and the biotinylated ACE2-64 peptides are purified by a commercially available kit and mixed with streptavidin-coated magnetic microbeads.

[0407] A commercial monoclonal antibody against the COVID-19 S protein ("S-Ab") is biotinylated *in vitro* and mixed with streptavidin-coated magnetic microbeads.

[0408] Beads with immobilized ACE2-64 or immobilized S-Ab are washed and equilibrated in an inert Tris buffer (*e.g.*, 20 mM Tris pH 8.0, 150 mM NaCl).

[0409] Recombinant cells expressing VLPs from msDNA-VLPs, such as the eukaryotic cells of Example 2(D), are lysed.

[0410] Beads with immobilized ACE2-64 or immobilized S-Ab and the cell lysate containing VLPs are added to a microfluidic device and mixed. VLPs captured by the

ACE2-64 or S-Ab coated beads are separated from the cell lysate. The beads are then washed three times with a buffer of moderate salinity (*e.g.*, 20 mM Tris pH 8.0, 300 mM NaCl). The VLPs are then purified in a buffer of high salinity (*e.g.*, 20 mM Tris pH 8.0, 1.5 M NaCl), which results in the dissociation of VLPs from the beads. The purified VLPs are collected. Quality control assays, such as agarose gel electrophoresis to detect RNA and episomal DNA, qPCR to assess gDNA levels, and electron microscopy, are performed to confirm the identity and purity of the VLPs.

Example 6

Production of targeting ligands for nanoparticle formulations

[0411] A peptide library is derived from the conserved regions of coronavirus S protein and produced by peptide synthesis. Exemplary peptides are SEQ ID NOs:76-99.

[0412] Recombinant ACE2 protein is purchased from a commercial source.

[0413] The following portion of the COVID-19 S protein is provided as a control for binding to ACE2, with the bolded and underlined residues being directly involved in ACE2 binding:
RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASF STFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTG**K**IADYNYKLPDDF TGCVIAWNSNNLDSKVGGN**Y**NYL**Y**RLFRKSNLKPFERDISTEIYQAGSTPCNGV**E** G**FN**CYFPL**Q**SYGF**Q**P**TN**GVG**Y**Q (SEQ ID NO:100).

[0414] An *in vitro* fluorescence polarization (FP) assay or similar technique is performed according to standard procedures to determine the affinity of each peptide to the recombinant ACE2 protein.

[0415] Ligands (*i.e.*, peptides) with the strongest affinities to ACE2 receptor are selected and attached to nanoparticles (*e.g.*, LNPs).

[0416] The ability of single ligand and dual-ligand nanoparticles to target ACE2 receptor is determined. For example, the targeting ability of nanoparticles containing the ligand with the highest affinity to ACE2 receptor is compared to nanoparticles containing two different ligands having the highest affinities to ACE2 receptor.

[0417] Multiple ligand targeting is also tested using nanoparticles with one ligand that targets ACE2 receptor (*e.g.*, to facilitate ACE2 receptor-mediated endocytosis) and a

second ligand that is a nuclear localization signal (NLS) (*e.g.*, to facilitate proper intracellular delivery via nuclear targeting).

SEQUENCES

SEQ ID NO:1 membrane protein, amino acid sequence

MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIKLIFLWLLWPVTLACF
VLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILLNVPLHGTILT
RPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYKLGASQRVAGD
SGFAAYSRYRIGNYKLNTDHSSSSDNIALLVQ

SEQ ID NO:2 membrane protein, nucleic acid sequence

atggcagattccaacggtactattaccgttgaagagcttaaaaagctccttgaacaatggaacct
agtaataggtttcctattccttacatggatttgtcttctacaatttgcctatgccaacaggaa
taggtttttgtatataattaagttaattttcctctggctgttatggccagtaactttagcttgtt
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acatcacgaacgctttcttattacaaattgggagcttcgcagcgtgtagcaggtgactcaggttt
tgctgcatacagtcgctacaggattggcaactataaattaaacacagaccattccagtagcagtg
acaatattgctttgcttgtacagtaa

SEQ ID NO:3 envelope protein, amino acid sequence

MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYSRVKNL
NSSRVPDLLV

SEQ ID NO:4 envelope protein, nucleic acid sequence

atgtactcattcgtttcggaagagacaggtacgttaatagttaatagcgtacttctttttcttgc
tttcgtggtattcttgctagttacactagccatccttactgcgcttcgattgtgtgcgtactgct
gcaatattgttaacgtgagtcttgtaaaaccttcttttacgtttactctcgtgttaaaaatctg
aattcttctagagttcctgatcttctggtctaa

SEQ ID NO:5 spike protein, amino acid sequence

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWF
HAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKV
CEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFK
NIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTA
GAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTES
IVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDL
CFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRL
FRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHA
PATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEIL

DITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL
IGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIP
TNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQE
VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIA
ARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIG
VTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISS
VLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV
DFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVT
QRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDIS
GINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLC
CMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT

SEQ ID NO:6 spike protein, nucleic acid sequence

atgtttgtttttcttgttttattgccactagtctctagtcagtgtgttaatcttacaaccagaac
tcaattacccccctgcatacactaattctttcacacgtggtgtttattaccctgacaaagtttt
cagatcctcagttttacattcaactcaggacttgttcttacctttcttttccaatgttacttggt
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tttgcatctgtttatgcttggaacaggaagagaatcagcaactgtgttgctgattattctgtcct
atataattccgcatc

SEQ ID NO:7 internal fusion peptide, amino acid sequence

SFIEDLLFNKVTLADAGF

SEQ ID NO:8 internal fusion peptide, nucleic acid sequence

tcatttattgaagatctacttttcaacaaagtgacacttgcagatgctggcttc

SEQ ID NO:9 receptor-binding domain, amino acid sequence

PNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTN
VYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKS
NLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATV
CGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLE

SEQ ID NO:10 receptor-binding domain, nucleic acid sequence

cctaatattacaaacttgtgccctttggtgaagttttaacgccaccagatttgcatctgttta
tgcttggaacaggaagagaatcagcaactgtgttgctgattattctgtcctatataattccgcat
cattttccacttttaagtgttatggagtgtctcctactaaattaaatgatctctgctttactaat
gtctatgcagattcatttgtaattagaggtgatgaagtcagacaaatcgctccagggcaaactgg
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aacaggcacaggtgttcttactgagtctaacaaaaagtttctgcctttccaacaatttggcagag
acattgctgacactactgatgctgtccgtgatccacagacacttgag

SEQ ID NO:11 immunogenic sequence, amino acid sequence

PNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTN
VYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKS
NLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAP

SEQ ID NO:12 conserved amino acid sequence

SFIEDL

SEQ ID NO:13 conserved amino acid sequence

GVYYP

SEQ ID NO:14 conserved amino acid sequence

FLPF

SEQ ID NO:15 conserved amino acid sequence

VLPF

SEQ ID NO:16 conserved amino acid sequence

SLLI

SEQ ID NO:17 conserved amino acid sequence

LPIGI

SEQ ID NO:18 conserved amino acid sequence

AAYYV

SEQ ID NO:19 conserved amino acid sequence

TFLL

SEQ ID NO:20 conserved amino acid sequence

AVDC

SEQ ID NO:21 conserved amino acid sequence

IVRFP

SEQ ID NO:22 conserved amino acid sequence

ISNC

SEQ ID NO:23 conserved amino acid sequence

LCFT

SEQ ID NO:24 conserved amino acid sequence

YNYKL

SEQ ID NO:25 conserved amino acid sequence

IAWN

SEQ ID NO:26 conserved amino acid sequence

VVVLSF

SEQ ID NO:27 conserved amino acid sequence

CVNF

SEQ ID NO:28 conserved amino acid sequence

GLTG

SEQ ID NO:29 conserved amino acid sequence

VAVLY

SEQ ID NO:30 conserved amino acid sequence

GCLI

SEQ ID NO:31 conserved amino acid sequence

GICA

SEQ ID NO:32 conserved amino acid sequence

FTIS

SEQ ID NO:33 conserved amino acid sequence

SVDC

SEQ ID NO:34 conserved amino acid sequence

YGSFC

SEQ ID NO:35 conserved amino acid sequence

FNFS

SEQ ID NO:36 conserved amino acid sequence

RDLICAQ

SEQ ID NO:37 conserved amino acid sequence

VLPPLL

SEQ ID NO:38 conserved amino acid sequence

IPFA

SEQ ID NO:39 conserved amino acid sequence

YRFN

SEQ ID NO:40 conserved amino acid sequence

KLQDVVN

SEQ ID NO:41 conserved amino acid sequence

GAISS

SEQ ID NO:42 conserved amino acid sequence

EVQIDRLI

SEQ ID NO:43 conserved amino acid sequence

YVTQQL

SEQ ID NO:44 conserved amino acid sequence

HLMSF

SEQ ID NO:45 conserved amino acid sequence

GVVHLF

SEQ ID NO:46 conserved amino acid sequence

WFVT

SEQ ID NO:47 conserved amino acid sequence

INAS

SEQ ID NO:48 conserved amino acid sequence

LLQF

SEQ ID NO:49 conserved amino acid sequence

LWLLWP

SEQ ID NO:50 conserved amino acid sequence

LMWL

SEQ ID NO:51 conserved amino acid sequence

SFRLF

SEQ ID NO:52 conserved amino acid sequence

FNPETN

SEQ ID NO:53 conserved amino acid sequence

ITVA

SEQ ID NO:54 conserved amino acid sequence

LRLC

SEQ ID NO:55 recombinant spike protein, amino acid sequence

PNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTN
VYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKS
NLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPGGG
GGGSFIEDLLFNKVTLADAGFGGGGGGWPWYIWLGFIAGLIAIVMVTIML

SEQ ID NO:56 recombinant spike protein, nucleic acid sequence

ccaaacattaccaacctgtgcccttcggcgaggtgttcaacgccacacggttcgccagcgtgta
cgcctggaacagaaagcggatcagcaactgcgtggccgactacagtgtcctgtataactccgcca
gcttttctacattcaagtgctacggcgtctcccctaccaagctgaacgacctgtgcttcaccaat

gtgtacgccgattctttcgtgatcagaggcgacgaggtgcggcagatcgcccctggccagaccgg
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cggtggcggcggcggctggccttggtacatctggctgggcttcatcgccggactgatcgccatcg
tgatggtcaccatcatgctgtga

SEQ ID NO:57 single open reading frame for coronavirus VLP, amino acid sequence

MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYSRVKNL
NSSRVPDLLVATNFSLLKQAGDVEENPGPMADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQ
FAYANRNRFLYIIKLIFLWLLWPVTLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFR
LFARTRSMWSFNPETNILLNVPLHGTILTRPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPK
EITVATSRTLSYYKLGASQRVAGDSGFAAYSRYRIGNYKLNTDHSSSSDNIALLVQATNFSLLKQ
AGDVEENPGPPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSP
TKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNY
NYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLS
FELLHAPGGGGGGSFIEDLLFNKVTLADAGFGGGGGGWPWYIWLGFIAGLIAIVMVTIML

SEQ ID NO:58 single open reading frame for coronavirus VLP, nucleic acid sequence

atgtactctttcgtgtctgaggaaaccggcaccctgatcgtgaacagcgtgctgctgtttctggc
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aattctagcagggtgcccgacctgctggtggccaccaacttcagcctgctgaaacaggctggcga
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agctgctggaacagtggaatcttgtgatcggcttcctgttcctgacctggatctgcctgctgcag
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gcccgtgaccctggcttgtttcgtgctggctgctgtttatagaatcaactggatcacaggcggca
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caaggtgaccctggccgacgccggttttggcggtggcggcggctggccttggtacatctggc
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SEQ ID NO:59 expression cassette for VLP, nucleic acid sequence

cgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgt
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SEQ ID NO:60 expression cassette for VLP, nucleic acid sequence

cgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgt
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tgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttc
ctacttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtac
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SEQ ID NO:61 expression cassette for VLP, nucleic acid sequence

cgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgt
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tccaaactcatcaatgtatctta

SEQ ID NO:62 expression cassette for VLP, nucleic acid sequence

cgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgt
caataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggag
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SEQ ID NO:63 expression vector with expression cassette for VLP, nucleic acid sequence

cccgggaggtaccgagctcttacgcgtgctagaattaaagtaacccaatcagcacacaattgcca
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SEQ ID NO:64 Forward Primer, envelope protein, nucleic acid sequence

actgctgcaacatcgtgaac

SEQ ID NO:65 Reverse Primer, envelope protein, nucleic acid sequence

tgctagaattcaggttcttcacc

SEQ ID NO:66 Forward Primer, membrane protein, nucleic acid sequence

ttcctgtggctgctgtgg

SEQ ID NO:67 Reverse Primer, membrane protein, nucleic acid sequence

atgaccagctcgctttccag

SEQ ID NO:68 Forward Primer, receptor-binding domain, nucleic acid sequence

atcagcacagagatctaccagg

SEQ ID NO:69 Reverse Primer, receptor-binding domain, nucleic acid sequence

agcaccaccactctgtaagg

SEQ ID NO:70 ACE2 receptor peptide, amino acid sequence

STIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMY

SEQ ID NO:71 BAP tag, amino acid sequence

GLNDIFEAQKIEWHE

SEQ ID NO:72 ACE2 receptor peptide with C-terminal BAP tag, amino acid sequence

STIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMY
GLNDIFEAQKIEWHE

SEQ ID NO:73 ACE2 receptor peptide with C-terminal BAP tag, nucleic acid sequence

tccactattgaagaacaggcaaagactttcttggacaaattcaaccacgaggccgaagacttgtt
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SEQ ID NO:74 ACE2 receptor peptide with N-terminal BAP tag, amino acid sequence

GLNDIFEAQKIEWHESTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDK
WSAFLKEQSTLAQMY

SEQ ID NO:75 ACE2 receptor peptide with N-terminal BAP tag, nucleic acid sequence

