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Yang et al.

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(54) **mRNA VACCINE ENCODING FUSION ANTIGEN AGAINST MPOX AND SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2**

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(51) **Int. Cl.**

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A61K 39/215	(2006.01)
A61K 39/275	(2006.01)
A61K 47/69	(2017.01)
C07K 14/005	(2006.01)

(52) **U.S. Cl.**
CPC ***A61K 39/275*** (2013.01); ***A61K 9/5015*** (2013.01); ***A61K 39/215*** (2013.01); ***C07K 14/005*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

An mRNA molecule is disclosed. The mRNA molecule contains a polynucleotide encoding an M1R antigen of Mpox and a polynucleotide encoding an RBD antigen of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and further contains a polynucleotide encoding an A35R antigen of Mpox. An application of the mRNA molecule in the preparation of an mRNA vaccine against Mpox or SARS-CoV-2 is further disclosed. Compared to an mRNA vaccine encoding separately corresponding antigens, the provided mRNA vaccine encoding a fusion antigen can induce considerable or even higher-level neutralizing antibody responses against Mpox and SARS-CoV-2, and provides 100% immune protection against the lethal challenge of ectromelia virus. The vaccine is obtained by synthesizing a single mRNA molecule and encapsulating the single mRNA within lipid nanoparticles. Therefore, the single-component fusion mRNA vaccine has a wider application prospect than multivalent mRNA vaccine compositions.

18 Claims, 10 Drawing Sheets

Specification includes a Sequence Listing.

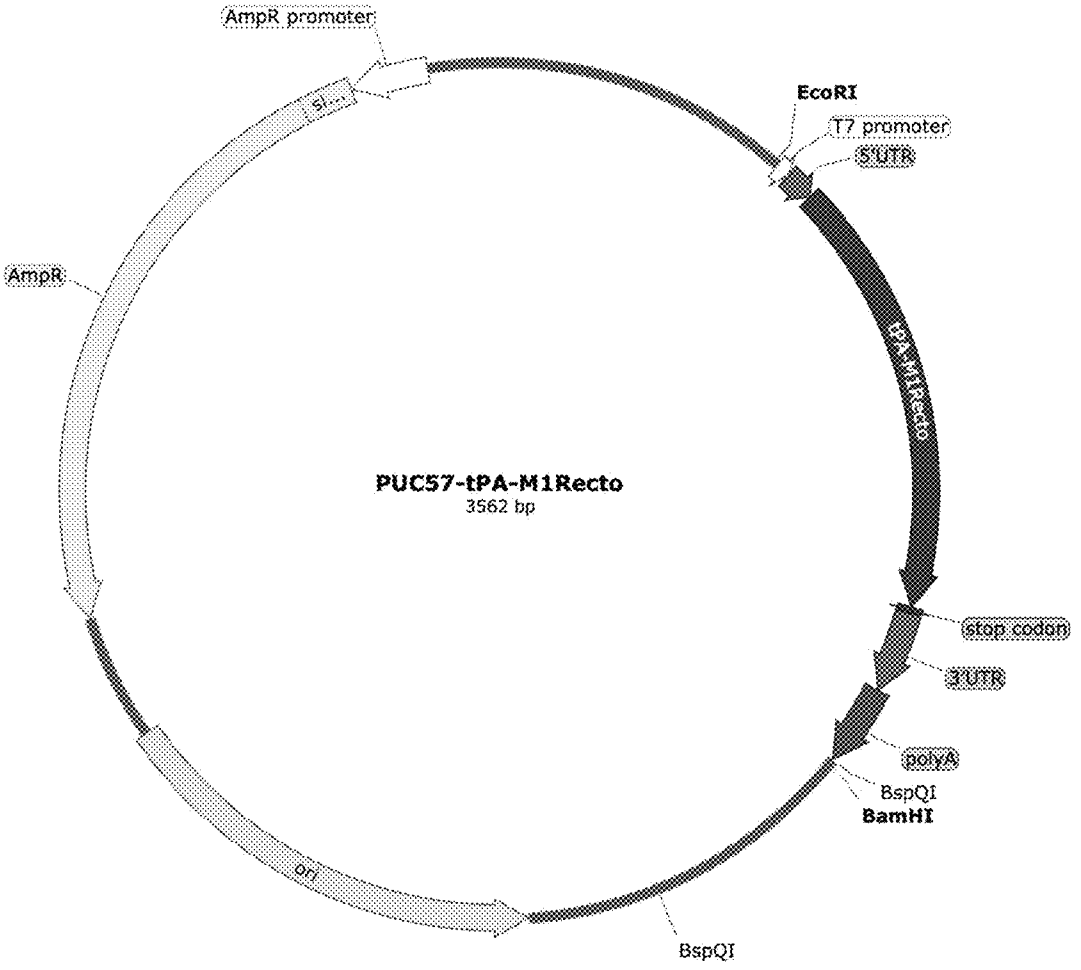
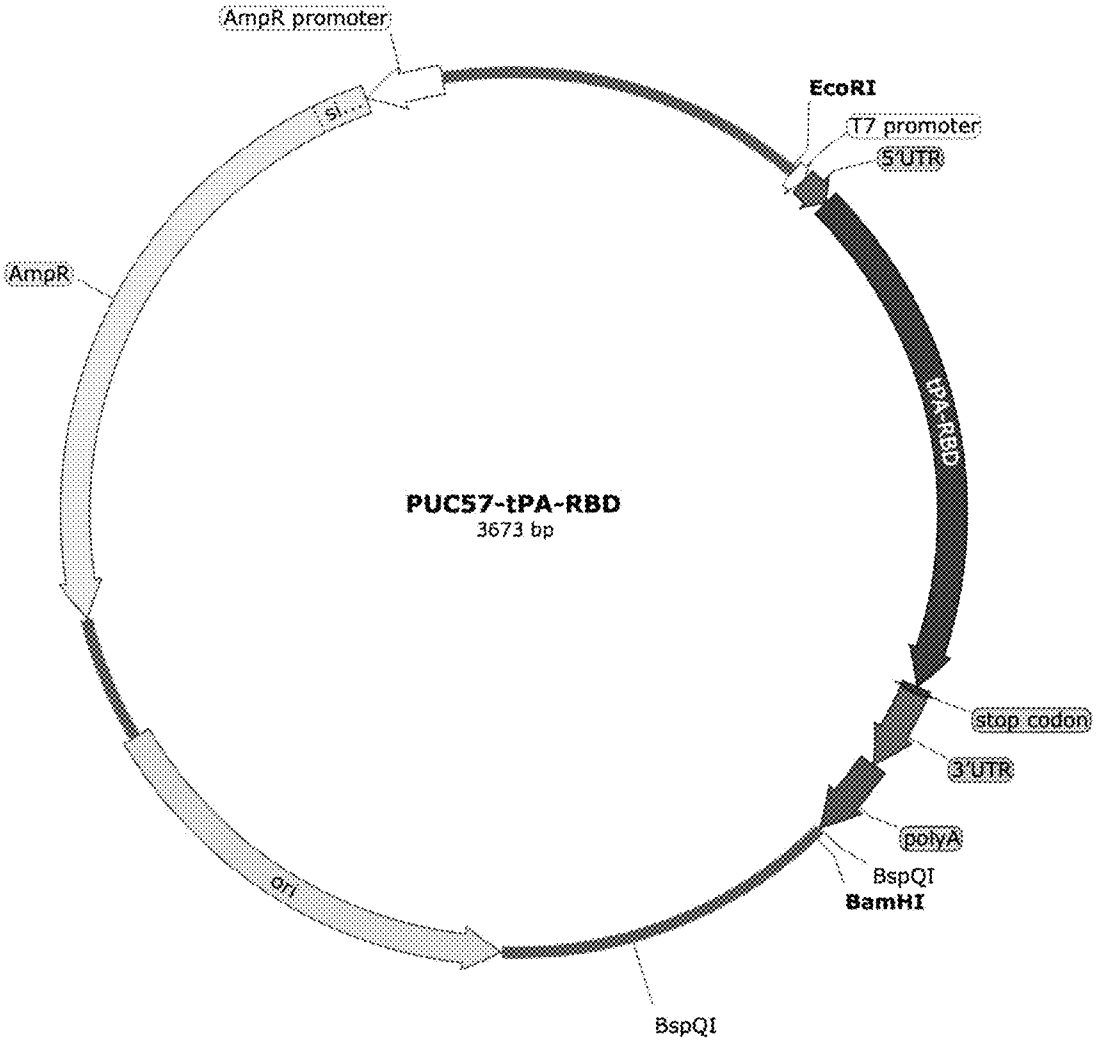

