

## IMMUNOGENIC COMPOSITION

### BACKGROUND OF THE INVENTION

#### 1. Field of the Invention

5 [0001] The present disclosure relates to an immunogenic composition and in particular to an immunogenic composition against Coronavirus.

#### 2. Description of the Related Art

[0002] Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by  
10 Severe Acute Respiratory Syndrome Coronavirus 2 (hereinafter referred to as SARS-CoV-2). The emergence of SARS-CoV-2 has raised serious concerns due to the virus has rapidly distributed worldwide and resulted in the World Health Organization (WHO) declaring a global health emergency on 30 January 2020. Therefore, there is an urgent need to develop an effective vaccine for the preparedness against the  
15 COVID-19.

[0003] SARS-CoV-2 are positive-sense single-stranded RNA viruses belonging to the betacoronavirus (beta-CoVs) of the subfamily Orthocoronavirinae, in the family Coronaviridae of the order Nidovirales ((2018). Arch. Virol. 163, 2601–2631).

Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) also belong to the beta-CoVs (N Engl J Med. 2003 May 15; 348(20):1967-76.; N Engl J Med. 2012 Nov 8; 367(19):1814-20.). Especially, both SARS-CoV and SARS-CoV-2 viruses entry into  
5 the host cells mediated by interaction of the receptor-binding domain (RBD) in S protein located on virus outer-membrane and angiotensin-converting enzyme 2 (ACE2) on cell.

#### BRIEF SUMMARY OF THE INVENTION

10 [0004] An object of the present invention is to provide an immunogenic composition, the immunogenic composition comprises a recombinant protein including a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and any polypeptide encoded by a polynucleotide which is at least 80% homologous with SEQ ID NO: 1-4, wherein the  
15 recombinant protein contains an IgG1 Fc protein fragment having a length of at least 6 amino acids; or a nucleic acid molecule encoding the recombinant protein.

[0005] The immunogenic composition described above, wherein the IgG1 Fc protein fragment has a length of between 12-18 amino acids.

[0006] The immunogenic composition described above, wherein the recombinant protein fuses with a secreted signal peptide.

[0007] The immunogenic composition described above further comprises immunogenic compounds, adjuvants, pharmaceutically acceptable carriers, stabilisers or preservatives.

[0008] To achieve the above and other objects, the present invention provides a SARS-CoV-2 vaccine. The SARS-CoV-2 vaccine comprises a recombinant protein including a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and any polypeptide encoded by a polynucleotide which is at least 80% homologous with SEQ ID NO: 1-4, wherein the recombinant protein contains an IgG1 Fc protein fragment having a length of at least 6 amino acids; or a nucleic acid molecule encoding the recombinant protein.

[0009] The vaccine described above, wherein the IgG1 Fc protein fragment has a length of between 12-18 amino acids.

[0010] The vaccine described above, wherein the recombinant protein fuses with nucleocapsid phosphoprotein protein of SARS-CoV-2, envelope protein of SARS-CoV-2 or membrane protein of SARS-CoV-2.

[0011] The vaccine described above, wherein the recombinant protein encoded by

the nucleic acid molecule fuses with nucleocapsid phosphoprotein protein of SARS-CoV-2, envelope protein of SARS-CoV-2 or membrane protein of SARS-CoV-2.

[0012] The vaccine described above further comprises immunogenic compounds, adjuvants, pharmaceutically acceptable carriers, stabilisers or preservatives.

5 [0013] To achieve the above and other objects, the present invention provides a vector. The vector comprises a nucleic acid molecule encoding a recombinant protein including a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and any polypeptide encoded by the polynucleotide which is at least 80% homologous with SEQ ID NO: 1-4, wherein the  
10 recombinant protein contains an IgG1 Fc protein fragment having a length of at least 6 amino acids.

[0014] The vector described above, wherein the recombinant protein encoded by the nucleic acid molecule fuses with nucleocapsid phosphoprotein protein of SARS-CoV-2, envelope protein of SARS-CoV-2 or membrane protein of SARS-CoV-2.