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SEQ ID NO:76 ACE2 binding peptide, amino acid sequence

QSYGFQPTN

SEQ ID NO:77 ACE2 binding peptide, amino acid sequence

LQSYGFQPTN

SEQ ID NO:78 ACE2 binding peptide, amino acid sequence

QSYGFQPTNGVGY

SEQ ID NO:79 ACE2 binding peptide, amino acid sequence

QPTNGVGY

SEQ ID NO:80 ACE2 binding peptide, amino acid sequence

FQPTNGVGY

SEQ ID NO:81 ACE2 binding peptide, amino acid sequence

QPTN

SEQ ID NO:82 ACE2 binding peptide, amino acid sequence

FQPTN

SEQ ID NO:83 ACE2 binding peptide, amino acid sequence

FQPTNGV

SEQ ID NO:84 ACE2 binding peptide, amino acid sequence

TNGVGY

SEQ ID NO:85 ACE2 binding peptide, amino acid sequence

FNCYFPLQ

SEQ ID NO:86 ACE2 binding peptide, amino acid sequence

GFNCYFPLQ

SEQ ID NO:87 ACE2 binding peptide, amino acid sequence

EGFN

SEQ ID NO:88 ACE2 binding peptide, amino acid sequence

VEGFNCY

SEQ ID NO:89 ACE2 binding peptide, amino acid sequence

EGFNCYFPLQ

SEQ ID NO:90 ACE2 binding peptide, amino acid sequence

YNYLY

SEQ ID NO:91 ACE2 binding peptide, amino acid sequence

NYNYLYR

SEQ ID NO:92 ACE2 binding peptide, amino acid sequence

SFIEDLLFNKVTLADAGF

SEQ ID NO:93 ACE2 binding peptide, amino acid sequence

SFIEDLLFNKVTLADAGFMKQYGCGKKKK

SEQ ID NO:94 ACE2 binding peptide, amino acid sequence

SFIEDLLF

SEQ ID NO:95 ACE2 binding peptide, amino acid sequence

SFIEDLLFGCGKKKK

SEQ ID NO:96 ACE2 binding peptide, amino acid sequence

SFIEDLLFNKVTLADAGFMKQY

SEQ ID NO:97 ACE2 binding peptide, amino acid sequence

SFIEDAAAGCGKKKK

SEQ ID NO:98 ACE2 binding peptide, amino acid sequence

SFIEDAAA

SEQ ID NO:99 ACE2 binding peptide, amino acid sequence

TRYYYLNYNYTTGY

SEQ ID NO:100 ACE2 binding control peptide, amino acid sequence

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVS
PTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGN
YNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQ

SEQ ID NO:101 immunogenic sequence, nucleic acid sequence

cctaatattacaaacttgtgccctttggtgaagttttaacgccaccagatttgcatctgttta
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SEQ ID NO:102 transmembrane domain, amino acid sequence

WPWYIWLGFIAGL

SEQ ID NO:103 transmembrane domain, nucleic acid sequence

tggccatggtacatttggctaggttttatagctggcttga

SEQ ID NO:104 bacterial sequence-free vector, nucleic acid sequence

cgcgcgtagtatgctgaaataggtgactagaagttcctatactttctagagaataggaacttcat
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* * *

[0418] The disclosure is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the disclosure in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Other embodiments are within the following claims.

WHAT IS CLAIMED IS:

1. An expression vector comprising:

an expression cassette that comprises a nucleic acid sequence encoding a recombinant protein comprising a conserved amino acid sequence from a virus fused to an immunogenic amino acid sequence,

a target sequence for a first recombinase flanking each side of the expression cassette, and

one or more additional target sequences for one or more additional recombinases integrated within non-binding regions of the target sequence for the first recombinase,

wherein protein expressed intracellularly from the expression cassette is capable of forming a virus-like particle (VLP).

2. The expression vector of claim 1, wherein the immunogenic amino acid sequence is from the same virus as the conserved amino acid sequence.

3. The expression vector of claim 1 or 2, wherein the conserved amino acid sequence is from a viral glycoprotein.

4. The expression vector of claim 3, wherein the immunogenic amino acid sequence is from the same viral glycoprotein.

5. The expression vector of any one of claims 1 to 4, wherein the expression cassette further comprises a nucleic acid sequence encoding a viral envelope protein and/or a nucleic acid sequence encoding a viral matrix protein.

6. The expression vector of claim 5, wherein the viral envelope protein and/or the viral matrix protein are from the same virus as the conserved amino acid sequence.

7. The expression vector of any one of claims 1 to 6, wherein the conserved amino acid sequence, the immunogenic amino acid sequence, the viral envelope protein, and/or the viral matrix protein is a consensus sequence.

8. The expression vector of any one of claims 1 to 7, wherein the recombinant protein is capable of stimulating an immune response against the virus comprising neutralizing antibodies.

9. The expression vector of any one of claims 1 to 8, wherein the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against the virus.

10. The expression vector of claim 8 or 9, wherein the immune response is cross-reactive to a related virus or strain.

11. The expression vector of any one of claims 1 to 10, wherein the recombinant protein excludes amino acid sequences from the virus that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

12. The expression vector of any one of claims 1 to 11, wherein the expression cassette comprises a single open reading frame comprising a nucleic acid sequence encoding a self-cleaving peptide between each nucleic acid sequence encoding a protein.

13. The expression vector of any one of claims 1 to 12, wherein the virus is a coronavirus, an influenza virus, a human immunodeficiency virus, a human papillomavirus, a hepatitis virus, or an oncolytic virus.

14. The expression vector of claim 13, wherein the virus is a coronavirus.

15. The expression vector of claim 14, wherein the coronavirus is COVID-19.

16. The expression vector of claim 14 or 15, wherein the expression cassette comprises nucleic acid sequences encoding a coronavirus Membrane (M) protein, a coronavirus Envelope (E) protein, and a recombinant protein comprising a conserved amino acid sequence and an immunogenic amino acid sequence from a coronavirus Spike (S) protein.

17. The expression vector of claim 16, wherein the conserved amino acid sequence is from the S protein S2' cleavage site and internal fusion peptide (IFP).

18. The expression vector of claim 16 or 17, wherein the conserved amino acid sequence comprises SEQ ID NO:12.

19. The expression vector of any one of claims 16 to 18, wherein the immunogenic amino acid sequence is from the S protein receptor-binding domain (RBD).

20. The expression vector of any one of claims 16 to 19, wherein the immunogenic amino acid sequence is at least about 90% identical to SEQ ID NO:11.

21. The expression vector of any one of claims 16 to 20, wherein the recombinant protein further comprises a transmembrane (TM) domain sequence from the S protein.

22. The expression vector of any one of claims 16 to 21, wherein the recombinant protein excludes amino acid sequences from the S protein that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

23. The expression vector of any one of claims 16 to 21, wherein the amino acid sequence of the recombinant protein is at least about 90% identical to SEQ ID NO:55.

24. The expression vector of any one of claims 16 to 23, wherein the expression cassette comprises a single open reading frame translated as an amino acid sequence at least about 90% identical to SEQ ID NO:57.

25. The expression vector of any one of claims 16 to 24, wherein the recombinant protein is capable of stimulating an immune response against COVID-19.

26. The expression vector of any one of claims 16 to 25, wherein the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against COVID-19.

27. The expression vector of claim 25 or 26, wherein the immune response is cross-reactive to other coronaviruses.

28. The expression vector of claim 27, wherein the immune response is cross-reactive to other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses.

29. The expression vector of any one of claims 1 to 28, wherein the target sequence for the first recombinase and the one or more additional target sequences for the one or more additional recombinases are selected from the group consisting of the PY54 pal site, the N15 telRL site, the loxP site, φK02 telRL site, the FRT site, the phiC31 attP site, and the λ attP site.

30. The expression vector of claim 29, wherein the expression vector comprises each of the target sequences.

31. The expression vector of claim 30, wherein the expression vector comprises the Tel recombinase pal site and the telRL, loxP, and FRT recombinase target binding sequences integrated within the pal site.

32. The expression vector of any one of claims 1 to 31, wherein the expression vector is for producing a bacterial sequence-free vector.

33. The expression vector of claim 32, wherein the bacterial sequence-free vector has circular covalently closed ends.

34. The expression vector of claim 32, wherein the bacterial sequence-free vector has linear covalently closed ends.

35. The expression vector of any one of claims 1 to 34, further comprising at least one enhancer sequence flanking each side of the target sequence for the first recombinase.

36. The expression vector of claim 35, wherein the at least one enhancer sequence is at least two enhancer sequences.

37. The expression vector of claim 35 or 36, wherein at least one enhancer sequence is a SV40 enhancer sequence.

38. A vector production system comprising recombinant cells designed to encode at least a first recombinase under the control of an inducible promoter, wherein the cells comprise the expression vector of any one of claims 1 to 37.

39. The vector production system of claim 38, wherein the inducible promoter is thermally-regulated, chemically-regulated, IPTG regulated, glucose-regulated, arabinose inducible, T7 polymerase regulated, cold-shock inducible, pH inducible, or combinations thereof.

40. The vector production system of claim 38 or 39, wherein the first recombinase is selected from telN and tel, and the expression vector incorporates the target sequence for at least the first recombinase.

41. The vector production system of any one of claims 38 to 40, wherein the recombinant cells have been further designed to encode a nuclease genome editing system, and wherein the expression vector further comprises a backbone sequence containing a cleavage site for the nuclease genome editing system.

42. The vector production system of claim 41, wherein the nuclease genome editing system is a CRISPR nuclease system comprising a Cas nuclease and gRNA, and the expression vector comprises a target sequence for the gRNA within the backbone sequence.

43. A method of producing a bacterial sequence-free vector comprising incubating the vector production system of any one of claims 38 to 42 under suitable conditions for expression of the first recombinase.

44. A method of producing a bacterial sequence-free vector comprising incubating the vector production system of claims 41 or 42 under suitable conditions for expression of the first recombinase and the nuclease genome editing system.

45. The method of claim 43 or 44, further comprising harvesting the bacterial sequence-free vector.

46. A bacterial sequence-free vector produced by the method of any of claims 43 to 45.

47. A bacterial sequence-free vector comprising an expression cassette that comprises a nucleic acid sequence encoding a recombinant protein comprising a conserved amino acid sequence from a virus fused to an immunogenic amino acid sequence, wherein protein expressed intracellularly from the expression cassette is capable of forming a VLP.

48. The bacterial sequence-free vector of claim 47, wherein the immunogenic amino acid sequence is from the same virus as the conserved amino acid sequence.

49. The bacterial sequence-free vector of claim 47 or 48, wherein the conserved amino acid sequence is from a viral glycoprotein.

50. The bacterial sequence-free vector of claim 49, wherein the immunogenic amino acid sequence is from the same viral glycoprotein.

51. The bacterial sequence-free vector of any one of claims 47 to 50, wherein the expression cassette further comprises a nucleic acid sequence encoding a viral envelope protein and/or a nucleic acid sequence encoding a viral matrix protein.

52. The bacterial sequence-free vector of claim 51, wherein the viral envelope protein and/or the viral matrix protein are from the same virus as the conserved amino acid sequence.

53. The bacterial sequence-free vector of any one of claims 47 to 52, wherein the conserved amino acid sequence, the immunogenic amino acid sequence, the viral envelope protein, and/or the viral matrix protein is a consensus sequence.

54. The bacterial sequence-free vector of any one of claims 47 to 53, wherein the recombinant protein is capable of stimulating an immune response against the virus comprising neutralizing antibodies.

55. The bacterial sequence-free vector of any one of claims 47 to 54, wherein the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against the virus.

56. The bacterial sequence-free vector of claim 54 or 55, wherein the immune response is cross-reactive to a related virus or strain.

57. The bacterial sequence-free vector of any one of claims 47 to 56, wherein the recombinant protein excludes amino acid sequences from the virus that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

58. The bacterial sequence-free vector of any one of claims 47 to 57, wherein the expression cassette comprises a single open reading frame comprising a nucleic acid sequence encoding a self-cleaving peptide between each nucleic acid sequence encoding a protein.

59. The bacterial sequence-free vector of any one of claims 47 to 58, wherein the virus is a coronavirus, an influenza virus, a human immunodeficiency virus, a human papillomavirus, a hepatitis virus, or an oncolytic virus.

60. The bacterial sequence-free vector of claim 59, wherein the virus is a coronavirus.

61. The bacterial sequence-free vector of claim 60, wherein the coronavirus is COVID-19.

62. The bacterial sequence-free vector of claim 60 or 61, wherein the expression cassette comprises nucleic acid sequences encoding a coronavirus M protein, a coronavirus E protein, and a recombinant protein comprising a conserved amino acid sequence and an immunogenic amino acid sequence from a coronavirus S protein.

63. The bacterial sequence-free vector of claim 62, wherein the conserved amino acid sequence is from the S protein S2' cleavage site and IFP.

64. The bacterial sequence-free vector of claim 62 or 63, wherein the conserved amino acid sequence comprises SEQ ID NO:12.

65. The bacterial sequence-free vector of any one of claims 62 to 64, wherein the immunogenic amino acid sequence is from the S protein RBD.

66. The bacterial sequence-free vector of any one of claims 62 to 65, wherein the immunogenic amino acid sequence is at least about 90% identical to SEQ ID NO:11.

67. The bacterial sequence-free vector of any one of claims 62 to 66, wherein the recombinant protein further comprises a TM domain sequence from the S protein.

68. The bacterial sequence-free vector of any one of claims 62 to 67, wherein the recombinant protein excludes amino acid sequences from the S protein that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

69. The bacterial sequence-free vector of any one of claims 62 to 68, wherein the amino acid sequence of the recombinant protein is at least about 90% identical to SEQ ID NO:55.

70. The bacterial sequence-free vector of any one of claims 62 to 69, wherein the expression cassette comprises a single open reading frame translated as an amino acid sequence at least about 90% identical to SEQ ID NO:57.