AmpR promoter
EcoRI
T7 promoter
5'UTR
tPA-M1Recto
stop codon
3'UTR
polyA
BspQI
BamHI
BspQI
ori
AmpR
PUC57-tPA-M1Recto
3562 bp

FIG. 1


AmpR promoter
SI...
EcoRI
T7 promoter
5'UTR
tPA-RBD
stop codon
3'UTR
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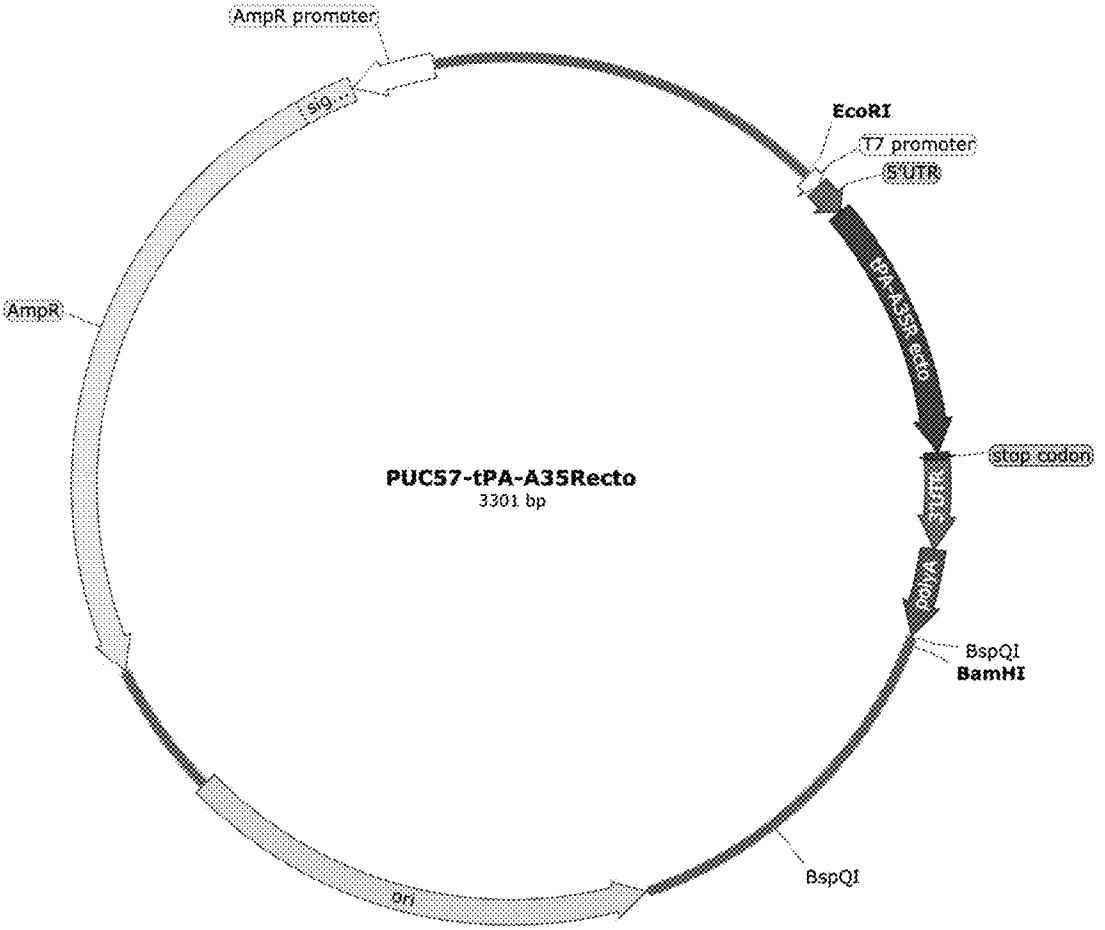
BspQI
BamHI
BspQI
ori
AmpR
PUC57-tPA-RBD
3673 bp

FIG. 2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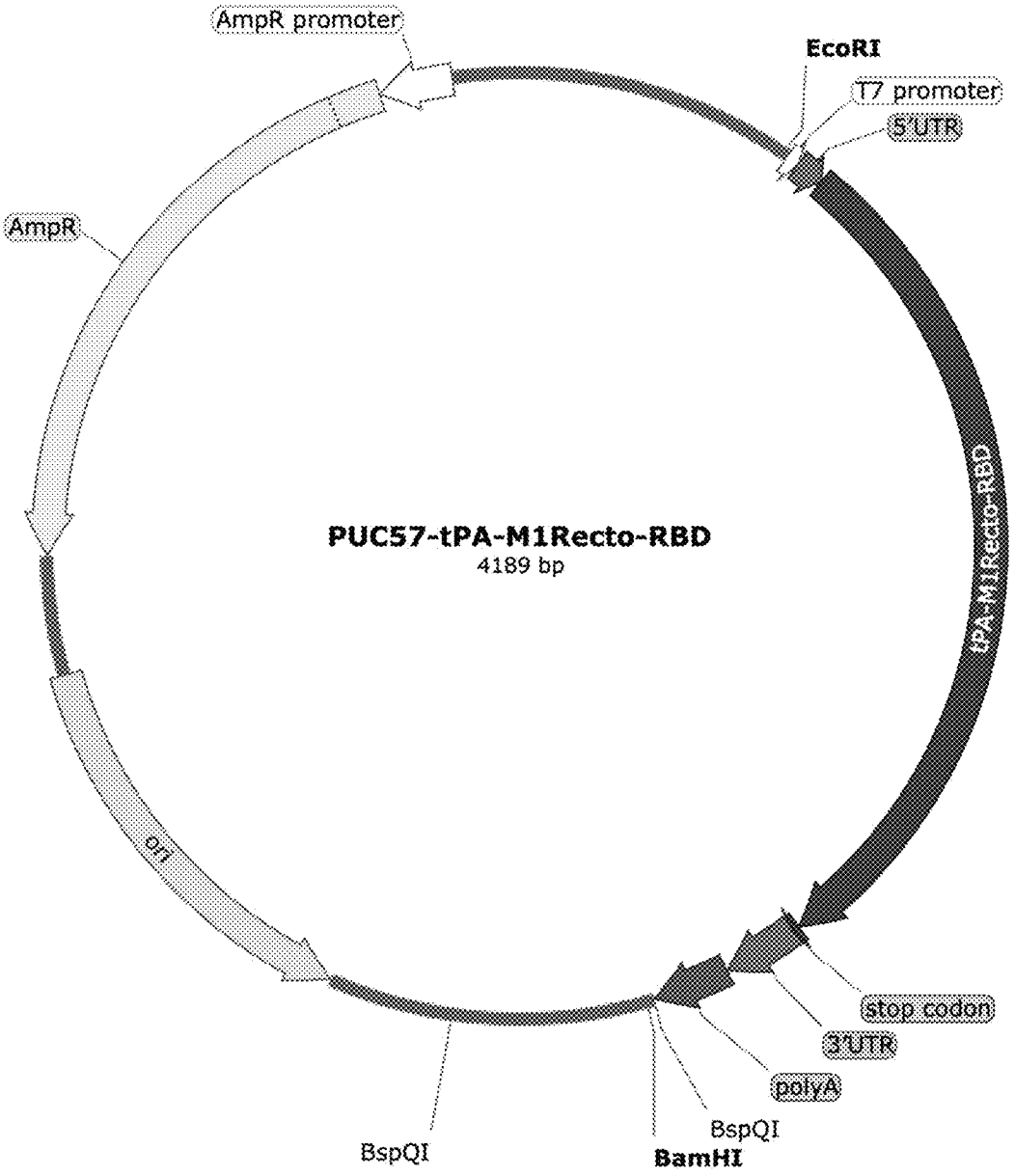
EcoRI
T7 promoter
5'UTR
stop codon
BspQI
BamHI
BspQI
AmpR
PUC57-tPA-A35Recto
3301 bp