15 [0015] The vector described above, wherein the vector is selected from the group consisting of baculovirus, vaccinia virus, avipoxvirus, adenovirus, alphavirus, rhabdovirus, and herpesvirus.

[0016] The vector described above, wherein the vector is baculovirus.

[0017] To achieve the above and other objects, the present invention provides a immunogenic composition comprising a nucleic acid molecule including a sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

5 [0018] The above immunogenic composition, vaccine and vector can induce neutralizing antibodies against SARS-CoV-2 virus and be used against the coronavirus disease 2019 (COVID-19).

#### BRIEF DESCRIPTION OF THE DRAWINGS

10 [0019] FIG. 1 shows the amino acid sequence of RBD-Fc (SEQ ID NO: 1).

[0020] FIG. 2 shows the amino acid sequence of S2△TM-Fc (SEQ ID NO: 2).

[0021] FIG. 3 shows the amino acid sequence of S1-Fc (SEQ ID NO: 3).

[0022] FIG. 4 shows the amino acid sequence of S△TM-Fc (SEQ ID NO: 4).

[0023] FIG. 5 shows the amino acid sequence of secreted signal peptide (SEQ ID  
15 NO: 5).

[0024] FIG. 6 shows the DNA gel electrophoresis image of RBD-Fc, S2△TM-Fc, S1-Fc and S△TM-Fc.

[0025] FIG. 7 shows the trend of cell expansion infected with viruses.

[0026] FIG. 8 shows the expression levels of vaccine candidates in western blot image at 3rd day, 4th day and 5th day after infection. (A) RBD-Fc (B) S2△TM-Fc (C) S1-Fc (D) S△TM-Fc.

[0027] FIG. 9 shows the SDS-PAGE (left) and western blot (right) image of purified vaccine candidates. Arrows indicates the expected position of expressed protein. (A) RBD-Fc (B) S2△TM-Fc (C) S1-Fc (D) S△TM-Fc (S) sample liquid (FT) flow through liquid (W) wash liquid (E) elute.

[0028] FIG. 10 shows the neutralizing antibody titers in sera which are blood sampled from the immunized mice.

[0029] FIG. 11 shows the result of plaque reduction neutralization test (PRNT) of the antisera from immunized mice against SARS-CoV-2 virus.

[0030] FIG. 12 shows the amino acid sequence of nucleocapsid phosphoprotein protein (SEQ ID NO: 10), envelope protein (SEQ ID NO: 11) and membrane protein (SEQ ID NO: 12) of SARS-CoV-2.

[0031] FIG. 13 shows the DNA sequence of RBD-Fc (SEQ ID NO: 6).

[0032] FIG. 14 shows the DNA sequence of S2△TM-Fc (SEQ ID NO: 7).

[0033] FIG. 15 shows the DNA sequence of S1-Fc (SEQ ID NO: 8).

[0034] FIG. 16 shows the DNA sequence of S△TM-Fc (SEQ ID NO: 9).

## DETAILED DESCRIPTION OF THE INVENTION

[0035] To facilitate understanding of the object, characteristics and effects of this present disclosure, embodiments together with the attached drawings for the detailed description of the present disclosure are provided.

[0036] Expression of vaccine candidates

[0037] First, DNA sequences of four vaccine candidates which encode recombinant protein RBD-Fc, S2 $\Delta$ TM-Fc, S1-Fc and S $\Delta$ TM-Fc are designed. FIG. 13 shows the DNA sequence of RBD-Fc. FIG. 14 shows the DNA sequence of S2 $\Delta$ TM-Fc. FIG. 15 shows the DNA sequence of S1-Fc. FIG. 16 shows the DNA sequence of S $\Delta$ TM-Fc.

