

Fully synthetic, long-chain nucleic acid for vaccine production to protect against coronaviruses.

Summary

In one aspect, the present disclosure provides a fully synthetic, long-chain nucleic acid with at least 4,000 bases, characterized in that the nucleic acid comprises a) at least two of the four sequence parts A-D in any arrangement, wherein i) Sequence part A comprises a sequence as defined in SEQ. ID. 50 or a sequence having at least 98.5% sequence identity to the sequence as defined in SEQ. ID. 50; ii) Sequence part C comprises a sequence as defined in SEQ. ID. 49 or a sequence having at least 97.2% sequence identity to the sequence as defined in SEQ. ID. 49; iii) Sequence part B comprises a sequence as defined in SEQ. ID. 48 or a sequence having at least 98.3% sequence identity to the sequence as defined in SEQ. ID. 48; iv) Sequence part D comprises a sequence as defined in SEQ. ID. 17 or a sequence having at least 98.5 % sequence identity to the sequence as defined in SEQ. ID. 17; or encompasses a ribonucleic acid sequence corresponding to the deoxyribonucleic acid sequence according to the sequence parts A-D; and b) does not comprise 1.) a nucleic acid sequence part that encodes an amino acid sequence having the function of a SARS-CoV-2 amino acid sequence encoded by ORF7a; and/or 2.) a nucleic acid sequence part that encodes an amino acid sequence having the function of a SARS-CoV-2 amino acid sequence encoded by ORF3a.

In another aspect, the present disclosure provides a vector comprising the nucleic acid as disclosed herein, wherein preferably the vector comprises the sequences defined by the SEQ. ID. 46 and SEQ. ID. 47.

In another aspect, the present disclosure provides a kit comprising two or more nucleic acids as disclosed herein, wherein preferably the nucleic acids are present in at least one plasmid, preferably in two or more plasmids.

In another aspect, the present disclosure provides a biotechnological production unit comprising at least one vector as disclosed herein.

In another aspect, the present disclosure provides a virus envelope, a fragment of a virus envelope and/or virus envelope protein obtainable by gene expression using at least one nucleic acid as disclosed herein, using the vector as disclosed herein, using the kit as disclosed herein, or the biotechnological production unit as disclosed herein, wherein the virus envelope, the fragment of a virus envelope and/or the virus envelope protein package the at least one nucleic acid as disclosed herein.

In another aspect, the present disclosure provides a vaccine against the coronavirus SARS-CoV-2 comprising at least one nucleic acid as disclosed herein and products obtainable by gene expression using at least one nucleic acid as disclosed herein, using the vector as disclosed herein, using the kit as disclosed herein in a production organism, in particular comprising the virus envelope, the fragment of a virus envelope and/or the virus envelope protein as disclosed herein.

In another aspect, the present disclosure provides a method for the production of the vaccine a vaccine against the coronavirus SARS-CoV-2 comprising the successive steps of a) introducing the nucleotide acid sequence as disclosed herein into a biotechnological production unit, in particular a cell line, wherein, the nucleic acid-based mRNA coding for at least two of the protein components selected from the group consisting of the protein components a, b1, b2, c1, c2, d1 or d2 are prepared by translation; b) obtaining protein components from the biotechnological production unit in step a); and c) purifying the obtained protein components to obtain the vaccine against the coronavirus SARS-CoV-2.

In another aspect, the present disclosure provides a method for the production of a vaccine against the coronavirus SARS-CoV-2 comprising the virus envelope, the fragment of a virus envelope and/or the virus envelope protein as disclosed herein comprising the successive steps of: a) introducing the nucleotide acid sequence as disclosed herein into a biotechnological production unit, wherein the biotechnological production unit comprises a nucleotide acid coding for at least one of the protein components selected from the group consisting of the protein components a, b1, c1, and d1; b) obtaining a fragment of a virus envelope and/or virus envelope protein from the biotechnological production unit in step a); and c) purifying the obtained protein components to obtain the vaccine against the coronavirus SARS-CoV-2 comprising the virus envelope, the fragment of a virus envelope and/or the virus envelope protein as disclosed herein.

In another aspect, the present disclosure provides a method for the production of a vaccine against the coronavirus SARS-CoV-2 comprising the successive steps of: a) introducing the vector as disclosed herein into an amplifying biotechnological production unit; b) amplifying the nucleotide acid as disclosed herein in the amplifying biotechnological production unit; c) obtaining the nucleotide acid amplified in step b); d) obtaining the vaccine against the coronavirus SARS-CoV-2 by using method as disclosed herein.