71. The bacterial sequence-free vector of any one of claims 62 to 70, wherein the recombinant protein is capable of stimulating an immune response against COVID-19.

72. The bacterial sequence-free vector of any one of claims 62 to 71, wherein the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against COVID-19.

73. The bacterial sequence-free vector of claim 71 or 72, wherein the immune response is cross-reactive to other coronaviruses.

74. The bacterial sequence-free vector of claim 73, wherein the immune response is cross-reactive to other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses.

75. The bacterial sequence-free vector of any one of claims 47 to 74, further comprising at least one enhancer sequence flanking each side of the expression cassette.

76. The bacterial sequence-free vector of claim 75, wherein the at least one enhancer sequence is at least two enhancer sequences.

77. The bacterial sequence-free vector of claim 75 or 76, wherein at least one enhancer sequence is a SV40 enhancer sequence.

78. The bacterial sequence-free vector of any one of claims 47 to 77, comprising circular covalently closed ends.

79. The bacterial sequence-free vector of any one of claims 47 to 77, comprising linear covalently closed ends.

80. A polynucleotide encoding an amino acid sequence at least about 90% identical to SEQ ID NO:57.

81. A recombinant cell comprising the expression vector of any one of claims 1 to 37 or the bacterial sequence-free vector of any one of claims 46 to 79.

82. A method of producing a VLP, comprising culturing the recombinant cell of claim 81 under suitable conditions for production of the VLP from the expression vector or the bacterial sequence-free vector.

83. The method of claim 82, further comprising isolating the VLP.

84. The method of claim 83, wherein the isolating is by affinity purification.

85. The method of any one of claims 82 to 84, wherein the VLP is produced by the expression vector of any one of claims 14 to 37 or the bacterial sequence-free vector of any one of claims 60 to 79.

86. The method of claim 85, wherein the affinity purification comprises an angiotensin-converting enzyme 2 (ACE2) receptor peptide or an anti-S protein monoclonal antibody.

87. The method of claim 86, wherein the ACE2 receptor peptide comprises an amino acid sequence that is at least about 90% identical to the amino acid sequence of SEQ ID NO:70.

88. The method of claim 86 or 87, wherein the ACE2 receptor peptide comprises a biotin acceptor peptide (BAP) tag at the C-terminus or N-terminus of the peptide.

89. The method of claim 88, wherein the BAP tag comprises an amino acid sequence at least about 90% identical to the amino acid sequence of SEQ ID NO:71.

90. The method of any one of claims 86 to 89, wherein the ACE2 receptor peptide or anti-S protein monoclonal antibody is biotinylated and immobilized on a streptavidin-coated bead.

91. The method of any one of claims 84 to 90, wherein the affinity purification comprises microfluidics and/or chromatography.

92. A VLP produced by the method of any one of claims 82 to 91.

93. A VLP comprising a recombinant protein comprising a conserved amino acid sequence from a virus fused to an immunogenic amino acid sequence.

94. The VLP of claim 93, wherein the immunogenic amino acid sequence is from the same virus as the conserved amino acid sequence.

95. The VLP of claim 93 or 94, wherein the conserved amino acid sequence is from a viral glycoprotein.

96. The VLP of any one of claims 93 to 95, wherein the immunogenic amino acid sequence is from the same viral glycoprotein.

97. The VLP of any one of claims 93 to 96, further comprising a viral envelope protein and/or a viral matrix protein.

98. The VLP of claim 97, wherein the viral envelope protein and/or the viral matrix protein are from the same virus as the conserved amino acid sequence.

99. The VLP of any one of claims 93 to 98, wherein the conserved amino acid sequence, the immunogenic amino acid sequence, the viral envelope protein, and/or the viral matrix protein is a consensus sequence.

100. The VLP of any one of claims 93 to 99, wherein the recombinant protein is capable of stimulating an immune response against the virus comprising neutralizing antibodies.

101. The VLP of any one of claims 93 to 100, wherein the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against the virus.

102. The VLP of claim 100 or 101, wherein the immune response is cross-reactive to a related virus or strain.

103. The VLP of any one of claims 93 to 102, wherein the recombinant protein excludes amino acid sequences from the virus that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

104. The VLP of any one of claims 93 to 103, wherein the virus is a coronavirus, an influenza virus, a human immunodeficiency virus, a human papillomavirus, a hepatitis virus, or an oncolytic virus.

105. The VLP of claim 104, wherein the virus is a coronavirus.

106. The VLP of claim 105, wherein the coronavirus is COVID-19.

107. The VLP of claim 105 or 106, comprising a coronavirus Membrane (M) protein, a coronavirus Envelope (E) protein, and a recombinant protein comprising a conserved amino acid sequence and an immunogenic amino acid sequence from a coronavirus Spike (S) protein.

108. The VLP of claim 107, wherein the conserved amino acid sequence is from the S protein S2' cleavage site and internal fusion peptide (IFP).

109. The VLP of claim 107 or 108, wherein the conserved amino acid sequence comprises SEQ ID NO:12.

110. The VLP of any one of claims 107 to 109, wherein the immunogenic amino acid sequence is from the S protein receptor-binding domain (RBD).

111. The VLP of any one of claims 107 to 110, wherein the immunogenic amino acid sequence is at least about 90% identical to SEQ ID NO:11.

112. The VLP of any one of claims 107 to 111, wherein the recombinant protein further comprises a transmembrane (TM) domain sequence from the S protein.

113. The VLP of any one of claims 107 to 112, wherein the recombinant protein excludes amino acid sequences from the S protein that stimulate an immune response comprising non-neutralizing antibodies and/or that stimulate a Th2 cell-mediated immune response.

114. The VLP of any one of claims 107 to 113, wherein the amino acid sequence of the recombinant protein is at least about 90% identical to SEQ ID NO:55.

115. A VLP comprising a recombinant protein at least about 90% identical to SEQ ID NO:55, an M protein at least about 90% identical to SEQ ID NO:1, and an E protein at least about 90% identical to SEQ ID NO:3.

116. The VLP of any one of claims 107 to 115, wherein the recombinant protein is capable of stimulating an immune response against COVID-19.

117. The VLP of any one of claims 107 to 116, wherein the recombinant protein is capable of stimulating a Th1 cell-mediated immune response against COVID-19.

118. The VLP of claim 116 or 117, wherein the immune response is cross-reactive to other coronaviruses.

119. The VLP of claim 118, wherein the immune response is cross-reactive to other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses.

120. A composition comprising the expression vector of any one of claims 1 to 37, the bacterial sequence-free vector of any one of claims 46 to 79, or the virus-like particle of any one of claims 92 to 119.

121. The composition of claim 120, further comprising a delivery agent.

122. The composition of claim 121, wherein the delivery agent is a nanoparticle.

123. The composition of claim 121 or 122, wherein the delivery agent comprises a targeting ligand.

124. The composition of claim 123, wherein the targeting ligand comprises a S protein peptide.

125. The composition of claim 124, wherein the S protein peptide comprises an amino acid sequence at least about 90% identical to any one of SEQ ID NOs:76-99.

126. A method of treating a viral infection in a subject, comprising administering to the subject the expression vector of any one of claims 1 to 37, the bacterial sequence-free vector of any one of claims 46 to 79, the VLP of any one of claims 92 to 119, or the composition of any one of claims 120 to 125, wherein intracellular expression of the expression vector or the bacterial sequence-free vector produces a VLP.

127. The method of claim 126, wherein the administering is by parenteral or non-parenteral administration.

128. The method of claim 127, wherein the administering is by oral, pulmonary, intranasal, intravenous, epidermal, transdermal, subcutaneous, intramuscular, or intraperitoneal administration or by inhalation.

129. The method of any one of claims 126 to 128, wherein the VLP stimulates an immune response in the subject comprising neutralizing antibodies against the viral infection.

130. The method of any one of claims 126 to 129, wherein the VLP stimulates a Th1 cell-mediated immune response in the subject against the viral infection.

131. The method of claims 129 or 130, wherein the immune response is cross-reactive to a related virus or strain.

132. The method of any one of claims 126 to 131, wherein the VLP does not stimulate an immune response comprising non-neutralizing antibodies in the subject and/or does not stimulate a Th2 cell-mediated immune response in the subject.

133. The method of any one of claims 126 to 132, wherein the VLP cross-competes with the infecting virus for binding to a viral receptor.

134. The method of claim 133, wherein the VLP cross-competes with a related virus or strain for binding to the viral receptor.

135. The method of any one of claims 126 to 134, wherein the viral infection is a coronavirus, an influenza virus, a human immunodeficiency virus, a human papillomavirus, a hepatitis virus, or an oncolytic virus.

136. The method of claim 135, wherein the viral infection is a coronavirus.

137. The method of claim 136, wherein the viral infection is COVID-19.

138. The method of claim 137, wherein the VLP stimulates an immune response in the subject comprising neutralizing antibodies against COVID-19.

139. The method of any one of claims 137 or 138, wherein the VLP stimulates a Th1 cell-mediated immune response in the subject against COVID-19.

140. The method of claim 138 or 139, wherein the immune response is cross-reactive to other coronaviruses.

141. The method of claim 140, wherein the immune response is cross-reactive to other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses.

142. The method of any one of claims 137 to 141, wherein the VLP does not stimulate an immune response comprising non-neutralizing antibodies in the subject and/or does not stimulate a Th2 cell-mediated immune response in the subject.

143. The method of any one of claims 137 to 142, wherein the administering is by inhalation.

144. The method of any one of claims 137 to 143, wherein the VLP cross-competes with COVID-19 for binding to ACE2 receptor, neuropilin-1, or other receptors.

145. The method of claim 144, wherein the VLP cross-competes with other coronaviruses for binding to ACE2 receptor, neuropilin-1, and/or other receptors.

146. The method of claim 145, wherein the VLP cross-competes with other severe acute respiratory syndrome coronaviruses and/or human betacoronaviruses for binding to ACE2 receptor, neuropilin-1, and/or other receptors.

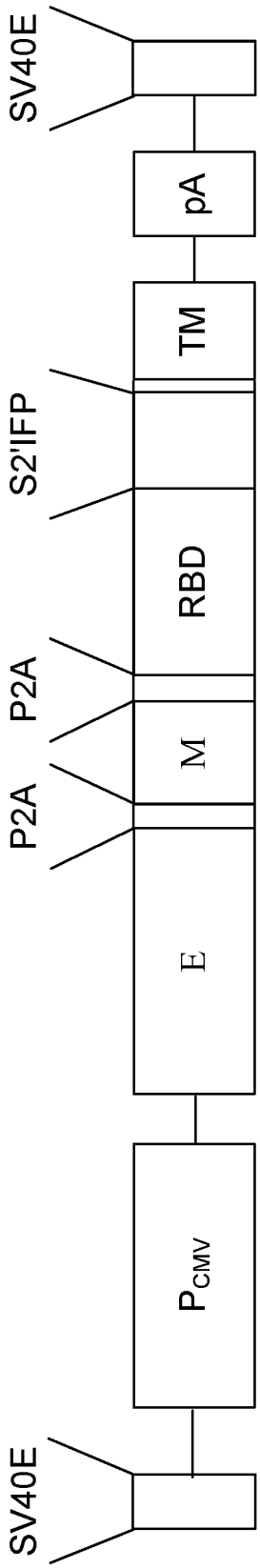
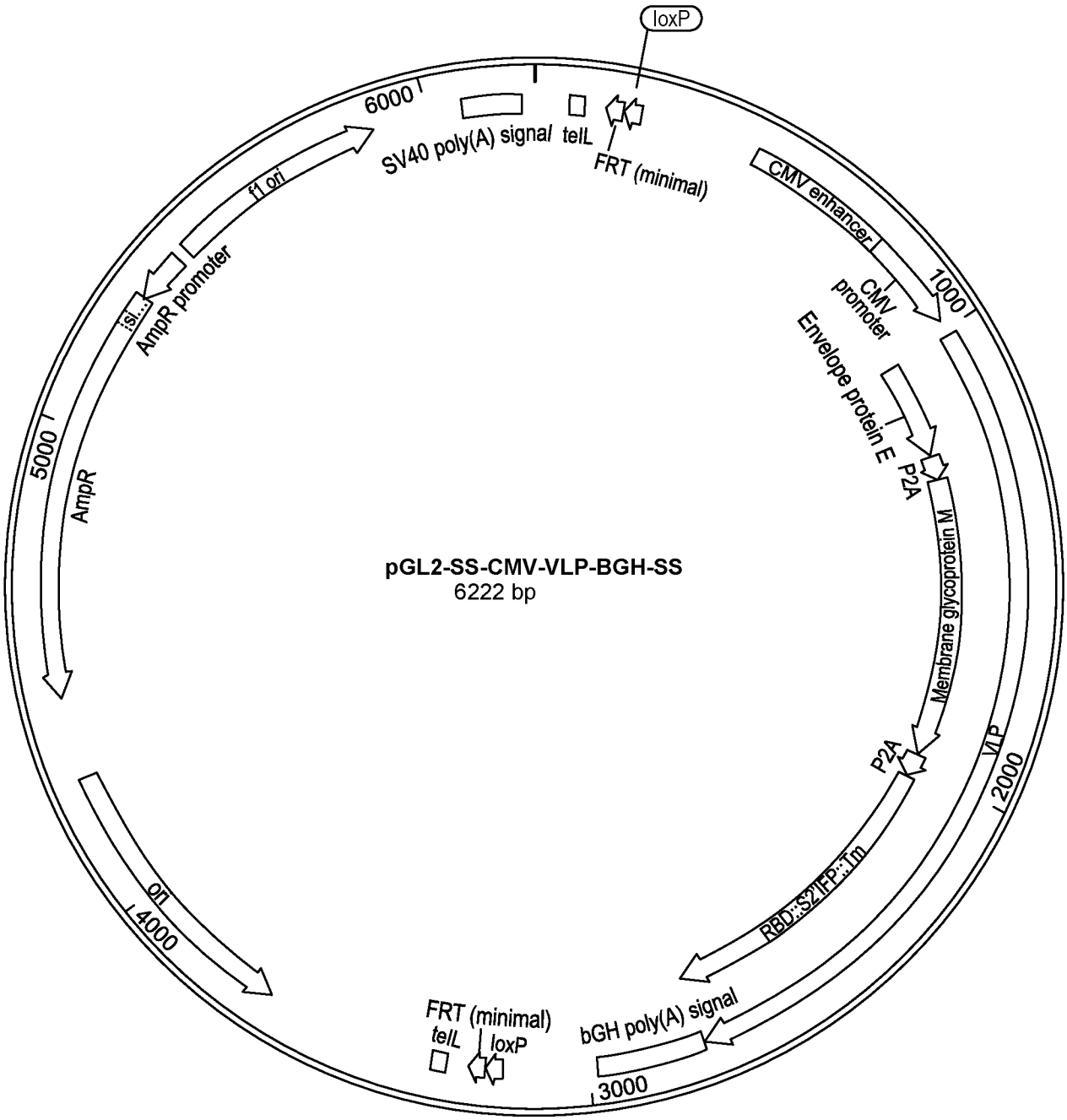