[0038] The vaccine candidates are designed according to the amino acid sequences of SARS-CoV-2 spike protein (Accession number: NC\_045512). Herein, RBD-Fc represents the receptor binding domain of spike protein of SARS-CoV-2 fused with human IgG1 constant heavy chain 2 and constant heavy chain 3 (Fc protein), the amino acid sequence of RBD-Fc is shown in figure 1; S2 $\Delta$ TM-Fc represents the S2 subunit of spike protein of SARS-CoV-2 deleting the transmembrane protein and fused with Fc protein, the amino acid sequence of S2 $\Delta$ TM-Fc is shown in figure 2;

S1-Fc represents the S1 subunit of spike protein of SARS-CoV-2 fused with Fc protein, the amino acid sequence of S1-Fc is shown in figure 3; S $\Delta$ TM-Fc represents the spike protein of SARS-CoV-2 deleting the transmembrane protein and fused with Fc protein, the amino acid sequence of S $\Delta$ TM-Fc is shown in figure 4. For the purpose of vaccine production, all the constructs of the vaccine candidates are fused with a secreted signal peptide, the secreted signal peptide is located at the N-terminal end of the recombinant protein and the Fc sequence is located at the C-terminal end of the recombinant protein. The amino acid sequence of secreted signal peptide is shown in figure 5. However, in other embodiments, the secreted signal peptide is not necessary.

[0039] Next, the above DNA sequences of four vaccine candidates are cloned into pFastBac 1 vector separately. In other embodiments, the vector may be selected from vaccinia virus, avipoxvirus, adenovirus, alphavirus, rhabdovirus, herpesvirus or other known suitable vector system.

[0040] Four pFastBac 1 vectors containing each of the above DNA sequences of vaccine candidates separately are transformed into the E. coli strain DH10Bac for production of the recombinant Bacmid DNA which contains the above DNA sequences of four vaccine candidates. The recombinant Bacmid DNA is identified by

PCR analysis. As shown in figure 6, “M” indicates the molecular weight marker, the numerals “1”, “2” and “3” indicate the serial number of E. coli colonies therefrom the above recombinant Bacmid DNA is obtained, the theoretic recombinant Bacmid DNA sizes of RBD-Fc, S2 $\Delta$ TM-Fc, S1-Fc and S $\Delta$ TM-Fc are 3,809 bps, 4,700 bps, 5,159 bps and 6,743 bps respectively. The determined recombinant Bacmid DNA sizes of RBD-Fc, S2 $\Delta$ TM-Fc, S1-Fc and S $\Delta$ TM-Fc are 3,900 bps, 4,800 bps, 5,200 bps and 6,800 bps respectively, corresponding to the above theoretic recombinant Bacmid DNA sizes. The above transformation and PCR analysis of the recombinant Bacmid DNA are performed according to Bac-to-Bac™ Baculovirus Expression System USER GUIDE.

[0041] Then, the recombinant Bacmid DNA which contains each of the above DNA sequences of four vaccine candidates are separately transfected into S. frugiperda cells (sf9 cell) for baculovirus production. The baculovirus is identified by DNA sequencing. The above transfection of the recombinant Bacmid DNA is performed according to Bac-to-Bac™ Baculovirus Expression System USER GUIDE.

[0042] Finally, Sf9 cells are infected with baculovirus containing the above DNA sequences of four vaccine candidates with multiplicity of infection (MOI) of 0.1, 0.001 and 0.0001 separately for five days, the trend of cell expansion infected with

viruses are shown in figure 7. At the time point of 3rd day, 4th day and 5th day post infection, the infected Sf9 cells are collected for checking the expression levels of vaccine candidates, proteins synthesized in the infected Sf9 cells are detected by western blot, the result is shown in figure 8, the location of protein standards and their size in kilodaltons (kda) indicated that the 55 kda (A: RBD-Fc), 110 kda(B: S2  $\Delta$ TM-Fc), 130 kda(C: S1-Fc) and 170 kda (D: S  $\Delta$ TM-Fc) recombinant protein portion are the major proteins being synthesized in the cells. The above western blotting is performed according to the reference “A Guide to Polyacrylamide Gel Electrophoresis and detection (Bio-Rad)”.