FIG. 3


AmpR promoter
EcoRI
T7 promoter
5'UTR
AmpR
PUC57-tPA-M1Recto-RBD
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tPA-M1Recto-RBD
ori
stop codon
3'UTR
polyA
BspQI
BamHI
BspQI

FIG. 4

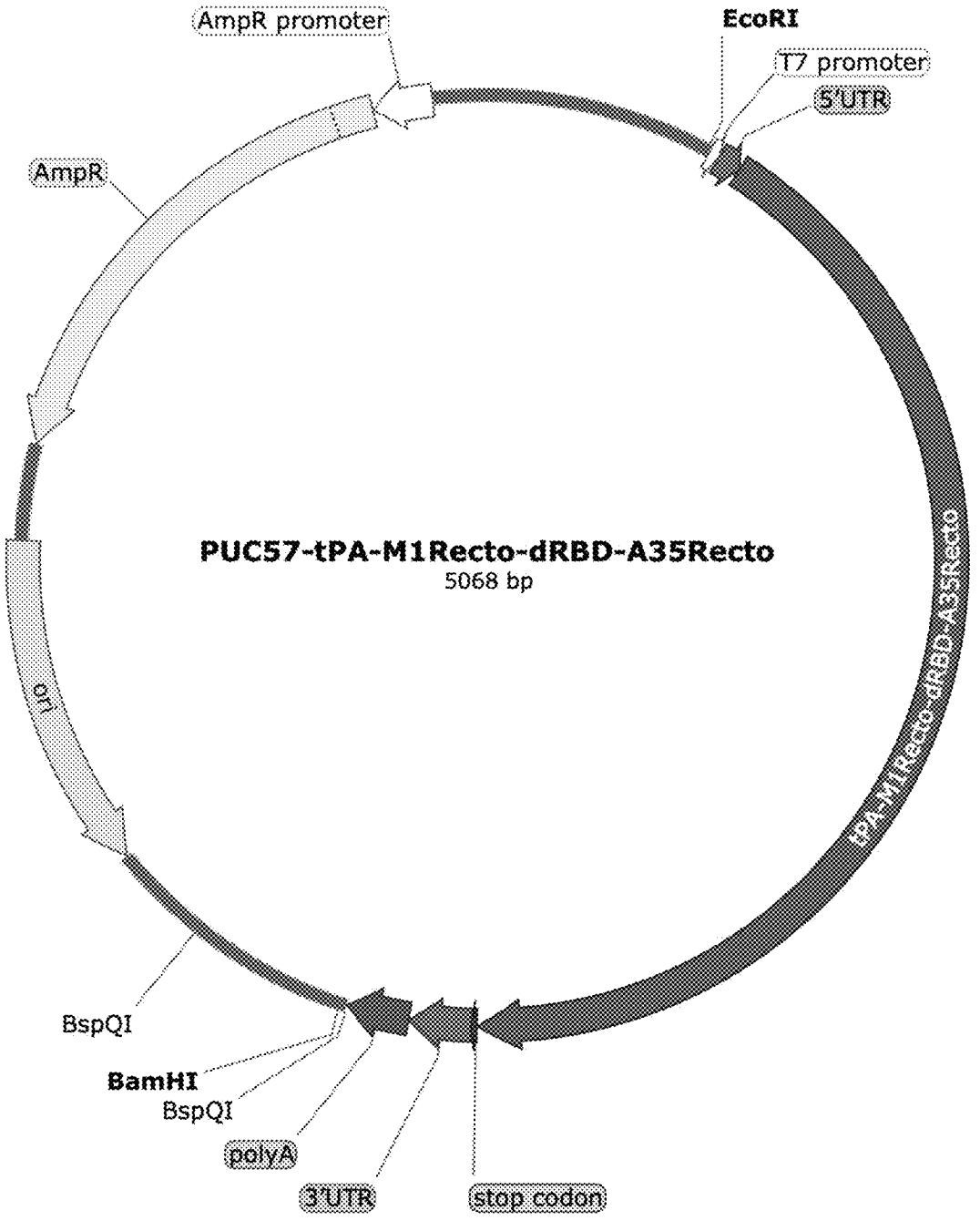

AmpR promoter
EcoRI
T7 promoter
5'UTR
AmpR
PUC57-tPA-M1Recto-dRBD-A35Recto
5068 bp
ori
tPA-M1Recto-dRBD-A35Recto
BspQI
BamHI
BspQI
polyA
3'UTR
stop codon

