

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, ϕ K02 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.