10 [0043] Production of vaccine candidates

[0044] Sf9 cells are prepared, sf9 cells are grown at 27°C to a density of  $3.5 \sim 5 \times 10^6$  cells/mL in insect-XPRESS Protein-free media (Lonza Ltd). sf9 cells are infected by the baculovirus expression vectors containing the above DNA sequences of four vaccine candidates at a multiplicity of infection (MOI) of 0.1~0.001 for production of four vaccine candidates. During infection of sf9 cells, the vaccine candidates are produced under the transcriptional control of the baculovirus polyhedrin promoter. Sf9 cells are harvested 72~96 hours post-infection by centrifugation for 15 minutes at 6,000 x g. The supernatant of Sf9 cells is stored at 4° C.

[0045] Purification of vaccine candidate RBD-Fc

[0046] In this embodiment, the purification of RBD-Fc which is non-denatured and suitable as a component of a coronavirus vaccine for human use being performed by protein A affinity chromatography. The following procedure is used to purify the

5 RBD-Fc from sf9 cells infected with the recombinant baculovirus.

[0047] 2 L culture broth of the infected sf9 cells containing RBD-Fc is prepared

according to the above procedure of “expression of vaccine candidates”, the initial

cell concentration of the sf9 cells to be infected is  $1.0 \times 10^6$  cells/mL, the sf9 cells

are then infected for three days until the infected cell concentration of the sf9 cells

10 achieve  $4.0 \times 10^6$  cells/mL (viability 40-60%, MOI=0.1). The culture broth of the

infected sf9 cells is centrifuged with  $9000 \times g$  for 20 minutes to obtain the

supernatant, and the supernatant is filtered with a filter (0.22  $\mu\text{m}$  pore size) to obtain

a sample liquid.

[0048] A pharmacia XK 16/40 column filled with 20 mL Protein A resin (Eshmuno

15 A, Merck Millipore) is prepared and mounted on AKTA Pure chromatography system.

The above sample liquid is applied to the columns at a flow rate of 20 mL/minute

(PreC: 0.30 Mpa), the flow through liquid is collected. After loading the above

sample liquid into the column, the column is further washed with PBS (pH 7.3), until

the UV absorption at 280 nm of the wash liquid returns to baseline. The wash liquid through the column is collected. After washing the column, Citric acid solution (0.3 M) as the elute is loaded into the column to obtain the elute containing RBD-Fc which binds to Protein A.

5 [0049] Since the contaminated protein also flows through the column into the elute, the previous elute containing RBD-Fc is eluted again from the Protein A resin column with approximately 5 fold column volume of 100 mM citrate acid (pH 3.0), and the eluent is neutralized with 1M Tris-HCl (pH 9.0). The sample liquid, the flow through liquid, the wash liquid and the elute are analyzed by SDS-PAGE as follows. The  
10 proteins in the sample liquid, the flow through liquid, the wash liquid and the elute are disrupted in a boiling water bath for 10 minutes in the presence of 2% sodium dodecyl sulfate and 5% beta-mercaptoethanol, and then analyzed by SDS-PAGE (10% polyacrylamide gel in the presence of 0.1% SDS), finally stained with Coomassie blue. The SDS-PAGE analysis is performed according to the reference "A  
15 Guide to Polyacrylamide Gel Electrophoresis and detection (Bio-Rad)".

[0050] The purification of S2 $\Delta$ TM-Fc, S1-Fc and S $\Delta$ TM-Fc is performed by the same procedure as the purification of RBD-Fc.

[0051] The result of purification of four vaccine candidates is shown in figure 9.

The purified vaccine candidates are obtained by the above procedure.

[0052] In other embodiment, other known technologies of protein purification are utilized to purify the above vaccine candidates.

[0053] Plaque reduction neutralization test (PRNT) of the antisera from immunized

5 mice against SARS-CoV-2 virus

[0054] First, twelve groups of C57BL/6 mice (7 weeks old, female, weight is 18~22

gram) are prepared, each group contains ten mice, five groups of mice are immunized

three times by intramuscular injection of 20  $\mu$ g of vaccine candidates in 50  $\mu$ l of PBS;

five groups of mice are immunized three times by intramuscular injection of 20  $\mu$ g

10 of vaccine candidates in 50  $\mu$ l of PBS containing aluminum hydroxide as an adjuvant;

one group of mice are injected 50  $\mu$ l of PBS containing aluminum hydroxide three

times by intramuscular injection; one group of mice are merely injected 50  $\mu$ l of PBS

three times by intramuscular injection. The immunized condition is listed in the table

1.