FIG. 5

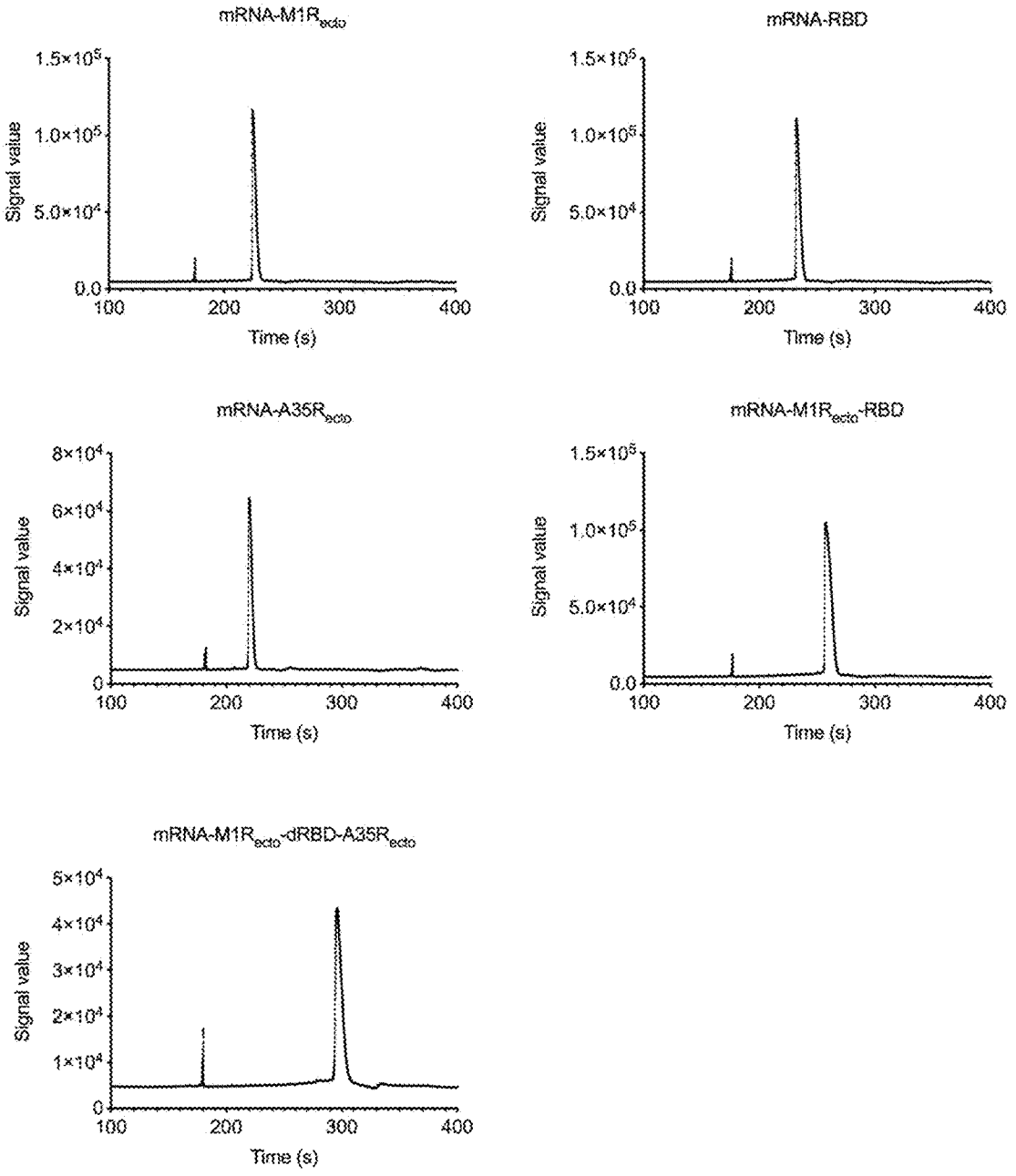

mRNA-M1R$_{ecto}$
Signal value
1.5×10^5
1.0×10^5
5.0×10^4
0.0
100
200
300
400
Time (s)
mRNA-RBD
Signal value
1.5×10^5
1.0×10^5
5.0×10^4
0.0
100
200
300
400
Time (s)
mRNA-A35R$_{ecto}$
Signal value
8×10^4
6×10^4
4×10^4
2×10^4
0
100
200
300
400
Time (s)
mRNA-M1R$_{ecto}$-RBD
Signal value
1.5×10^5
1.0×10^5
5.0×10^4
0.0
100
200
300
400
Time (s)
mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$
Signal value
5×10^4
4×10^4
3×10^4
2×10^4
1×10^4
0
100
200
300
400
Time (s)

FIG. 6

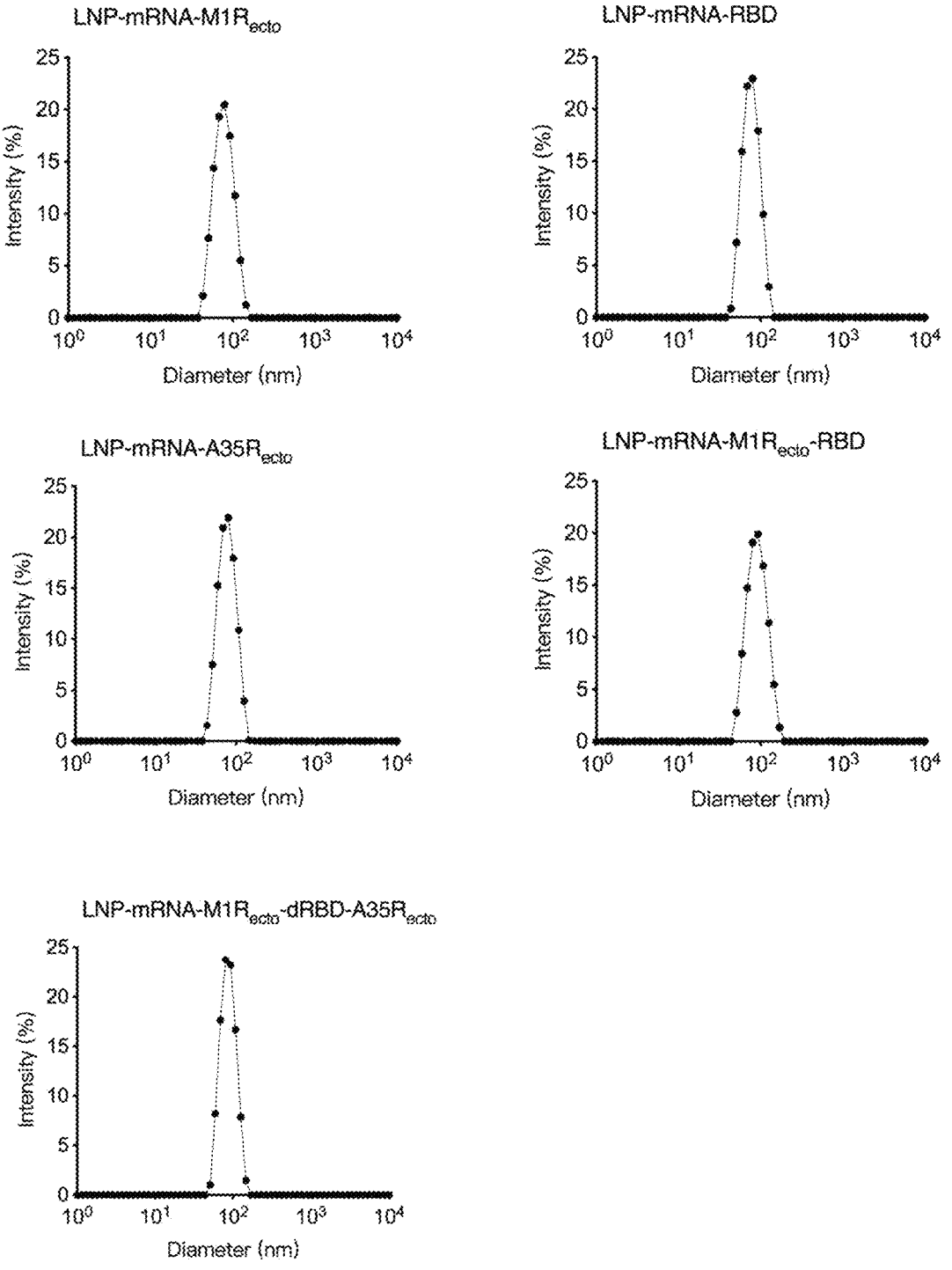

LNP-mRNA-M1R$_{ecto}$
LNP-mRNA-RBD
LNP-mRNA-A35R$_{ecto}$
LNP-mRNA-M1R$_{ecto}$-RBD
LNP-mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$
Intensity (%)
Diameter (nm)
0
5
10
15
20
25
10^0
10^1
10^2
10^3
10^4