FIG. 1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CMV promoter
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P2A
Membrane glycoprotein M
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VLP
2000
RBD::S2IFP::Tm
bGH poly(A) signal
3000
FRT (minimal)
telL
loxP
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6222 bp

FIG. 2

FIG. 3A

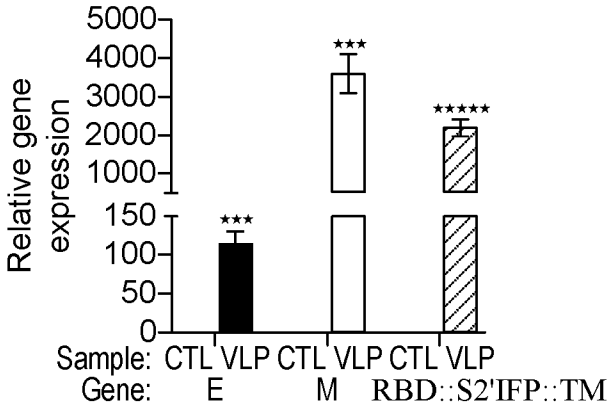


FIG. 3B

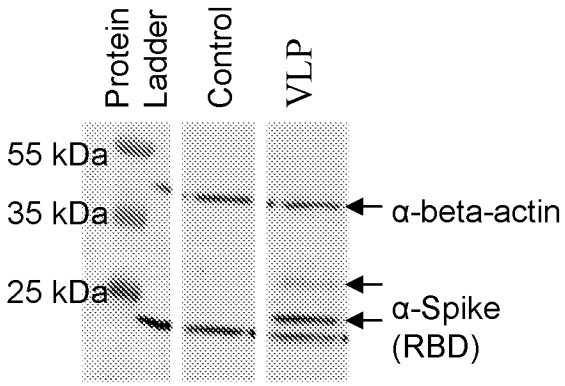
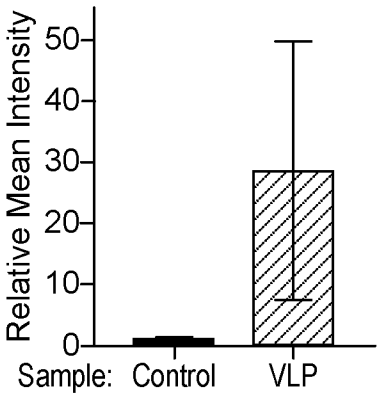
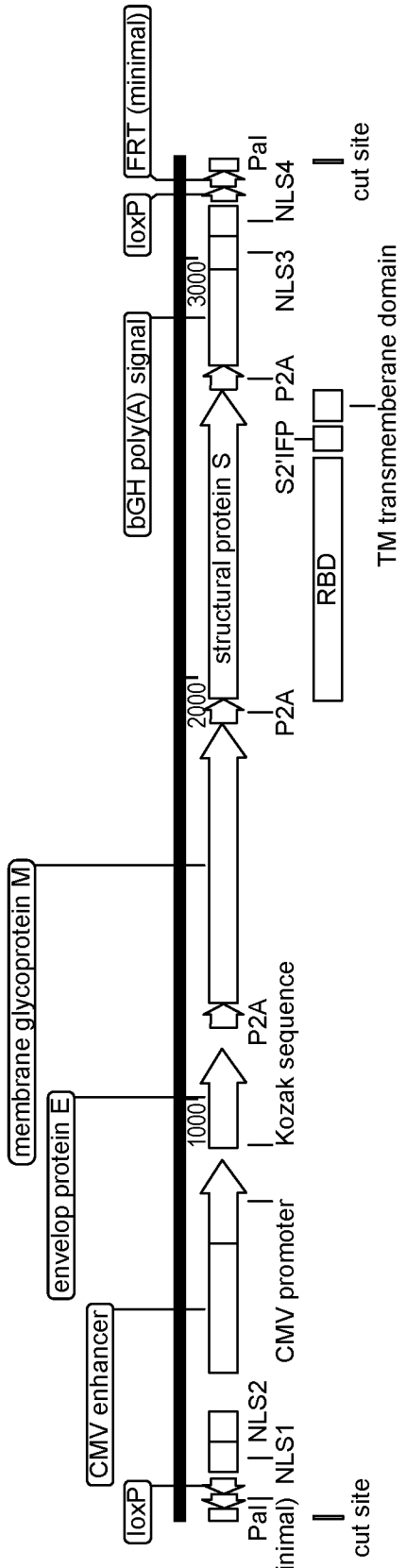


FIG. 3C

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CMV promoter
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RBD
S2'IFP
P2A
TM transmemberane domain
bGH poly(A) signal
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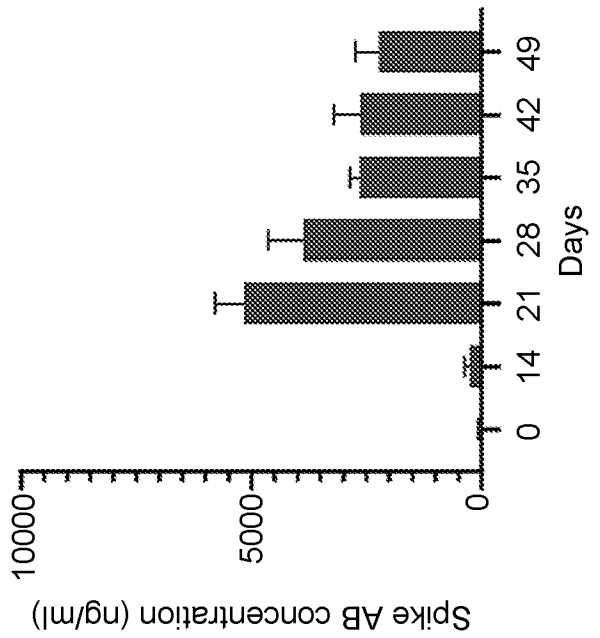
msDNA VLP Cov 19-BGH poly
3239 bp

FIG. 4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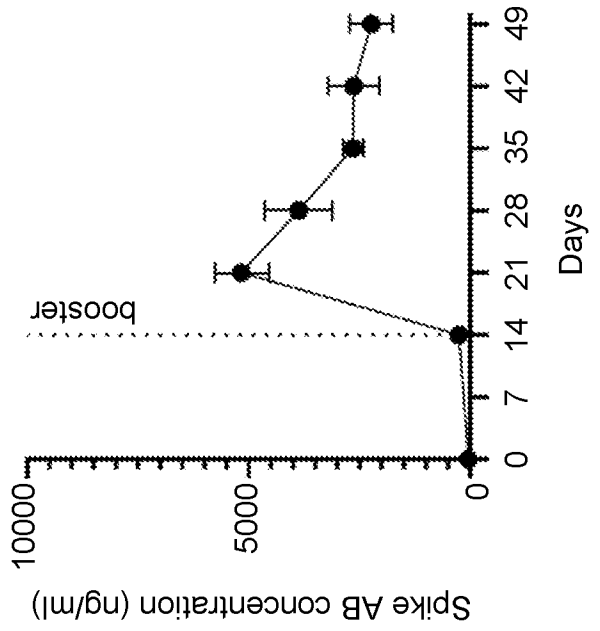
28
35
42
49
Days

FIG. 5B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Days

FIG. 5A

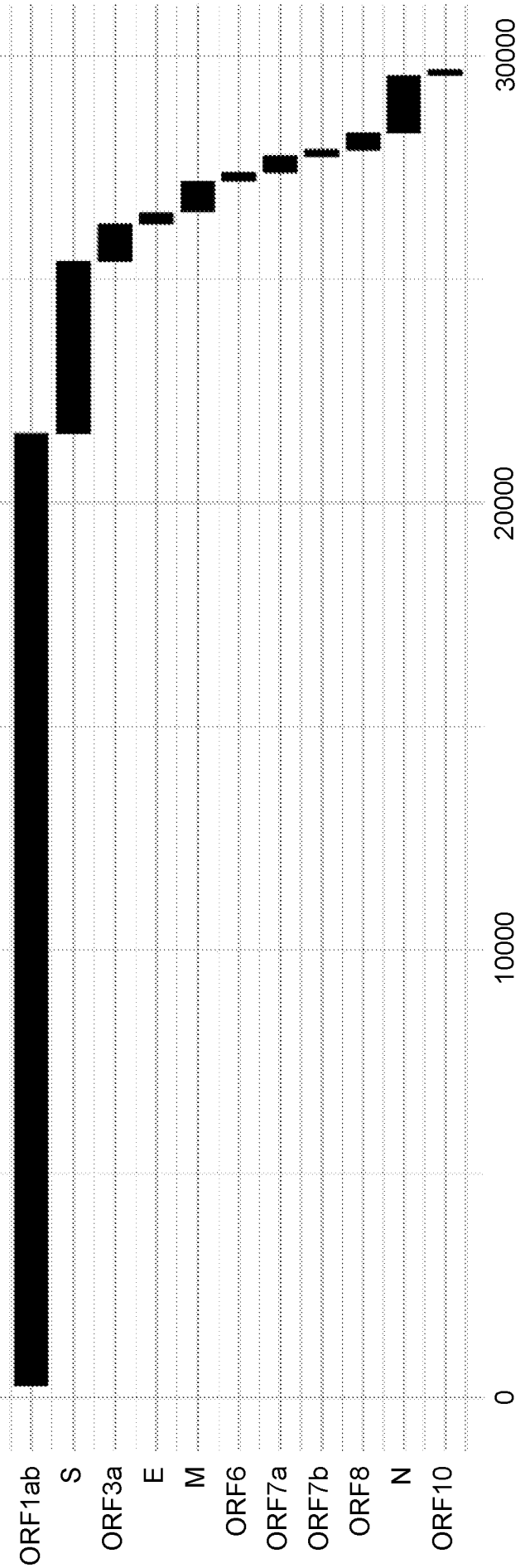
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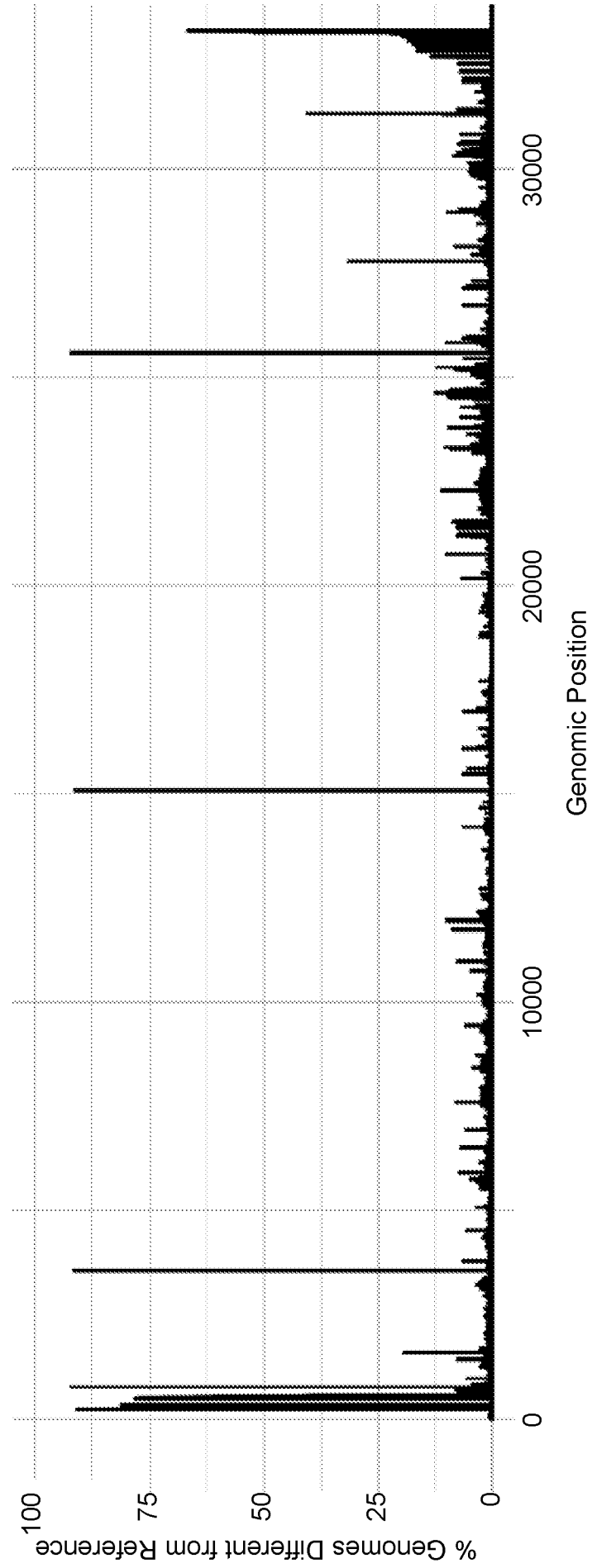
FIG. 6A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Genomic Position