Description

The present invention relates to a fully synthetic, long-chain nucleic acid according to the independent claim 1. The invention further relates to a kit comprising two or more of these nucleic acids and a biotechnological production unit comprising at least one plasmid comprising the nucleic acid. The invention further relates to a virus envelope, a fragment of a virus envelope and/or a virus envelope protein obtainable by gene expression using said nucleic acid. Furthermore, the invention concerns a vaccine comprising products obtainable by gene expression using the nucleic acid, in particular a vaccine against the coronavirus SARS-CoV-2, as well as a method for producing the vaccine.

The rapid development and availability of vaccines is crucial in combating many viruses and bacteria. The production of suitable vaccines is a multi-stage, complex process and is not always successful despite often high investments. Typically, the development of a suitable vaccine takes years. These long development times consist of a major problem, especially with regard to new emerging pathogens, or mutated pathogens, as from an epidemiological point of view it is only possible to react too late, if at all, to the emergence of new diseases. In contrast, the analysis, identification and further detection of new or heavily mutated pathogens are now possible within weeks or even days, which is a huge improvement over the last century.

In this context, viruses are of special interest, as they harbor high mutation rates causing the spread from other species to humans. Rapid spreading of these viruses makes them a major challenge for modern medicine. The usual time today (2020) between the detection/identification of a newly emerging virus and the development of a vaccine is typically years. In a few cases, with sufficient prior knowledge, experimental vaccines could be provided within months. However, this time span is much longer than the typical time until thousands or millions of people are infected. Such rapid spread is also a direct consequence of the high mobility of today's society.

Ideally, immediately after the identification of a new virus, a vaccine would be available in sufficient quantity and of the highest quality and would allow for a nationwide

Claims

1. Fully synthetic, long-chain nucleic acid with at least 4,000 bases, characterized in that the nucleic acid comprises
 - a) at least two of the four sequence parts A-D in any arrangement, wherein
 - i) Sequence part A comprises a sequence as defined in SEQ. ID. 50 or a sequence having at least 98.5% sequence identity to the sequence as defined in SEQ. ID. 50;
 - ii) Sequence part C comprises a sequence as defined in SEQ. ID. 49 or a sequence having at least 97.2% sequence identity to the sequence as defined in SEQ. ID. 49;
 - iii) Sequence part B comprises a sequence as defined in SEQ. ID. 48 or a sequence having at least 98.3% sequence identity to the sequence as defined in SEQ. ID. 48;
 - ~~iii) Sequence part C comprises a sequence as defined in SEQ. ID. 49 or a sequence having at least 97.2% sequence identity to the sequence as defined in SEQ. ID. 49;~~
 - iv) Sequence part D comprises a sequence as defined in SEQ. ID. 17 or a sequence having at least 98.5 % sequence identity to the sequence as defined in SEQ. ID. 17; orencompasses a ribonucleic acid sequence corresponding to the deoxyribonucleic acid sequence according to the sequence parts A-D; and
 - b) does not comprise
 - 1.) a nucleic acid sequence part that encodes an amino acid sequence having the function of a SARS-CoV-2 amino acid sequence encoded by ORF7a; and/or
 - 2.) a nucleic acid sequence part that encodes an amino acid sequence having the function of a SARS-CoV-2 amino acid sequence encoded by ORF3a.

2. The nucleic acid according to claim 1, characterized in that the nucleic acid

a) comprises at least sequence parts A and C in any arrangement or encompasses a ribonucleic acid sequence corresponding to the deoxyribonucleic acid sequence according to the sequence parts A and C, wherein

i) Sequence part A comprises a sequence as defined in SEQ. ID. 50 or a sequence having at least 98.5% sequence identity to the sequence as defined in SEQ. ID. 50; and

ii) Sequence part C comprises a sequence as defined in SEQ. ID. 49 or a sequence having at least 97.2% sequence identity to the sequence as defined in SEQ. ID. 49; and

b) does not comprise

1) a nucleic acid sequence part that encodes an amino acid sequence having the function of a SARS-CoV-2 amino acid sequence encoded by ORF7a; and/or

2) a nucleic acid sequence part that encodes an amino acid sequence having the function of a SARS-CoV-2 amino acid sequence encoded by ORF3a.