15 [0055] Table 1

| Group numbers | Antigens          | $\mu$ g/dose | Adjuvant |
|---------------|-------------------|--------------|----------|
| 1             | RBD-Fc            | 20           | -        |
| 2             | S2 $\Delta$ TM-Fc |              | -        |
| 3             | S1-Fc             |              | -        |

|    |                           |  |                      |
|----|---------------------------|--|----------------------|
| 4  | RBD-Fc+ S2 $\Delta$ TM-Fc |  | -                    |
| 5  | S2 $\Delta$ TM-Fc+S1-Fc   |  | -                    |
| 6  | -                         |  | -                    |
| 7  | RBD-Fc                    |  | Al(OH) <sup>3*</sup> |
| 8  | S2 $\Delta$ TM-Fc         |  | Al(OH) <sup>3*</sup> |
| 9  | S1-Fc                     |  | Al(OH) <sup>3*</sup> |
| 10 | RBD-Fc+S2 $\Delta$ TM-Fc  |  | Al(OH) <sup>3*</sup> |
| 11 | S2 $\Delta$ TM-Fc+S1-Fc   |  | Al(OH) <sup>3*</sup> |
| 12 | -                         |  | Al(OH) <sup>3*</sup> |

\* The quantity of adjuvant is 500  $\mu$ g/dose

[0056] Next, at the time point of immunization, mice of groups 1, 6 and 7 are blood sampled to detect neutralizing antibody titers. The result is shown in figure 10, mice of group 7 which are administered by RBD-Fc and an adjuvant has the higher titer.

5 [0057] Finally, twelve groups of vero E6 cells monolayer are pre-seeded in twelve wells of a 24-well plate as the experimental groups. 0.1 mL of twelve test sera (5120-fold dilution) obtained from the above mice that are immunized three times are separately mixed with 100 PFU/0.1 mL of SARS-CoV-2 virus (provided by Professor Sui-Yuan Chang, Department of Clinical Laboratory Sciences and Medical

10 Biotechnology, College of Medicine, National Taiwan University) and co-incubated at 37°C for 60 minutes. Then, twelve mixtures of SARS-CoV-2 and test sera are separately added into twelve experimental groups. Meanwhile, two positive control

wells (vero E6 cells are co-incubated with 100 PFU of SARS-CoV-2 virus without the above sera at 37°C for 60 minutes) are established for each assay as the positive control group to ensure infectivity of the cell monolayer. Subsequently, the medium of twelve experimental groups and two positive control groups are removed, twelve experimental groups and two positive control groups are then added with overlay medium. The 24-well plate with the experimental groups and positive control groups is incubated at 37°C in 5% CO<sub>2</sub> for six days and plaques were counted on the 6th day. The result of plaque reduction neutralization test is shown in figure 11; it demonstrates that when the experimental group contains the RBD domain fused with Fc protein (groups 1, 3-5, 7 and 9-10) it can effectively induce neutralizing antibodies against SARS-CoV-2 virus, the efficacy of the experimental groups containing the RBD domain fused with Fc protein and adjuvant are better, and the combination of S2  $\Delta$  TM-Fc fused with Fc protein and adjuvant can also induce neutralizing antibodies against SARS-CoV-2 virus.

[0058] Furthermore, a SARS-CoV-2 recombinant protein fragment used for SARS-CoV-2 is able to be designed at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to the RBD, S1 protein, S2 protein or spike protein; a Fc protein fragment used for

SARS-CoV-2 is able to be designed to has a length of at least 6 amino acids, and preferably has a length of between 12 and 18 amino acids. The length of Fc protein fragment may be 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 amino acids.

[0059] FIG. 12 shows the amino acid sequence of nucleocapsid phosphoprotein protein, envelope protein and membrane protein of SARS-CoV-2. For the use of COVID-19 vaccine, the nucleocapsid phosphoprotein protein, envelope protein or membrane protein of SARS-CoV-2 are also able to be designed as a part of COVID-19 vaccine, that is, nucleocapsid phosphoprotein protein, envelope protein and membrane protein of SARS-CoV-2 can be fused with the above vaccine candidates.