FIG. 6B

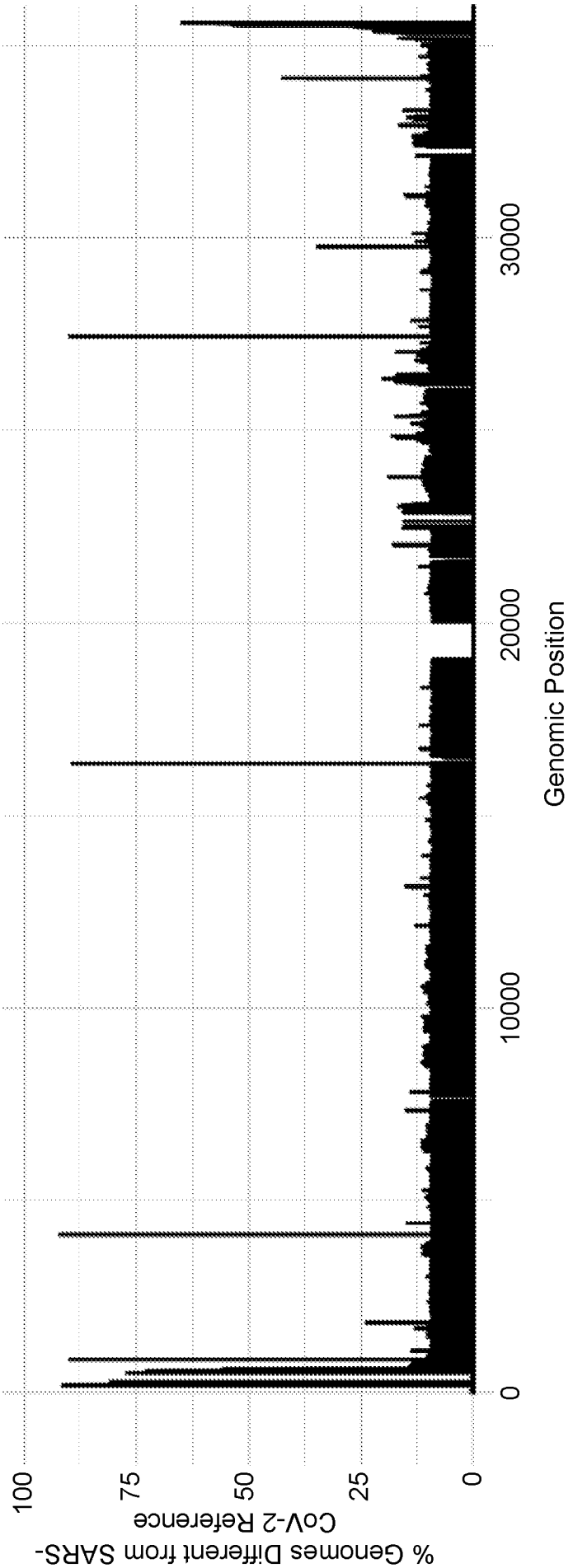
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FIG. 7

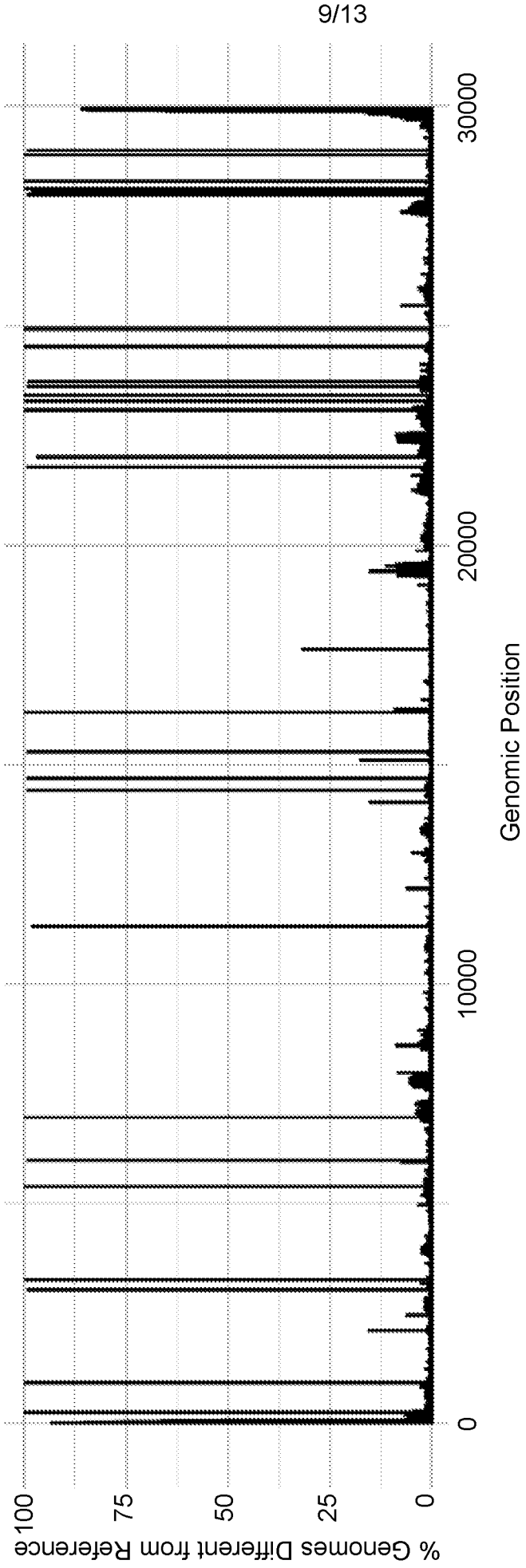
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FIG. 8A

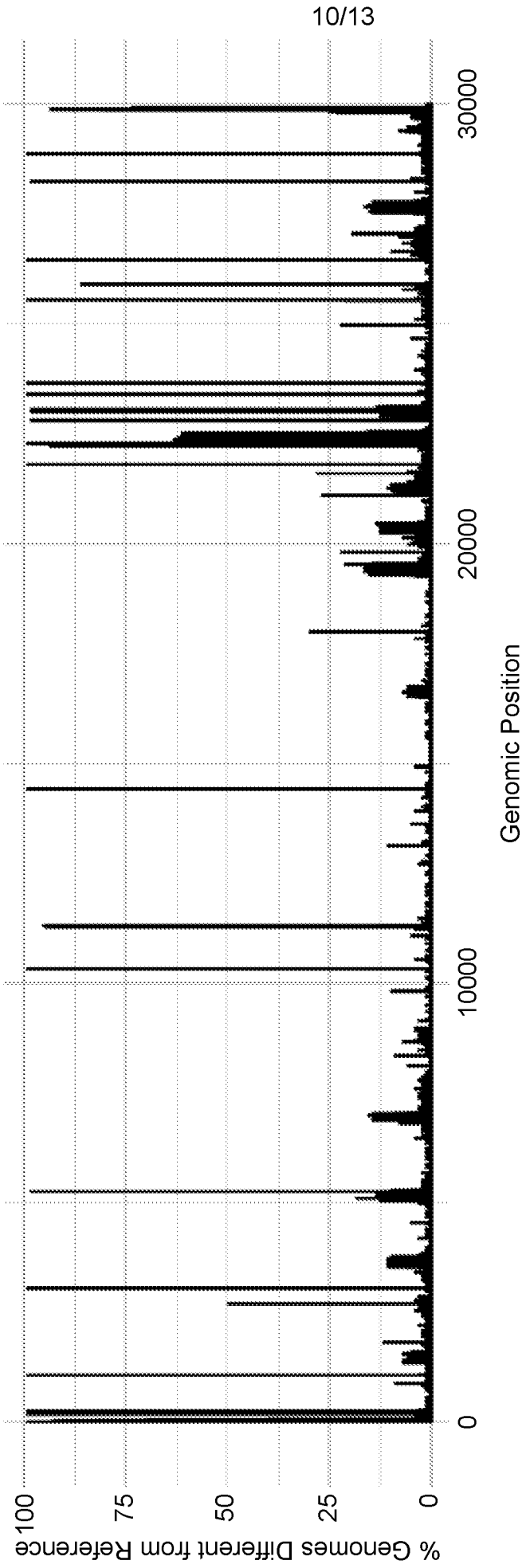
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FIG. 8B

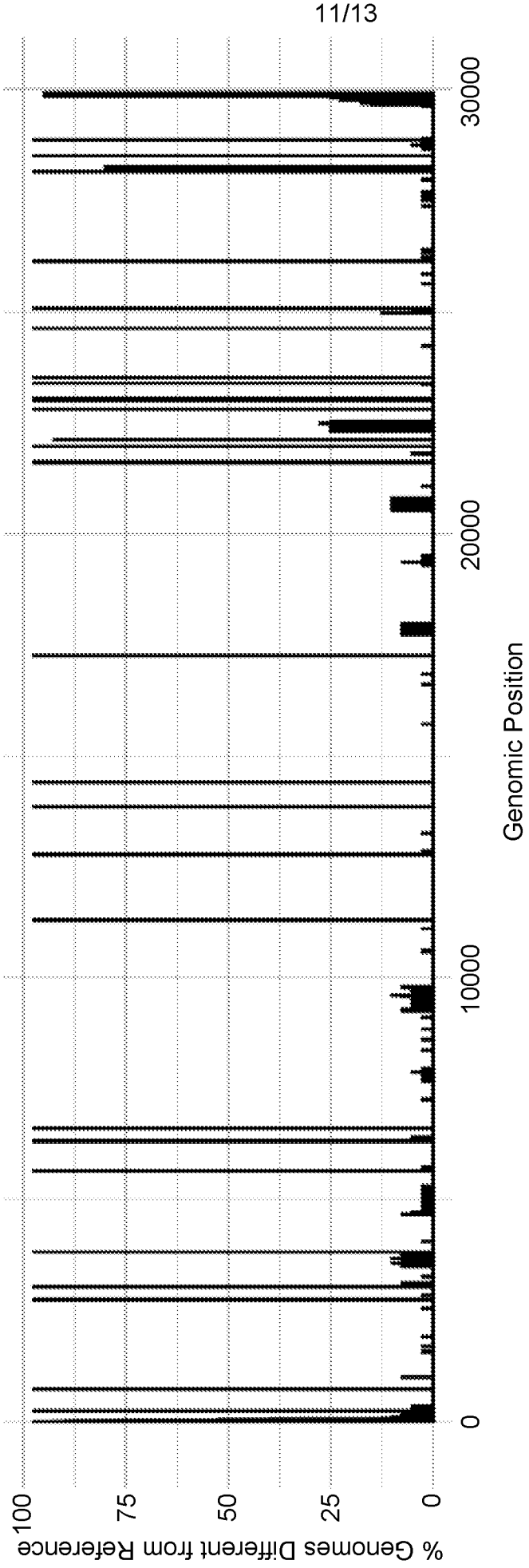
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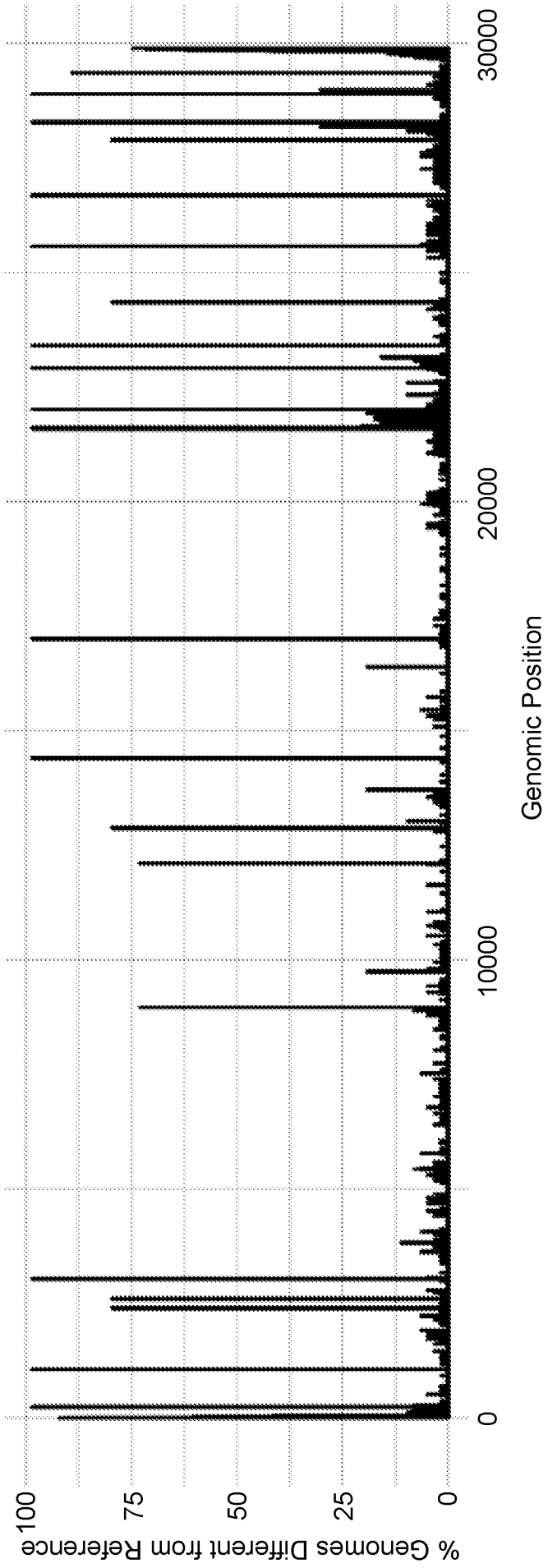
FIG. 8C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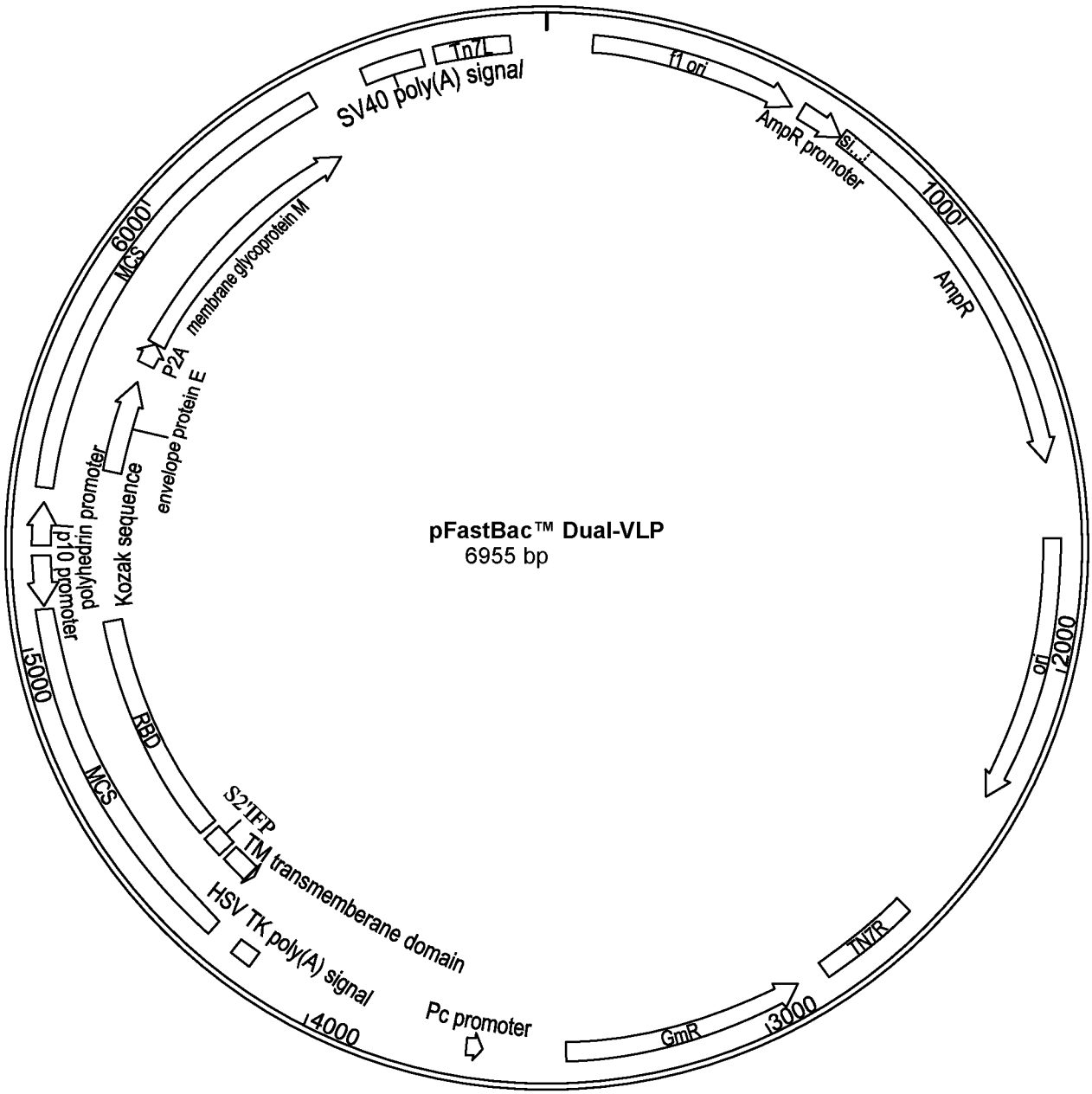
Genomic Position