2.3. The nucleic acid according to claim 1 or 2, characterized in that it has at least 8'000 bases, preferably at least 20'000 bases, in a defined sequence.

3.4. The nucleic acid according to claim 1, 2 or 23, characterized in that the nucleic acid comprises not more than one or no ORF-associated nucleic acid sequence parts, wherein the ORF-associated nucleic acid sequence part encodes an amino acid sequence having the function of a SARS-CoV-2 amino acid sequence encoded by ORF6 or ORF8;

4. The nucleic acid according to claim 3, wherein preferably the nucleic acid comprises no ORF-associated nucleic acid sequence part, wherein the ORF-associated nucleic acid sequence part encodes an amino acid sequence having the function of a SARS-CoV-2 amino acid sequence encoded by ORF6 or ORF8.

5. The nucleic acid according to one of the preceding claims, wherein the nucleic acid additionally comprises
 - a) 1.) an ORF1ab sequence defined by the SEQ. ID. 51 or a sequence having at least 98,5% sequence identity to SEQ. ID. 51; or
 - 2.) i) an ORF1b sequence defined by the SEQ. ID. 59 or a sequence having at least 98,5% sequence identity to SEQ. ID. 59; and
 - ii) a n ORF1a sequence defined by the SEQ. ID. 58 or a sequence having at least 98,6% sequence identity to SEQ. ID. 58; and
 - b) an ORF3a sequence defined by the SEQ. ID. 52 or a sequence having at least 99% sequence identity to SEQ. ID 52.
6. The nucleic acid according to claim 5, wherein the nucleic acid additionally comprises
 - a) an ORF6 sequence defined by the SEQ. ID. 53 or a sequence having at least 94,1% sequence identity to SEQ. ID 53; and/or
 - b) an ORF8 sequence defined by the SEQ. ID. 55 or a sequence having at least 99% sequence identity to SEQ. ID 55.
7. The nucleic acid according to any of the preceding claims, characterized in that sequence parts A to C correspond to the sequence according to SEQ. ID. 19 or the corresponding ribonucleic acid sequence.
8. The nucleic acid according to any of the preceding claims, characterized in that the nucleic acid comprises in any arrangement at least three of the four sequence parts A-D or at least three of four sequence parts with a ribonucleic acid sequence corresponding to the deoxyribonucleic acid sequence according to the sequence parts A-D.
9. The nucleic acid according to any of the preceding claims, characterized in that the nucleic acid comprises in any arrangement the four sequence parts A-D or four sequence parts with a ribonucleic acid sequence corresponding to the deoxyribonucleic acid sequence according to the sequence parts A-D.
10. The nucleic acid according to any one of claims 1 or 3 to 6, characterized in that the nucleic acid comprises two or three of the four sequence parts A-D, wherein preferably-

- ~~11.~~ The nucleic acid according to ~~claim 10~~, characterized in that the nucleic acid comprises three of the four sequence parts A-D.
- ~~12.11.~~ The nucleic acid according to any of the preceding claims, characterized in that the nucleic acid additionally comprises SEQ. ID. 28 and/or SEQ. ID. 29 or the corresponding ribonucleic acid sequence.
- ~~13.12.~~ The nucleic acid according to any of the preceding claims, characterized in that it has a maximum size of 1'000'000 bases, preferably a maximum size of 200'000 bases.
- ~~14.13.~~ A vector comprising the nucleic acid according to one of the preceding claims:
- ~~15.~~ The vector according to ~~claim 14~~, wherein preferably the vector comprises the sequences defined by the SEQ. ID. 46 and SEQ. ID. 47.
- ~~16.~~ The vector according to any one of claims 14 to 15, wherein the vector is a plasmid vector.
- ~~17.14.~~ A kit comprising two or more nucleic acids according to any of claims 1 to ~~13~~12.
- ~~18.~~ The kit according to ~~claim 17~~, wherein preferably the nucleic acids are present in at least one plasmid, preferably in two or more plasmids.
- ~~19.15.~~ A biotechnological production unit comprising at least one vector according to ~~claims 14 to 16~~13.
- ~~20.16.~~ A virus envelope, a fragment of a virus envelope and/or virus envelope protein obtainable by gene expression using at least one nucleic acid according to one of claims 1 to ~~34~~, using the vector according to ~~one of claims 14 to 16~~13, using the kit according to ~~one of claims 17 or 18~~14, or the biotechnological production unit according to ~~claim 19~~15, wherein the virus envelope, the fragment of a virus envelope and/or the virus envelope protein package the at least one nucleic acid according to one of claims 1 to ~~13~~12.
- ~~21.17.~~ A vaccine against the coronavirus SARS-CoV-2 comprising at least one nucleic acid according to one of claims 1 to ~~13~~12 and products obtainable by gene expression using at least one nucleic acid according to one of the claims

1 to ~~12~~13, using the vector according to ~~one of claims 14 to 16~~13, using the kit according to ~~one of the claims 17 or 18~~14 in a production organism, in particular comprising the virus envelope, the fragment of a virus envelope and/or the virus envelope protein according to claim ~~20~~16.