[0060] As used herein, “percent homology” of two amino acid sequences is determined using the algorithm described in Karlin and Altschul, Proc, Natl. Acad. Sci. USA 87:2264-2268, 1990, modified as described in Karlin and Altschul, Proc, Natl. Acad. Sci. USA 90:5873-5877, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al., J. Mol. Biol. 215:403-410, 1990. BLAST protein searches are performed with the XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to a reference polypeptide. To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul et al., Nucleic Acids Res. 25:3389-3402, 1997.

When utilizing the BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) are used. See [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov).

[0061] As mentioned above, SARS-CoV-2 recombinant proteins fused with Fc protein can induce neutralizing antibodies against SARS-CoV-2 virus and it can be used as a vaccine against the coronavirus disease 2019 (COVID-19).

[0062] While the present disclosure has been described by means of specific embodiments, numerous modifications and variations could be made thereto by those skilled in the art without departing from the scope and spirit of the present disclosure set forth in the claims.

## CLAIMS

### WHAT IS CLAIMED IS:

1. A immunogenic composition comprising:

a recombinant protein including a sequence selected from the group consisting

5 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and any

polypeptide encoded by a polynucleotide which is at least 80% homologous with

SEQ ID NO: 1-4, wherein the recombinant protein contains an IgG1 Fc protein

fragment having a length of at least 6 amino acids; or

a nucleic acid molecule encoding the recombinant protein.

10 2. The immunogenic composition of claim 1, wherein the IgG1 Fc protein fragment

has a length of between 12-18 amino acids.

3. The immunogenic composition of claim 1, wherein the recombinant protein fuses

with a secreted signal peptide.

4. The immunogenic composition of claim 1, further comprising immunogenic

15 compounds, adjuvants, pharmaceutically acceptable carriers, stabilisers or

preservatives.

5. A SARS-CoV-2 vaccine comprising:

a recombinant protein including a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and any polypeptide encoded by a polynucleotide which is at least 80% homologous with SEQ ID NO: 1-4, wherein the recombinant protein contains an IgG1 Fc protein

5 fragment having a length of at least 6 amino acids; or

a nucleic acid molecule encoding the recombinant protein.

6. The vaccine of claim 5, wherein the IgG1 Fc protein fragment has a length of between 12-18 amino acids.

7. The vaccine of claim 5, wherein the recombinant protein fuses with nucleocapsid  
10 phosphoprotein protein of SARS-CoV-2, envelope protein of SARS-CoV-2 or membrane protein of SARS-CoV-2.

8. The vaccine of claim 5, wherein the recombinant protein encoded by the nucleic acid molecule fuses with nucleocapsid phosphoprotein protein of SARS-CoV-2, envelope protein of SARS-CoV-2 or membrane protein of SARS-CoV-2.

15 9. The vaccine of claim 5, further comprising immunogenic compounds, adjuvants, pharmaceutically acceptable carriers, stabilisers or preservatives.

10.A vector comprising a nucleic acid molecule encoding a recombinant protein including a sequence selected from the group consisting of SEQ ID NO: 1, SEQ

ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and any polypeptide encoded by the polynucleotide which is at least 80% homologous with SEQ ID NO: 1-4, wherein the recombinant protein contains an IgG1 Fc protein fragment having a length of at least 6 amino acids.

5 11. The vector of claim 10, wherein the recombinant protein encoded by the nucleic acid molecule fuses with nucleocapsid phosphoprotein protein of SARS-CoV-2, envelope protein of SARS-CoV-2 or membrane protein of SARS-CoV-2.

12. The vector of claim 10, wherein the vector is selected from the group consisting of baculovirus, vaccinia virus, avipoxvirus, adenovirus, alphavirus, rhabdovirus,  
10 and herpesvirus.

13. The vector of claim 12, wherein the vector is baculovirus.

14. A immunogenic composition comprising:

a nucleic acid molecule including a sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

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