FIG. 8D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AmpR
ori
2000
TNZR
3000
GmR
Pc promoter
HSV TK poly(A) signal
4000
MCS
5000
p10 promoter
polyhedrin promoter
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RBD
S2IFP
TM transmemberane domain

FIG. 9

SEQUENCE LISTING

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<151> 2020-12-11

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<170> PatentIn version 3.5

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Phe Val Leu Ala Ala Val Tyr Arg Ile Asn Trp Ile Thr Gly Gly Ile
65                  70                  75                  80


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                85                  90                  95


Ile Ala Ser Phe Arg Leu Phe Ala Arg Thr Arg Ser Met Trp Ser Phe
            100                 105                 110


Asn Pro Glu Thr Asn Ile Leu Leu Asn Val Pro Leu His Gly Thr Ile
        115                 120                 125


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Leu Arg Gly His Leu Arg Ile Ala Gly His His Leu Gly Arg Cys Asp
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<223> Envelope Protein

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Met Tyr Ser Phe Val Ser Glu Glu Thr Gly Thr Leu Ile Val Asn Ser
1 5 10 15

Val Leu Leu Phe Leu Ala Phe Val Val Phe Leu Leu Val Thr Leu Ala
20 25 30

```
Ile Leu Thr Ala Leu Arg Leu Cys Ala Tyr Cys Cys Asn Ile Val Asn
        35                  40                  45

Val Ser Leu Val Lys Pro Ser Phe Tyr Val Tyr Ser Arg Val Lys Asn
    50                  55                  60

Leu Asn Ser Ser Arg Val Pro Asp Leu Leu Val
65                  70                  75
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<210> 4
<211> 228
<212> DNA
<213> Artificial Sequence

<220>
<223> Envelope Protein

<400> 4

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cttgctttcg tggtattctt gctagttaca ctagccatcc ttactgcgct tcgattgtgt      120

gcgtactgct gcaatattgt taacgtgagt cttgtaaaac cttcttttta cgtttactct      180

cgtgttaaaa atctgaattc ttctagagtt cctgatcttc tggtctaa                   228
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<210> 5
<211> 1273
<212> PRT
<213> Artificial Sequence

<220>
<223> Spike protein

<400> 5

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Met Phe Val Phe Leu Val Leu Leu Pro Leu Val Ser Ser Gln Cys Val
1               5                   10                  15

Asn Leu Thr Thr Arg Thr Gln Leu Pro Pro Ala Tyr Thr Asn Ser Phe
            20                  25                  30

Thr Arg Gly Val Tyr Tyr Pro Asp Lys Val Phe Arg Ser Ser Val Leu
        35                  40                  45

His Ser Thr Gln Asp Leu Phe Leu Pro Phe Phe Ser Asn Val Thr Trp
    50                  55                  60

Phe His Ala Ile His Val Ser Gly Thr Asn Gly Thr Lys Arg Phe Asp
65                  70                  75                  80

Asn Pro Val Leu Pro Phe Asn Asp Gly Val Tyr Phe Ala Ser Thr Glu
                85                  90                  95

Lys Ser Asn Ile Ile Arg Gly Trp Ile Phe Gly Thr Thr Leu Asp Ser
            100                 105                 110
```

```
Lys Thr Gln Ser Leu Leu Ile Val Asn Asn Ala Thr Asn Val Val Ile
        115                 120                 125

Lys Val Cys Glu Phe Gln Phe Cys Asn Asp Pro Phe Leu Gly Val Tyr
    130                 135                 140

Tyr His Lys Asn Asn Lys Ser Trp Met Glu Ser Glu Phe Arg Val Tyr
145                 150                 155                 160

Ser Ser Ala Asn Asn Cys Thr Phe Glu Tyr Val Ser Gln Pro Phe Leu
                165                 170                 175

Met Asp Leu Glu Gly Lys Gln Gly Asn Phe Lys Asn Leu Arg Glu Phe
            180                 185                 190

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

Pro Ile Asn Leu Val Arg Asp Leu Pro Gln Gly Phe Ser Ala Leu Glu
    210                 215                 220

Pro Leu Val Asp Leu Pro Ile Gly Ile Asn Ile Thr Arg Phe Gln Thr
225                 230                 235                 240

Leu Leu Ala Leu His Arg Ser Tyr Leu Thr Pro Gly Asp Ser Ser Ser
                245                 250                 255

Gly Trp Thr Ala Gly Ala Ala Ala Tyr Tyr Val Gly Tyr Leu Gln Pro
            260                 265                 270

Arg Thr Phe Leu Leu Lys Tyr Asn Glu Asn Gly Thr Ile Thr Asp Ala
        275                 280                 285

Val Asp Cys Ala Leu Asp Pro Leu Ser Glu Thr Lys Cys Thr Leu Lys
    290                 295                 300

Ser Phe Thr Val Glu Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg Val
305                 310                 315                 320

Gln Pro Thr Glu Ser Ile Val Arg Phe Pro Asn Ile Thr Asn Leu Cys
                325                 330                 335

Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val Tyr Ala
            340                 345                 350

Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser Val Leu
        355                 360                 365

Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser Pro
    370                 375                 380

Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp Ser Phe
```

385 390 395 400

Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln Thr Gly
405 410 415

Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr Gly Cys
420 425 430

Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly Gly Asn
435 440 445

Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys Pro Phe
450 455 460

Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr Pro Cys
465 470 475 480

Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser Tyr Gly
485 490 495

Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln Pro Tyr Arg Val Val Val
500 505 510

Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly Pro Lys
515 520 525

Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe Asn Phe Asn
530 535 540

Gly Leu Thr Gly Thr Gly Val Leu Thr Glu Ser Asn Lys Lys Phe Leu
545 550 555 560

Pro Phe Gln Gln Phe Gly Arg Asp Ile Ala Asp Thr Thr Asp Ala Val
565 570 575

Arg Asp Pro Gln Thr Leu Glu Ile Leu Asp Ile Thr Pro Cys Ser Phe
580 585 590

Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Thr Ser Asn Gln Val
595 600 605

Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Glu Val Pro Val Ala Ile
610 615 620

His Ala Asp Gln Leu Thr Pro Thr Trp Arg Val Tyr Ser Thr Gly Ser
625 630 635 640

Asn Val Phe Gln Thr Arg Ala Gly Cys Leu Ile Gly Ala Glu His Val
645 650 655

Asn Asn Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala
660 665 670

```
Ser Tyr Gln Thr Gln Thr Asn Ser Pro Arg Arg Ala Arg Ser Val Ala
        675                 680                 685

Ser Gln Ser Ile Ile Ala Tyr Thr Met Ser Leu Gly Ala Glu Asn Ser
    690                 695                 700

Val Ala Tyr Ser Asn Asn Ser Ile Ala Ile Pro Thr Asn Phe Thr Ile
705                 710                 715                 720

Ser Val Thr Thr Glu Ile Leu Pro Val Ser Met Thr Lys Thr Ser Val
                725                 730                 735

Asp Cys Thr Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ser Asn Leu
            740                 745                 750

Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Thr
        755                 760                 765

Gly Ile Ala Val Glu Gln Asp Lys Asn Thr Gln Glu Val Phe Ala Gln
    770                 775                 780

Val Lys Gln Ile Tyr Lys Thr Pro Pro Ile Lys Asp Phe Gly Gly Phe
785                 790                 795                 800

Asn Phe Ser Gln Ile Leu Pro Asp Pro Ser Lys Pro Ser Lys Arg Ser
                805                 810                 815

Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly
            820                 825                 830

Phe Ile Lys Gln Tyr Gly Asp Cys Leu Gly Asp Ile Ala Ala Arg Asp
        835                 840                 845

Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu
    850                 855                 860

Leu Thr Asp Glu Met Ile Ala Gln Tyr Thr Ser Ala Leu Leu Ala Gly
865                 870                 875                 880

Thr Ile Thr Ser Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile
                885                 890                 895

Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr
            900                 905                 910

Gln Asn Val Leu Tyr Glu Asn Gln Lys Leu Ile Ala Asn Gln Phe Asn
        915                 920                 925

Ser Ala Ile Gly Lys Ile Gln Asp Ser Leu Ser Ser Thr Ala Ser Ala
    930                 935                 940
```

```
Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn
945                 950                 955                 960


Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val
                965                 970                 975


Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln
            980                 985                 990


Ile Asp Arg Leu Ile Thr Gly Arg  Leu Gln Ser Leu Gln  Thr Tyr Val
        995                 1000                1005


Thr Gln  Gln Leu Ile Arg Ala  Ala Glu Ile Arg Ala  Ser Ala Asn
    1010                1015                1020


Leu Ala  Ala Thr Lys Met Ser  Glu Cys Val Leu Gly  Gln Ser Lys
    1025                1030                1035


Arg Val  Asp Phe Cys Gly Lys  Gly Tyr His Leu Met  Ser Phe Pro
    1040                1045                1050


Gln Ser  Ala Pro His Gly Val  Val Phe Leu His Val  Thr Tyr Val
    1055                1060                1065


Pro Ala  Gln Glu Lys Asn Phe  Thr Thr Ala Pro Ala  Ile Cys His
    1070                1075                1080


Asp Gly  Lys Ala His Phe Pro  Arg Glu Gly Val Phe  Val Ser Asn
    1085                1090                1095


Gly Thr  His Trp Phe Val Thr  Gln Arg Asn Phe Tyr  Glu Pro Gln
    1100                1105                1110


Ile Ile  Thr Thr Asp Asn Thr  Phe Val Ser Gly Asn  Cys Asp Val
    1115                1120                1125


Val Ile  Gly Ile Val Asn Asn  Thr Val Tyr Asp Pro  Leu Gln Pro
    1130                1135                1140


Glu Leu  Asp Ser Phe Lys Glu  Glu Leu Asp Lys Tyr  Phe Lys Asn
    1145                1150                1155


His Thr  Ser Pro Asp Val Asp  Leu Gly Asp Ile Ser  Gly Ile Asn
    1160                1165                1170


Ala Ser  Val Val Asn Ile Gln  Lys Glu Ile Asp Arg  Leu Asn Glu
    1175                1180                1185


Val Ala  Lys Asn Leu Asn Glu  Ser Leu Ile Asp Leu  Gln Glu Leu
    1190                1195                1200
```

```
Gly Lys  Tyr Glu Gln Tyr Ile  Lys Trp Pro Trp Tyr  Ile Trp Leu
    1205                 1210                 1215


Gly Phe  Ile Ala Gly Leu Ile  Ala Ile Val Met Val  Thr Ile Met
    1220                 1225                 1230


Leu Cys  Cys Met Thr Ser Cys  Cys Ser Cys Leu Lys  Gly Cys Cys
    1235                 1240                 1245


Ser Cys  Gly Ser Cys Cys Lys  Phe Asp Glu Asp Asp  Ser Glu Pro
    1250                 1255                 1260


Val Leu  Lys Gly Val Lys Leu  His Tyr Thr
    1265                 1270
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<210> 6
<211> 1118
<212> DNA
<213> Artificial Sequence

<220>
<223> Spike protein

<400> 6

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atgtttgttt ttcttgtttt attgccacta gtctctagtc agtgtgttaa tcttacaacc       60

agaactcaat tacccccctgc atacactaat tctttcacac gtggtgttta ttaccctgac      120

aaagttttca gatcctcagt tttacattca actcaggact tgttcttacc tttcttttcc      180

aatgttactt ggttccatgc tatacatgtc tctgggacca atggtactaa gaggtttgat      240

aaccctgtcc taccatttaa tgatggtgtt tattttgctt ccactgagaa gtctaacata      300

ataagaggct ggatttttgg tactacttta gattcgaaga cccagtccct acttattgtt      360

aataacgcta ctaatgttgt tattaaagtc tgtgaatttc aattttgtaa tgatccattt      420

ttgggtgttt attaccacaa aaacaacaaa agttggatgg aaagtgagtt cagagtttat      480

tctagtgcga ataattgcac ttttgaatat gtctctcagc cttttcttat ggaccttgaa      540

ggaaaacagg gtaatttcaa aaatcttagg gaatttgtgt ttaagaatat tgatggttat      600

tttaaaatat attctaagca cacgcctatt aatttagtgc gtgatctccc tcagggtttt      660

tcggctttag aaccattggt agatttgcca ataggtatta acatcactag gtttcaaact      720

ttacttgctt tacatagaag ttatttgact cctggtgatt cttcttcagg ttggacagct      780

ggtgctgcag cttattatgt gggttatctt caacctagga cttttctatt aaaatataat      840

gaaaatggaa ccattacaga tgctgtagac tgtgcacttg accctctctc agaaacaaag      900

tgtacgttga aatccttcac tgtagaaaaa ggaatctatc aaacttctaa ctttagagtc      960

caaccaacag aatctattgt tagatttcct aatattacaa acttgtgccc ttttggtgaa     1020

gtttttaacg ccaccagatt tgcatctgtt tatgcttgga acaggaagag aatcagcaac     1080

tgtgttgctg attattctgt cctatataat tccgcatc                             1118
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<210> 7

<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Internal Fusion Peptide

<400> 7

Ser Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala
1   5   10   15

Gly Phe

<210> 8
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Internal Fusion Peptide

<400> 8
tcatttattg aagatctact tttcaacaaa gtgacacttg cagatgctgg cttc   54

<210> 9
<211> 254
<212> PRT
<213> Artificial Sequence

<220>
<223> receptor-binding domain

<400> 9

Pro Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr
1   5   10   15

Arg Phe Ala Ser Val Tyr Ala Trp Asn Arg Lys Arg Ile Ser Asn Cys
            20   25   30

Val Ala Asp Tyr Ser Val Leu Tyr Asn Ser Ala Ser Phe Ser Thr Phe
        35   40   45

Lys Cys Tyr Gly Val Ser Pro Thr Lys Leu Asn Asp Leu Cys Phe Thr
    50   55   60

Asn Val Tyr Ala Asp Ser Phe Val Ile Arg Gly Asp Glu Val Arg Gln
65   70   75   80

Ile Ala Pro Gly Gln Thr Gly Lys Ile Ala Asp Tyr Asn Tyr Lys Leu
                85   90   95

Pro Asp Asp Phe Thr Gly Cys Val Ile Ala Trp Asn Ser Asn Asn Leu
            100   105   110

Asp Ser Lys Val Gly Gly Asn Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg
        115   120   125