~~22.18.~~ 18. The vaccine according to claim ~~21~~17 comprising at least two molecularly precisely defined protein components selected from the group consisting of the protein components a, b1, c1, or d1 wherein

(i) the protein component a comprises

a) the sequence according to SEQ. ID. 14 analogous to the S protein of SARS-CoV-2 or a sequence having at least 90% sequence identity to SEQ. ID. 14; or

b) the sequence according to SEQ. ID. 18 analogous to the S protein of SARS-CoV-2 or sequence having at least 90% sequence identity to SEQ. ID.18;

(ii) the protein component b1 comprises

a) the sequence according to SEQ. ID. 6 analogous to the envelope protein E of SARS-CoV-2 or a sequence having at least 90% sequence identity to SEQ. ID.6; or

b) the sequence according to SEQ. ID. 21 analogous to the envelope protein E of SARS-CoV-2 or a sequence having at least 90% sequence identity to SEQ. ID.21; and

(iii) the protein component c1 comprises

a) the sequence according to SEQ. ID. 10 analogous to the envelope protein M of SARS-CoV-2 or a sequence having at least 90% sequence identity to SEQ. ID. 10; or

b) the sequence according to SEQ. ID. 22 analogous to the membrane protein M of SARS-CoV-2 or a sequence having at least 90% sequence identity to SEQ. ID.22; and

(iv) the protein component d1 comprises

a) the sequence according to SEQ. ID. 2 analogous to the nucleocapsid phosphoprotein N of SARS-CoV-2 or a sequence having at least 90% sequence identity to SEQ. ID. 2; or

b) the sequence according to SEQ. ID. 26 analogous to the nucleocapsid phosphoprotein N of SARS-CoV-2 or a sequence having at least 90% sequence identity to SEQ. ID. 26.

~~23-19.~~ 19. A method for the production of the vaccine a vaccine against the coronavirus SARS-CoV-2 comprising the successive steps of

a) introducing the nucleotide acid sequence according to one of claims 1 to ~~13~~ 12 into a biotechnological production unit, in particular a cell line,

wherein, the nucleic acid-based mRNA coding for at least two of the protein components selected from the group consisting of the protein components a, b1, b2, c1, c2, d1 or d2 are prepared by translation;

b) obtaining protein components from the biotechnological production unit in step a); and

c) purifying the obtained protein components to obtain the vaccine against the coronavirus SARS-CoV-2.

~~24-20.~~ 20. A method for the production of a vaccine against the coronavirus SARS-CoV-2 comprising the virus envelope, the fragment of a virus envelope and/or the virus envelope protein according to claim ~~20-16~~ 16 comprising the successive steps of:

a) introducing the nucleotide acid sequence according to one of claims 1 to ~~13~~ 12 into a biotechnological production unit, wherein the biotechnological production unit comprises a nucleotide acid coding for at least one of the protein components selected from the group consisting of the protein components a, b1, c1, and d1;

b) obtaining a fragment of a virus envelope and/or virus envelope protein from the biotechnological production unit in step a); and

c) purifying the obtained protein components to obtain the vaccine against the coronavirus SARS-CoV-2 comprising the virus envelope, the fragment of a virus envelope and/or the virus envelope protein according to claim ~~20-16~~ 16.

~~25-21.~~ A method for the production of a vaccine against the coronavirus SARS-CoV-2 comprising the successive steps of:

- a) introducing the vector according to ~~one of claims 14 to 16~~13 into an amplifying biotechnological production unit;
- b) amplifying the nucleotide acid according to one of claims 1 to ~~13~~12 in the amplifying biotechnological production unit;
- c) obtaining the nucleotide acid amplified in step b); and
- d) obtaining the vaccine against the coronavirus SARS-CoV-2 by using method according to claim ~~23-19~~ or ~~24~~20.