FIG. 7

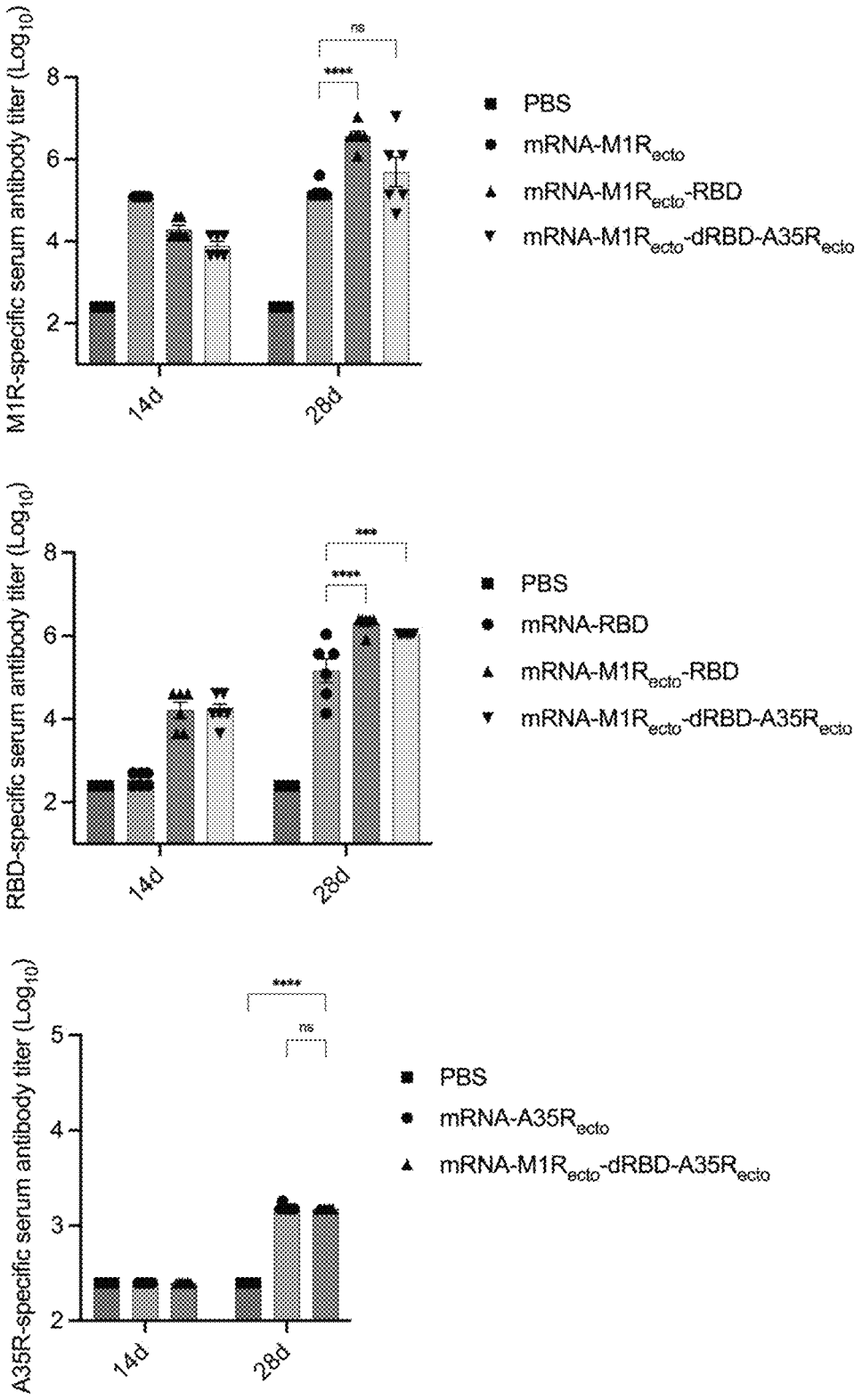
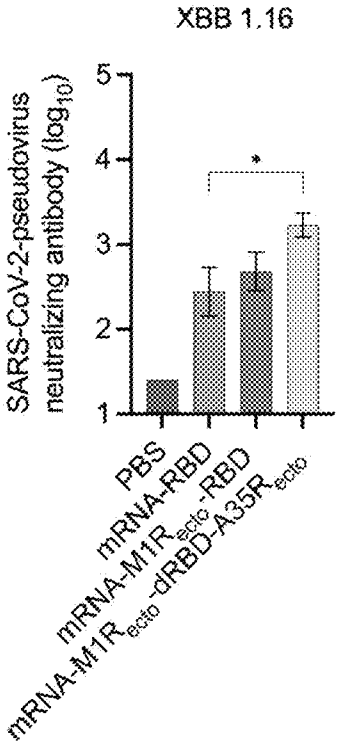

M1R-specific serum antibody titer (Log10)
8
6
4
2
ns
****
14d
28d
PBS
mRNA-M1Recto
mRNA-M1Recto-RBD
mRNA-M1Recto-dRBD-A35Recto
RBD-specific serum antibody titer (Log10)
8
6
4
2
***
****
14d
28d
PBS
mRNA-RBD
mRNA-M1Recto-RBD
mRNA-M1Recto-dRBD-A35Recto
A35R-specific serum antibody titer (Log10)
5
4
3
2
****
ns
14d
28d
PBS
mRNA-A35Recto
mRNA-M1Recto-dRBD-A35Recto

FIG. 8


XBB 1.16
SARS-CoV-2-pseudovirus neutralizing antibody (log_{10})
5
4
3
2
1
*
PBS
mRNA-RBD
mRNA-M1R$_{ecto}$-RBD
mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$

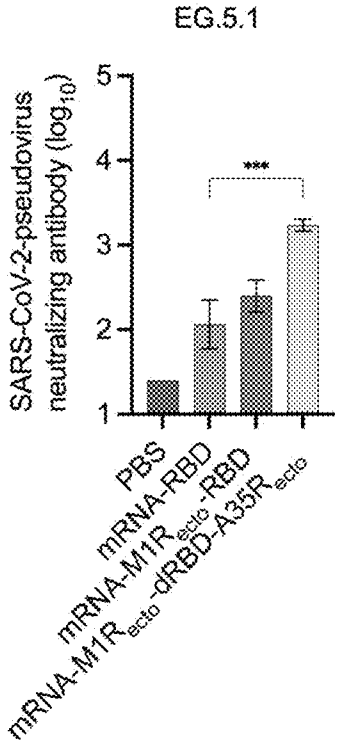

EG.5.1
SARS-CoV-2-pseudovirus neutralizing antibody (log_{10})
5
4
3
2
1
***
PBS
mRNA-RBD
mRNA-M1R$_{ecto}$-RBD
mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$