```
Lys Ser Asn Leu Lys Pro Phe Glu Arg Asp Ile Ser Thr Glu Ile Tyr
    130                 135                 140

Gln Ala Gly Ser Thr Pro Cys Asn Gly Val Glu Gly Phe Asn Cys Tyr
145                 150                 155                 160

Phe Pro Leu Gln Ser Tyr Gly Phe Gln Pro Thr Asn Gly Val Gly Tyr
                165                 170                 175

Gln Pro Tyr Arg Val Val Val Leu Ser Phe Glu Leu Leu His Ala Pro
            180                 185                 190

Ala Thr Val Cys Gly Pro Lys Lys Ser Thr Asn Leu Val Lys Asn Lys
        195                 200                 205

Cys Val Asn Phe Asn Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr
    210                 215                 220

Glu Ser Asn Lys Lys Phe Leu Pro Phe Gln Gln Phe Gly Arg Asp Ile
225                 230                 235                 240

Ala Asp Thr Thr Asp Ala Val Arg Asp Pro Gln Thr Leu Glu
                245                 250
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<210> 10
<211> 762
<212> DNA
<213> Artificial Sequence

<220>
<223> receptor-binding domain

<400> 10

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cctaatatta caaacttgtg ccctttggt gaagttttta acgccaccag atttgcatct       60

gtttatgctt ggaacaggaa gagaatcagc aactgtgttg ctgattattc tgtcctatat      120

aattccgcat cattttccac ttttaagtgt tatggagtgt ctcctactaa attaaatgat      180

ctctgcttta ctaatgtcta tgcagattca tttgtaatta gaggtgatga agtcagacaa      240

atcgctccag ggcaaactgg aaagattgct gattataatt ataaattacc agatgatttt      300

acaggctgcg ttatagcttg gaattctaac aatcttgatt ctaaggttgg tggtaattat      360

aattacctgt atagattgtt taggaagtct aatctcaaac cttttgagag agatatttca      420

actgaaatct atcaggccgg tagcacacct tgtaatggtg ttgaaggttt taattgttac      480

tttccttttac aatcatatgg tttccaaccc actaatggtg ttggttacca accatacaga      540

gtagtagtac tttcttttga acttctacat gcaccagcaa ctgtttgtgg acctaaaaag      600

tctactaatt tggttaaaaa caaatgtgtc aatttcaact tcaatggttt aacaggcaca      660

ggtgttctta ctgagtctaa caaaaagttt ctgcctttcc aacaatttgg cagagacatt      720

gctgacacta ctgatgctgt ccgtgatcca cagacacttg ag                         762
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<210> 11
<211> 192
<212> PRT
<213> Artificial Sequence

<220>
<223> immunogenic sequence

<400> 11

Pro Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr
1 5 10 15

Arg Phe Ala Ser Val Tyr Ala Trp Asn Arg Lys Arg Ile Ser Asn Cys
20 25 30

Val Ala Asp Tyr Ser Val Leu Tyr Asn Ser Ala Ser Phe Ser Thr Phe
35 40 45

Lys Cys Tyr Gly Val Ser Pro Thr Lys Leu Asn Asp Leu Cys Phe Thr
50 55 60

Asn Val Tyr Ala Asp Ser Phe Val Ile Arg Gly Asp Glu Val Arg Gln
65 70 75 80

Ile Ala Pro Gly Gln Thr Gly Lys Ile Ala Asp Tyr Asn Tyr Lys Leu
85 90 95

Pro Asp Asp Phe Thr Gly Cys Val Ile Ala Trp Asn Ser Asn Asn Leu
100 105 110

Asp Ser Lys Val Gly Gly Asn Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg
115 120 125

Lys Ser Asn Leu Lys Pro Phe Glu Arg Asp Ile Ser Thr Glu Ile Tyr
130 135 140

Gln Ala Gly Ser Thr Pro Cys Asn Gly Val Glu Gly Phe Asn Cys Tyr
145 150 155 160

Phe Pro Leu Gln Ser Tyr Gly Phe Gln Pro Thr Asn Gly Val Gly Tyr
165 170 175

Gln Pro Tyr Arg Val Val Val Leu Ser Phe Glu Leu Leu His Ala Pro
180 185 190

<210> 12
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 12

Ser Phe Ile Glu Asp Leu
1   5

<210> 13
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 13

Gly Val Tyr Tyr Pro
1   5

<210> 14
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 14

Phe Leu Pro Phe
1

<210> 15
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 15

Val Leu Pro Phe
1

<210> 16
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 16

Ser Leu Leu Ile
1

<210> 17
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 17

Leu Pro Ile Gly Ile
1   5

<210> 18
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 18

Ala Ala Tyr Tyr Val
1   5

<210> 19
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 19

Thr Phe Leu Leu
1

<210> 20
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 20

Ala Val Asp Cys
1

<210> 21
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 21

Ile Val Arg Phe Pro
1   5

<210> 22
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 22

Ile Ser Asn Cys
1

<210> 23
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 23

Leu Cys Phe Thr
1

<210> 24
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 24

Tyr Asn Tyr Lys Leu
1   5

<210> 25
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 25

Ile Ala Trp Asn
1

<210> 26
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 26

Val Val Val Leu Ser Phe
1   5

<210> 27
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 27

Cys Val Asn Phe
1

<210> 28
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 28

Gly Leu Thr Gly
1

<210> 29
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 29

Val Ala Val Leu Tyr
1   5

<210> 30
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 30

Gly Cys Leu Ile
1

<210> 31
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 31

Gly Ile Cys Ala
1

<210> 32
<211> 4
<212> PRT
<213> Artificial Sequence

<220>

<223> Conserved Amino Acid Sequence

<400> 32

Phe Thr Ile Ser
1

<210> 33
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 33

Ser Val Asp Cys
1

<210> 34
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 34

Tyr Gly Ser Phe Cys
1   5

<210> 35
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 35

Phe Asn Phe Ser
1

<210> 36
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 36

Arg Asp Leu Ile Cys Ala Gln
1   5

<210> 37
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 37

Val Leu Pro Pro Leu Leu
1   5

<210> 38
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 38

Ile Pro Phe Ala
1

<210> 39
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 39

Tyr Arg Phe Asn
1

<210> 40
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 40

Lys Leu Gln Asp Val Val Asn
1   5

<210> 41
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 41

Gly Ala Ile Ser Ser
1   5

<210> 42
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 42

Glu Val Gln Ile Asp Arg Leu Ile
1   5

<210> 43
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 43

Tyr Val Thr Gln Gln Leu
1   5

<210> 44
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 44

His Leu Met Ser Phe
1   5

<210> 45
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 45

Gly Val Val His Leu Phe
1   5

<210> 46
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 46

Trp Phe Val Thr
1

<210> 47
<211> 4
<212> PRT

<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 47

Ile Asn Ala Ser
1

<210> 48
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 48

Leu Leu Gln Phe
1

<210> 49
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 49

Leu Trp Leu Leu Trp Pro
1   5

<210> 50
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 50

Leu Met Trp Leu
1

<210> 51
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 51

Ser Phe Arg Leu Phe
1   5

<210> 52
<211> 6

<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 52

Phe Asn Pro Glu Thr Asn
1   5

<210> 53
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 53

Ile Thr Val Ala
1

<210> 54
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Conserved Amino Acid Sequence

<400> 54

Leu Arg Leu Cys
1

<210> 55
<211> 245
<212> PRT
<213> Artificial Sequence

<220>
<223> recombinant spike protein

<400> 55

Pro Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr
1   5   10   15

Arg Phe Ala Ser Val Tyr Ala Trp Asn Arg Lys Arg Ile Ser Asn Cys
            20   25   30

Val Ala Asp Tyr Ser Val Leu Tyr Asn Ser Ala Ser Phe Ser Thr Phe
        35   40   45

Lys Cys Tyr Gly Val Ser Pro Thr Lys Leu Asn Asp Leu Cys Phe Thr
    50   55   60

Asn Val Tyr Ala Asp Ser Phe Val Ile Arg Gly Asp Glu Val Arg Gln
65   70   75   80

```
Ile Ala Pro Gly Gln Thr Gly Lys Ile Ala Asp Tyr Asn Tyr Lys Leu
                85                  90                  95

Pro Asp Asp Phe Thr Gly Cys Val Ile Ala Trp Asn Ser Asn Asn Leu
            100                 105                 110

Asp Ser Lys Val Gly Gly Asn Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg
        115                 120                 125

Lys Ser Asn Leu Lys Pro Phe Glu Arg Asp Ile Ser Thr Glu Ile Tyr
    130                 135                 140

Gln Ala Gly Ser Thr Pro Cys Asn Gly Val Glu Gly Phe Asn Cys Tyr
145                 150                 155                 160

Phe Pro Leu Gln Ser Tyr Gly Phe Gln Pro Thr Asn Gly Val Gly Tyr
                165                 170                 175

Gln Pro Tyr Arg Val Val Val Leu Ser Phe Glu Leu Leu His Ala Pro
            180                 185                 190

Gly Gly Gly Gly Gly Gly Ser Phe Ile Glu Asp Leu Leu Phe Asn Lys
        195                 200                 205

Val Thr Leu Ala Asp Ala Gly Phe Gly Gly Gly Gly Gly Gly Trp Pro
    210                 215                 220

Trp Tyr Ile Trp Leu Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met
225                 230                 235                 240

Val Thr Ile Met Leu
                245
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<210> 56
<211> 738
<212> DNA
<213> Artificial Sequence

<220>
<223> recombinant spike protein

<400> 56

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aactccgcca gcttttctac attcaagtgc tacggcgtct cccctaccaa gctgaacgac      180

ctgtgcttca ccaatgtgta cgccgattct ttcgtgatca gaggcgacga ggtgcggcag      240

atcgcccctg gccagaccgg aaagatcgct gattacaact acaagctgcc tgatgacttc      300

accggctgcg tgatcgcctg gaactccaac aacctggaca gcaaggtggg gggcaactac      360

aactacctgt acagactgtt cagaaagagc aatctgaagc ctttcgagag agatatcagc      420

acagagatct accaggccgg cagcacccct tgtaatggcg ttgagggctt caattgctac      480
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tttccactgc agagctatgg ctttcagcct acaaacggcg tgggctacca accttacaga 540

gtggtggtgc tgtctttcga gctgctgcac gcccctggcg gaggaggagg cggatctttc 600

atcgaggacc tgctgttcaa caaggtgacc ctggccgacg ccggttttgg cggtggcggc 660

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gtcaccatca tgctgtga 738

<210> 57
<211> 580
<212> PRT
<213> Artificial Sequence

<220>
<223> single open reading frame for coronavirus VLP

<400> 57

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Val Leu Leu Phe Leu Ala Phe Val Val Phe Leu Leu Val Thr Leu Ala
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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 Ser Phe Tyr Val Tyr Ser Arg Val Lys Asn
50 55 60

Leu Asn Ser Ser Arg Val Pro Asp Leu Leu Val Ala Thr Asn Phe Ser
65 70 75 80

Leu Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala
85 90 95

Asp Ser Asn Gly Thr Ile Thr Val Glu Glu Leu Lys Lys Leu Leu Glu
100 105 110

Gln Trp Asn Leu Val Ile Gly Phe Leu Phe Leu Thr Trp Ile Cys Leu
115 120 125

Leu Gln Phe Ala Tyr Ala Asn Arg Asn Arg Phe Leu Tyr Ile Ile Lys
130 135 140

Leu Ile Phe Leu Trp Leu Leu Trp Pro Val Thr Leu Ala Cys Phe Val
145 150 155 160

Leu Ala Ala Val Tyr Arg Ile Asn Trp Ile Thr Gly Gly Ile Ala Ile
165 170 175

Ala Met Ala Cys Leu Val Gly Leu Met Trp Leu Ser Tyr Phe Ile Ala
180 185 190