FIG. 9

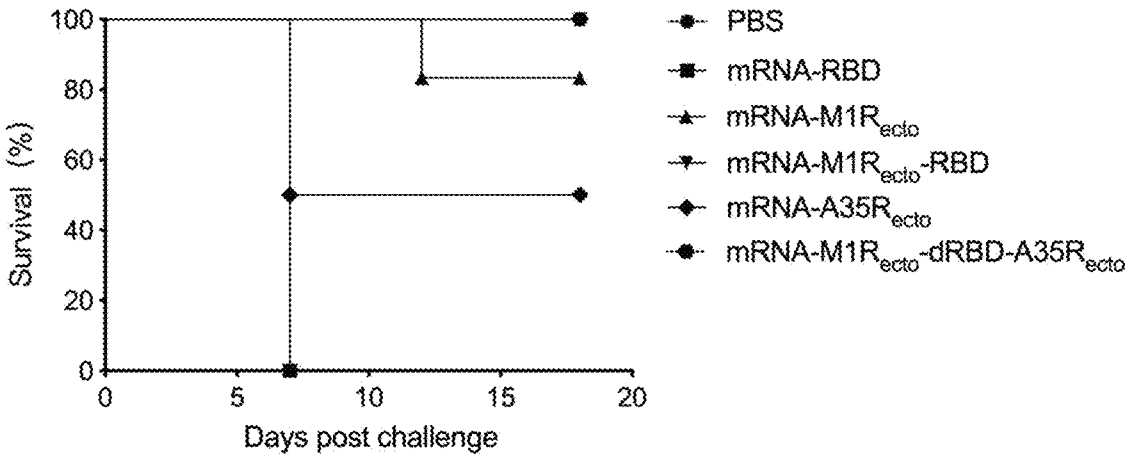

100
80
60
40
20
0
Survival (%)
0
5
10
15
20
Days post challenge
PBS
mRNA-RBD
mRNA-M1Recto
mRNA-M1Recto-RBD
mRNA-A35Recto
mRNA-M1Recto-dRBD-A35Recto

FIG. 10

mRNA VACCINE ENCODING FUSION ANTIGEN AGAINST MPOX AND SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2

CROSS REFERENCE TO THE RELATED APPLICATIONS

This application is based upon and claims priority to Chinese Patent Application No. 202410246021.5, filed on Mar. 5, 2024, the entire contents of which are incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. The XML copy, created on Mar. 17, 2025, is named "2025-03-17-Sequence listing-69705-H005US00.xml," and is 26,604 bytes in size.

TECHNICAL FIELD

The present disclosure relates to the technical field of biology engineering, and in particular, to an mRNA vaccine against Mpox and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

BACKGROUND

In the context of the long-term pandemic of COVID-19, the emergence of other emerging infectious diseases continuously poses new threats to humans. Orthopoxvirus includes smallpox virus, Mpox, vaccinia virus, ectromelia virus, etc., with a very close evolutionary distance. Although the current Mpox is mainly confined to certain specific populations, immune dysfunction caused by SARS-CoV-2 infection may lead to the expansion of the susceptible population of Mpox. At present, there have been cases of co-infection of SARS-CoV-2 and Mpox. Therefore, it is necessary to develop a single-component fusion vaccine against Mpox and SARS-CoV-2. The vaccine can enhance the immune protection against SARS-CoV-2 by boosting immunization while generating immune responses to the orthopoxvirus.

S protein is the most important protective antigen of SARS-CoV-2, as well as a core component of the recombinant vaccine against SARS-CoV-2. By contrast, Mpox has a more complex antigen spectrum. At present, most recombinant monkeypox vaccines adopt a multivalent strategy. Aiming at different antigens such as M1R, A35R, H3L, A29L, B6R, E8L, etc., a combinatorial design is carried out to form a multi-component vaccine. The mRNA vaccine has the technical advantages of rapid synthesis and high immunogenicity. An objective of the present disclosure is to provide an mRNA vaccine encoding a single-component fusion antigen based on mRNA technology, which can induce high-level antibody responses against antigens of Mpox and SARS-CoV-2 simultaneously.

SUMMARY

In view of this, the present disclosure first provides an mRNA molecule. The mRNA molecule contains an mRNA

encoding an MIR antigen of Mpox and an mRNA encoding an RBD antigen of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In a preferred embodiment, a sequence of a polypeptide encoded by the mRNA molecule is shown in SEQ ID NO: 2.

In a more preferred embodiment, a sequence of a polynucleotide encoded by the mRNA molecule is shown in SEQ ID NO: 1.

In another preferred embodiment of the present disclosure, the mRNA molecule further contains an mRNA encoding an A35R antigen of Mpox.

In a more preferred embodiment, a sequence of a polypeptide encoded by mRNA molecules is shown in SEQ ID NO: 4.

More preferably, a sequence of a polynucleotide encoded by the mRNA molecule is shown in SEQ ID NO: 3.

In a more preferred embodiment of the present disclosure, a 5' end of the mRNA molecule further contains a promoter and a 5'untranslated region (UTR), and a 3' end of the mRNA further contains a 3'UTR, a stop codon, poly (A), and BspQI restriction enzyme sites in series.

Especially preferably, a sequence of the promoter is shown in SEQ ID NO: 5, a sequence of the 5'UTR is shown in SEQ ID NO: 6, a sequence of the 3'UTR is shown in SEQ ID NO: 7, a sequence of the stop codon is TGATAATAG, a sequence of the BspQI restriction enzyme sites in series is GAAGAGC, and a length of poly (A) is 110 nucleotides.

In a preferred embodiment, a sequence of the mRNA molecule is shown in SEQ ID NO: 14 or SEQ ID NO: 15.

In a more preferred embodiment, the 5' end of the mRNA molecule further contains a Cap1 cap structure.

Secondly, the present disclosure provides a lipid nanoparticle encapsulating the mRNA molecule.

Thirdly, the present disclosure provides a preparation method for the lipid nanoparticle, including the following steps:

(a) forming a lipid mixture with heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate, 1, 2-distearoyl-sn-glycero-3-phosphocholine, methoxy polyethylene glycol-dimyristoyl glycerol, and cholesterol according to a molar ratio of 50:10:1.5:38.5, and preparing a mRNA solution containing the mRNA molecule; and

(b) mixing the lipid mixture with the mRNA solution obtained in step (a).

In a more preferred embodiment, in step (b), a mass ratio of the lipid mixture to the mRNA solution is 1:3.

Lastly, the present disclosure provides an application of the mRNA in preparing an mRNA vaccine against Mpox or SARS-CoV-2.

Compared to an mRNA vaccine encoding separately corresponding antigens, the mRNA vaccine encoding a fusion antigen provided by the present disclosure can induce considerable or even higher-level neutralizing antibody responses against Mpox and SARS-CoV-2, and provides 100% immune protection against the lethal challenge of the ectromelia virus. In addition, the preparation of the single-component fusion mRNA vaccine is simple, and the single-component fusion mRNA vaccine is obtained by only synthesizing a single mRNA molecule and encapsulating the single mRNA molecule within lipid nanoparticles. Therefore, the single-component fusion mRNA vaccine has a wider application prospect than multivalent mRNA vaccine compositions.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a schematic diagram of a template plasmid PUC57-tPA-M1R$_{ecto}$ for mRNA synthesis;

FIG. **2** is a schematic diagram of a template plasmid PUC57-tPA-RBD for mRNA synthesis;

FIG. **3** is a schematic diagram of a template plasmid PUC57-tPA-A35R$_{ecto}$ for mRNA synthesis;

FIG. **4** is a schematic diagram of a template plasmid PUC57-tPA-M1R$_{ecto}$-RBD for mRNA synthesis;

FIG. **5** is a schematic diagram of a template plasmid PUC57-tPA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$ for mRNA synthesis;

FIG. **6** is an electrophoretic pattern illustrating synthesized mRNA molecules characterized by capillary electrophoresis;

FIG. **7** is a particle size distribution chart illustrating lipid nanoparticles encapsulated-mRNA vaccines characterized by dynamic light scattering;

FIG. **8** is a chart illustrating levels of specific antibodies induced by mRNA candidate vaccines;

FIG. **9** is a chart illustrating neutralizing antibody levels against SARS-CoV-2 pseudovirus induced by mRNA candidate vaccines; and

FIG. **10** is a chart illustrating levels of the immune protection of mRNA candidate vaccines against the lethal challenge of the ectromelia virus.

DETAILED DESCRIPTION OF THE EMBODIMENTS

In the following, the advantages and characteristics of the present disclosure will be more clearly described in combination with the embodiments to further describe the present disclosure. However, these embodiments are merely exemplificative, which do not constitute any restriction on the scope of protection limited by the claims of the present disclosure.

Embodiment 1 Preparation of mRNA Vaccines

(1) Constructing Template Plasmids

The tPA signal peptide was fused to the N-terminus of encoded antigens M1R$_{ecto}$, RBD, A35R$_{ecto}$, M1R$_{ecto}$-RBD, and M1R$_{ecto}$-dRBD-A35R$_{ecto}$, respectively, through sequence optimization, the obtained target nucleotide sequences were SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 1, and SEQ ID NO: 3, respectively. According to the 5'→3' direction, a T7 promoter (SEQ ID NO: 5), a 5'UTR (SEQ ID NO: 6), target nucleotides, a 3'UTR (SEQ ID NO: 7), a stop codon (TGATAATAG), a poly (A) sequence of 110 nucleotides (nt), BspQI restriction enzyme sites (GAAGAGC) were connected and cloned into PUC57 plasmids, to obtain the template plasmids (FIGS. **1-5**).

(2) Linearization of the Template Plasmids

In a 200 μL reaction system containing 20 μg template plasmids, 10 μL BspQI enzyme (10 U/μL), and 20 μL 10×BspQ IBuffer and Nuclease-Free H_2O, a reaction was performed at 50° C. for 1 h. The linearized template plasmids were purified with the phenol-chloroform extraction method, an equal volume of phenol-chloroform (Tris saturated phenol:chloroform:isopentanol=25:24:1) was added to the DNA solution, and fully and evenly mixed; at room temperature, a centrifugal acceleration was adjusted to 12000 g for performing centrifuge for 10 min; the upper aqueous phase was extracted carefully, and an equal volume of chloroform solution (chloroform:isopentanol=24:1) was added, and fully and evenly mixed; and after centrifugation (ditto), the supernatant was extracted carefully and detected for DNA concentration.

(3) In Vitro Transcription of mRNA and Purification

In vitro transcription was performed on mRNA molecules encoding M1R$_{ecto}$, RBD, A35R$_{ecto}$, M1R$_{ecto}$-RBD, and M1R$_{ecto}$-dRBD-A35R$_{ecto}$, a modification ratio of N1-methylpseudourine to uracil was 100%. In a 100 μL reaction system containing 5 μg linearized plasmids, 10 μL T7 RNA Polymerase (50 U/μL), 5 μL inorganic pyrophosphatase (0.1 U/μL), L RNase Inhibitor (40 U/μL), 10 μL 10×Reaction buffer, 10 μL ATP (100 mM), 10 μL GTP (100 mM), 10 μL m1ψ/UTP (100 mM), and 10 μL CTP (100 mM) and Nuclease-Free H_2O (the above reagents were purchased from Nanjing Vazyme Biotech Co., Ltd.), after being fully and evenly mixed, a reaction was performed at 37° C. for 2 h. Subsequently, 5 μL DNase I (1 U/μL) was added to the reaction system for reacting at 37° C. for 15 min, to remove the DNA templates for transcription. The transcription products of mRNA were purified with the phenol-chloroform extraction method as described above.

(4) mRNA Capping and Purification

In a 100 μL reaction system containing 200 μg transcribed mRNA, 50 μL 10×Capping Reaction buffer, 25 μL GTP (10 mM), 25 μL SAM (4 mM), 25 μL Vaccinia Capping Enzyme (10 U/μL), and 25 μL 2'-O-Methyltransferase (50 U/μL) and Nuclease-Free H_2O (the above reagents were purchased from Nanjing Vazyme Biotech Co., Ltd.), after being fully and evenly mixed, a reaction was performed at 37° C. for 1 h, and the transcription products of mRNA were purified with phenol-chloroform extraction method as described above. The molecular integrity of the transcription products of mRNA was detected by capillary electrophoresis (FIG. **6**), and the results shows that the size of the prepared products including mRNA-M1R$_{ecto}$ (SEQ ID NO:11), mRNA-RBD (SEQ ID NO:12), RNA-A35R$_{ecto}$ (SEQ ID NO:13), mRNA-M1R$_{ecto}$-RBD (SEQ ID NO:14), and mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$ (SEQ ID NO:15) is in line with expectations, with a purity of more than 90%.

(5) the Encapsulation of mRNA within Lipid Nanoparticles

SM-102 (heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate, purchased from Xiamen Sinopeg Biotech Co., Ltd.), DSPC (1, 2-distearoyl-sn-glycero-3-phosphocholine, purchased from Xiamen Sinopeg Biotech Co., Ltd.), DMG-PEG2000 (methoxy polyethylene glycol-dimyristoyl glycerol, purchased from Xiamen Sinopeg Biotech Co., Ltd.), and cholesterol (purchased from AVT (Shanghai) Pharmaceutical Tech Co., Ltd.) were dissolved in ethanol at a molar ratio of 50:10:1.5:38.5, to prepare ethanol phase; and the mRNA molecules were dissolved in 50 mM sodium acetate buffer (pH=5.0), to prepare aqueous phase; when performing the encapsulation by microfluidics, the volume ratio of the ethanol phase to the aqueous phase was 1:3, and the total flow rate was 12 mL/min. After the encapsulation, ultrafiltration concentration and buffer exchange with PBS buffer were performed, after the encapsulation rate and effective concentration were detected, the obtained products were stored at 4° C. Dynamic light scattering (DLS) measurements show that the mRNA vaccines encapsulated within lipid nanoparticles have a uniform particle size distribution (FIG. **7**), the average size of LNP-mRNA-M1R$_{ecto}$, LNP-mRNA-RBD, LNP-mRNA-A35R$_{ecto}$, LNP-mRNA-M1R$_{ecto}$-RBD, and LNP-mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$ were 74.40 nm, 73.82 nm, 73.27 nm, 85.91 nm, and 83.93 nm, respectively, and the dispersion coefficients were all less than 0.05.

Embodiment 2 Immune Response of the mRNA Vaccines

In a BALB/c mouse model, 5 μg of five candidate vaccines including mRNA-M1R$_{ecto}$, mRNA-RBD, mRNA-

A35R$_{ecto}$, mRNA-M1R$_{ecto}$-RBD, mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$ were inoculated on day 0 and day 14 by intramuscular injection, respectively (with 6 mice in each group), blood samples were collected for serum collection on the 14 th and 28 th day, the specific IgG antibody and neutralizing antibody levels were detected, and the lethal challenge of the ectromelia virus was carried out on day 28.

(1) Specific IgG Antibody Response

The recombinant proteins of M1R (Sino Biological, Inc. (China), 40904-V07H), A35R (Sino Biological, Inc. (China), 40886-V07E), and RBD (Sino Biological, Inc. (China), 40592-V08H136) were diluted to the concentration of 1 μg/mL, which was coated in 96-well plates overnight, after blocking, the IgG antibody titer was detected by enzyme-linked immunosorbent assay, and statistically analyzed by two-way ANOVA with Šidák's multiple comparison test.