```
Ser Phe Arg Leu Phe Ala Arg Thr Arg Ser Met Trp Ser Phe Asn Pro
        195                 200                 205

Glu Thr Asn Ile Leu Leu Asn Val Pro Leu His Gly Thr Ile Leu Thr
    210                 215                 220

Arg Pro Leu Leu Glu Ser Glu Leu Val Ile Gly Ala Val Ile Leu Arg
225                 230                 235                 240

Gly His Leu Arg Ile Ala Gly His His Leu Gly Arg Cys Asp Ile Lys
                245                 250                 255

Asp Leu Pro Lys Glu Ile Thr Val Ala Thr Ser Arg Thr Leu Ser Tyr
            260                 265                 270

Tyr Lys Leu Gly Ala Ser Gln Arg Val Ala Gly Asp Ser Gly Phe Ala
        275                 280                 285

Ala Tyr Ser Arg Tyr Arg Ile Gly Asn Tyr Lys Leu Asn Thr Asp His
    290                 295                 300

Ser Ser Ser Ser Asp Asn Ile Ala Leu Leu Val Gln Ala Thr Asn Phe
305                 310                 315                 320

Ser Leu Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Pro
                325                 330                 335

Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Arg
            340                 345                 350

Phe Ala Ser Val Tyr Ala Trp Asn Arg Lys Arg Ile Ser Asn Cys Val
        355                 360                 365

Ala Asp Tyr Ser Val Leu Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys
    370                 375                 380

Cys Tyr Gly Val Ser Pro Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn
385                 390                 395                 400

Val Tyr Ala Asp Ser Phe Val Ile Arg Gly Asp Glu Val Arg Gln Ile
                405                 410                 415

Ala Pro Gly Gln Thr Gly Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro
            420                 425                 430

Asp Asp Phe Thr Gly Cys Val Ile Ala Trp Asn Ser Asn Asn Leu Asp
        435                 440                 445

Ser Lys Val Gly Gly Asn Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys
    450                 455                 460
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Ser Asn Leu Lys Pro Phe Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln
465                 470                 475                 480

Ala Gly Ser Thr Pro Cys Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe
                485                 490                 495

Pro Leu Gln Ser Tyr Gly Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln
            500                 505                 510

Pro Tyr Arg Val Val Val Leu Ser Phe Glu Leu Leu His Ala Pro Gly
        515                 520                 525

Gly Gly Gly Gly Gly Ser Phe Ile Glu Asp Leu Leu Phe Asn Lys Val
    530                 535                 540

Thr Leu Ala Asp Ala Gly Phe Gly Gly Gly Gly Gly Gly Trp Pro Trp
545                 550                 555                 560

Tyr Ile Trp Leu Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val
                565                 570                 575

Thr Ile Met Leu
            580
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<210> 58
<211> 1743
<212> DNA
<213> Artificial Sequence

<220>
<223> single open reading frame for coronavirus VLP

<400> 58

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cgggtgaaga acctgaattc tagcagggtg cccgacctgc tggtggccac caacttcagc      240

ctgctgaaac aggctggcga tgtggaagag aaccctggac ctatggccga tagcaacggc      300

accattacag tggaggaact caaaaagctg ctggaacagt ggaatcttgt gatcggcttc      360

ctgttcctga cctggatctg cctgctgcag ttcgcctacg ccaaccgcaa cagattcctg      420

tacatcatca aactgatctt cctgtggctg ctgtggcccg tgaccctggc ttgtttcgtg      480

ctggctgctg tttatagaat caactggatc acaggcggca tcgcaatcgc catggcctgt      540

ctggtgggcc tgatgtggct gagctacttc atcgccagct ttagactgtt cgctagaaca      600

agaagcatgt ggtcctttaa ccccgagaca aacatcctcc tgaatgtgcc actgcatggc      660

accatcctga caagacccct gctggaaagc gagctggtca tcggcgccgt gatcctgcgg      720

ggccacctga gaatcgctgg ccaccacctg ggcagatgtg acatcaagga cctgcccaag      780

gaaatcactg tggccacaag cagaaccctc agctactaca agctgggagc ctctcagaga      840
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tga 1743

<210> 59
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<212> DNA
<213> Artificial Sequence

<220>
<223> expression cassette for VLP

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atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc 180

aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta 240

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catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg actcacgggg 360

atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc aaaatcaacg 420

ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg gtaggcgtgt 480

acggtgggag gtctatataa gcagagctgg tttagtgaac cgtcagatcc gctagcgcca 540

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taacttgttt attgcagctt ataatggtta caaataaagc aatagcatca caaatttcac 2460

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tta 2523

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<211> 2510
<212> DNA
<213> Artificial Sequence

<220>
<223> expression cassette for VLP

<400> 60

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ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg gtaggcgtgt 480

acggtgggag gtctatataa gcagagctgg tttagtgaac cgtcagatcc gctagcgcca 540

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<211> 3273
<212> DNA
<213> Artificial Sequence

<220>
<223> expression cassette for VLP

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atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc 180

aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta 240

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<400> 65
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<210> 66
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<400> 66
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<210> 67
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atgaccagct cgctttccag 20

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agcaccacca ctctgtaagg 20

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Glu Ala Glu Asp Leu Phe Tyr Gln Ser Ser Leu Ala Ser Trp Asn Tyr
20 25 30

Asn Thr Asn Ile Thr Glu Glu Asn Val Gln Asn Met Asn Asn Ala Gly
35 40 45

Asp Lys Trp Ser Ala Phe Leu Lys Glu Gln Ser Thr Leu Ala Gln Met
50 55 60

Tyr
65

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<220>
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<400> 71

Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Glu
1 5 10 15

<210> 72
<211> 80
<212> PRT
<213> Artificial Sequence

<220>
<223> ACE2 receptor peptide with C-terminal BAP tag

<400> 72

Ser Thr Ile Glu Glu Gln Ala Lys Thr Phe Leu Asp Lys Phe Asn His
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Glu Ala Glu Asp Leu Phe Tyr Gln Ser Ser Leu Ala Ser Trp Asn Tyr
            20   25   30

Asn Thr Asn Ile Thr Glu Glu Asn Val Gln Asn Met Asn Asn Ala Gly
        35   40   45

Asp Lys Trp Ser Ala Phe Leu Lys Glu Gln Ser Thr Leu Ala Gln Met
    50   55   60

Tyr Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Glu
65   70   75   80

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<212> DNA
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<220>
<223> ACE2 receptor peptide with C-terminal BAP tag

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gttcagaata tgaacaatgc aggcgacaaa tggtccgcct ttttgaaaga acaaagtacc   180

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<212> PRT
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<220>
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<400> 74

Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Glu Ser
1   5   10   15

Thr Ile Glu Glu Gln Ala Lys Thr Phe Leu Asp Lys Phe Asn His Glu
            20   25   30

Ala Glu Asp Leu Phe Tyr Gln Ser Ser Leu Ala Ser Trp Asn Tyr Asn
        35   40   45

Thr Asn Ile Thr Glu Glu Asn Val Gln Asn Met Asn Asn Ala Gly Asp
    50   55   60

Lys Trp Ser Ala Phe Leu Lys Glu Gln Ser Thr Leu Ala Gln Met Tyr
65   70   75   80

<210> 75

<211> 240
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<220>
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tcccttgcga gttggaatta caatacgaat atcaccgaag aaaacgttca gaatatgaac 180

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<210> 76
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<400> 76

Gln Ser Tyr Gly Phe Gln Pro Thr Asn
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<210> 77
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<212> PRT
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Leu Gln Ser Tyr Gly Phe Gln Pro Thr Asn
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<210> 78
<211> 13
<212> PRT
<213> Artificial Sequence

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<400> 78

Gln Ser Tyr Gly Phe Gln Pro Thr Asn Gly Val Gly Tyr
1   5   10

<210> 79
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<220>
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<400> 79

Gln Pro Thr Asn Gly Val Gly Tyr
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Phe Gln Pro Thr Asn Gly Val Gly Tyr
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<210> 82
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<400> 82

Phe Gln Pro Thr Asn
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Thr Asn Gly Val Gly Tyr
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<210> 85
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Phe Asn Cys Tyr Phe Pro Leu Gln
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<210> 86
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Gly Phe Asn Cys Tyr Phe Pro Leu Gln
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<210> 87
<211> 4
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<400> 87

Glu Gly Phe Asn
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<210> 88
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<400> 88

Val Glu Gly Phe Asn Cys Tyr
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<210> 89
<211> 10
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<400> 89

Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln
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<400> 90

Tyr Asn Tyr Leu Tyr
1   5

<210> 91
<211> 7
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<400> 91

Asn Tyr Asn Tyr Leu Tyr Arg
1   5

<210> 92
<211> 18
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Ser Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala
1   5   10   15

Gly Phe

<210> 93
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<400> 93

Ser Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala
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Gly Phe Met Lys Gln Tyr Gly Cys Gly Lys Lys Lys Lys
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<210> 94
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<400> 94

Ser Phe Ile Glu Asp Leu Leu Phe
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<210> 95
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<212> PRT
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<400> 95

Ser Phe Ile Glu Asp Leu Leu Phe Gly Cys Gly Lys Lys Lys Lys
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<210> 96
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<210> 97
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<210> 99
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<212> PRT
<213> Artificial Sequence

<220>
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<400> 99

Thr Arg Tyr Tyr Tyr Leu Asn Tyr Asn Tyr Thr Thr Gly Tyr
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<211> 188
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<220>
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<400> 100

Arg Val Gln Pro Thr Glu Ser Ile Val Arg Phe Pro Asn Ile Thr Asn
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Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val
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Tyr Ala Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser
        35   40   45

Val Leu Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val
    50   55   60

Ser Pro Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp
65   70   75   80

Ser Phe Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln
                85   90   95

Thr Gly Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr
            100   105   110

Gly Cys Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly
        115   120   125

Gly Asn Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys
    130   135   140

Pro Phe Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr
145   150   155   160

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Pro Cys Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser
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Tyr Gly Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln
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<213>  Artificial Sequence

<220>
<223>  immunogenic sequence

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aattccgcat cattttccac ttttaagtgt tatggagtgt ctcctactaa attaaatgat      180

ctctgcttta ctaatgtcta tgcagattca tttgtaatta gaggtgatga agtcagacaa      240

atcgctccag ggcaaactgg aaagattgct gattataatt ataaattacc agatgatttt      300

acaggctgcg ttatagcttg gaattctaac aatcttgatt ctaaggttgg tggtaattat      360

aattacctgt atagattgtt taggaagtct aatctcaaac cttttgagag agatatttca      420

actgaaatct atcaggccgg tagcacacct tgtaatggtg ttgaaggttt taattgttac      480

tttcctttac aatcatatgg tttccaaccc actaatggtg ttggttacca accatacaga      540

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<210>  102
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<212>  PRT
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<220>
<223>  transmembrane domain

<400>  102

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<210>  103
<211>  40
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  transmembrane domain

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<213>  Artificial Sequence
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<220>
<223> bacterial sequence-free vector

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actaattgag atgcatgctt tgcatacttc tgcctgctgg ggagcctggg gactttccac 180

acctggttgc tgactaattg agatgcatgc tttgcatact tctgcctgct ggggagcctg 240

gggactttcc acacccctga ttctgtggat aaccgtatta ccgccatgca ttagttatta 300

atagtaatca attacggggt cattagttca tagcccatat atggagttcc gcgttacata 360

acttacggta aatggcccgc ctggctgacc gcccaacgac ccccgcccat tgacgtcaat 420

aatgacgtat gttcccatag taacgccaat agggactttc cattgacgtc aatgggtgga 480

gtatttacgg taaactgccc acttggcagt acatcaagtg tatcatatgc caagtacgcc 540

ccctattgac gtcaatgacg gtaaatggcc cgcctggcat tatgcccagt acatgacctt 600

atgggacttt cctacttggc agtacatcta cgtattagtc atcgctatta ccatggtgat 660

gcggttttgg cagtacatca atgggcgtgg atagcggttt gactcacggg gatttccaag 720

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ctttcgtgtc tgaggaaacc ggcaccctga tcgtgaacag cgtgctgctg tttctggcct 960

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agacaatagc aggcatgctg gggatgcggt gggctctatg gaagcttacg cgtggccgct 2880

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