In the specific antibody response against M1R, 14 days after a single immunization with mRNA-M1R$_{ecto}$-RBD and mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$, the geometry mean values of IgG antibody titer were 19454 and 7798, respectively, and the antibody titer was significantly increased 14 days after boosting immunization (28 days after the first immunization), the geometric mean values were 3647529 and 486407, respectively, which were 20 times ($P<0.0001$) and 3 times higher than mRNA-M1R$_{ecto}$ antibody titer (162181).

In the specific antibody response against RBD, 14 days after a single immunization with mRNA-M1R$_{ecto}$-RBD and mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$, both geometry mean values of IgG antibody titer were 16218, and the antibody titer was significantly increased 14 days after boosting immunization (28 days after the first immunization), the geometric mean values were 2023019 and 1093956, respectively, which were 14 times ($P<0.0001$) and 7 times ($P=0.0002$) higher than the mRNA-RBD antibody titer (145881).

In the specific antibody response against A35R, the geometric mean value of IgG antibody titer 28 days after boosting immunization with mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$ was 1500, which was not statistically different from the mRNA-A35R$_{ecto}$, but significantly higher than the PBS control group ($P<0.001$). (FIG. **8**).

(2) SARS-CoV-2-Pseudovirus Neutralizing Antibody Response

Through infection of 293 cells stably express human ACE2 with SARS-CoV-2-pseudovirus, neutralizing antibody levels in serum against SARS-CoV-2-pseudovirus were detected 14 days after boosting immunization (28 days after the first immunization). For the pseudovirus of SARS-CoV-2 Omicron variants XBB.1.16, the geometry mean values of neutralizing antibody titers against SARS-CoV-2-pseudovirus with mRNA-M1R$_{ecto}$-RBD and mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$ reached 480 and 1694, which were 2 times and 6 times ($p=0.027$) higher than the neutralizing antibody titer with mRNA-RBD (277), respectively. For the pseudovirus of SARS-CoV-2 Omicron variants EG.5.1, the geometry mean values of neutralizing antibody titers against SARS-CoV-2-pseudovirus with mRNA-M1R$_{ecto}$-RBD and mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$ reached 251 and 1714, which were 2 times (no statistical difference) and 15 times ($p=0.0004$) of the neutralizing antibody titer with mRNA-RBD (115), respectively. The above results indicate that the neutralizing antibody level against SARS-CoV-2, which was produced by activation of mRNA-M1R$_{ecto}$-RBD, is equivalent to that produced by activation of mRNA-RBD, while compared with mRNA-RBD, RNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$ can induce a higher neutralizing antibody level against SARS-CoV-2.

(3) Protection Against the Lethal Challenge of Ectromelia Virus

Through infection of BS-C-1 cells with ectromelia virus, the ectromelia virus (ATCC VR-1374) was amplified and cultured. 28 days after the first immunization, each mouse was challenged intraperitoneally with 200 PFU ectromelia virus, the survival of mice was monitored within 18 days, and statistically analyzed by Log-rank (Mantel-Cox) test. In a lethal challenge experiment, the survival rates of mRNA-M1R$_{ecto}$-RBD and mRNA-M1R$_{ecto}$-dRBD-A35R$_{ecto}$ immunized groups were both 100%, compared with the PBS control group (with a survival rate of 0%), which had significant immune protection ($p=0.0009$). The above results confirm that the mRNA vaccines encoding M1R$_{ecto}$-RBD and M1R$_{ecto}$-dRBD-A35R$_{ecto}$ can provide complete immune protection against the lethal challenge of orthopoxvirus. (FIG. **10**).

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aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa  2340
aaaaaaaaaa aaaaaaaaaa a                                            2361
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What is claimed is:

1. An mRNA molecule, wherein the sequence of a polypeptide encoded by the mRNA molecule is shown in SEQ ID NO: 2.

2. The mRNA molecule according to claim **1**, wherein the sequence of the mRNA molecule is shown in SEQ ID NO: 1.

3. An mRNA molecule, wherein the sequence of a polypeptide encoded by the mRNA molecule is shown in SEQ ID NO: 4.

4. The mRNA molecule according to claim **3**, wherein the sequence of the mRNA molecule is shown in SEQ ID NO: 3.

5. The mRNA molecule according to claim **1**, wherein the 5' end of the mRNA molecule contains a promoter and a 5' untranslated region (UTR), and the 3' end of the mRNA contains a 3'UTR, a stop codon, poly (A), and BspQI restriction enzyme sites in series.

6. The mRNA molecule according to claim **5**, wherein the sequence of the promoter is shown in SEQ ID NO: 5, a sequence of the 5'UTR is shown in SEQ ID NO: 6, the sequence of the 3'UTR is shown in SEQ ID NO: 7, the sequence of the stop codon is TGATAATAG, the sequence of the BspQI restriction enzyme sites in series is GAAGAGC, and the length of poly (A) is 110 nucleotides.

7. The mRNA molecule according to claim **6**, wherein the sequence of the mRNA molecule is shown in SEQ ID NO: 14 or SEQ ID NO: 15.

8. The mRNA molecule according to claim **7**, wherein the 5' end of the mRNA molecule is connected to a Cap1 cap structure.

9. A lipid nanoparticle encapsulating the mRNA molecule according to claim **8**.

10. A preparation method of the lipid nanoparticle according to claim 9 comprising the following steps:

(a) forming a lipid mixture with heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate, 1, 2-distearoyl-sn-glycero-3-phosphocholine, methoxy polyethylene glycol-dimyristoyl glycerol, and cholesterol according to a molar ratio of 50:10:1.5:38.5, and preparing a mRNA solution containing the mRNA molecule according to claim 6; and

(b) mixing the lipid mixture with the mRNA solution obtained in step (a).

11. The preparation method according to claim 10, wherein in step (b), a mass ratio of the lipid mixture to the mRNA solution is 1:3.

12. The mRNA molecule according to claim 3, wherein the 5' end of the mRNA molecule contains a promoter and a 5' untranslated region (UTR), and the 3' end of the mRNA contains a 3'UTR, a stop codon, poly (A), and BspQI restriction enzyme sites in series.

13. The mRNA molecule according to claim 12, wherein the sequence of the promoter is shown in SEQ ID NO: 5, the sequence of the 5'UTR is shown in SEQ ID NO: 6, the sequence of the 3'UTR is shown in SEQ ID NO: 7, the sequence of the stop codon is TGATAATAG, the sequence of the BspQI restriction enzyme sites in series is GAAGAGC, and the length of poly (A) is 110 nucleotides.

14. The mRNA molecule according to claim 13, wherein the sequence of the mRNA molecule is shown in SEQ ID NO: 14 or SEQ ID NO: 15.

15. The mRNA molecule according to claim 14, wherein the 5' end of the mRNA molecule is connected to a Cap1 cap structure.

16. A lipid nanoparticle encapsulating the mRNA molecule according to claim 15.

17. A preparation method of the lipid nanoparticle according to claim 16 comprising the following steps:

(a) forming a lipid mixture with heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate, 1, 2-distearoyl-sn-glycero-3-phosphocholine, methoxy polyethylene glycol-dimyristoyl glycerol, and cholesterol according to a molar ratio of 50:10:1.5:38.5, and preparing a mRNA solution containing the mRNA molecule according to claim 13; and

(b) mixing the lipid mixture with the mRNA solution obtained in step (a).

18. The preparation method according to claim 17, wherein in step (b), a mass ratio of the lipid mixture to the mRNA solution is 1:3.

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