

We claim:

1. A method of generating an optimized nucleotide sequence encoding an engineered influenza structural protein, the method comprising:
 - a) providing an amino acid sequence of the engineered influenza structural protein;
 - b) reverse-translating the amino acid sequence to generate a first nucleotide sequence that is a non-optimized parental nucleotide sequence;
 - c) identifying a second nucleotide sequence that encodes an influenza structural protein that shares at least 90% sequence identity with the engineered influenza structural protein, which first and second nucleotide sequences comprise one or more positions where codons are different from one another;
 - d) at every position where the codons in the first and second nucleotide sequences code for the same amino acid, changing codons in the first nucleotide sequence to match codons from the second nucleotide sequence; and
 - e) at every position where the codons in the first and second nucleotide sequences code for a different amino acid, changing codons in the first nucleotide sequence to match codons that are based on structural protein-specific influenza codon usage preferences to generate the optimized nucleotide sequence, wherein translation of the optimized nucleotide sequence and expression of the encoded engineered influenza structural protein are improved for one or more expression systems relative to the non-optimized parental nucleotide sequence.
2. The method of claim 1, wherein the influenza structural protein that shares a high degree of sequence identity with the engineered influenza structural protein is a wild-type influenza structural protein.
3. The method of claim 1 or 2, further comprising adding the 5' and 3' non-coding sequences from a high titer rescued strain to the optimized nucleotide sequence.
4. The method of claim 3, wherein in the high titer rescued strain is A/PuertoRico/8/34 (PR8).

5. The method of anyone of claims 1-4, wherein the amino acid sequence of the engineered influenza structural protein encoded by the optimized nucleotide sequence is the same as the amino acid sequence encoded by the first nucleotide sequence.
6. The method of any one of claims 1-5, wherein the optimized nucleotide sequence further comprises a nucleotide sequence encoding a signal peptide, a nucleotide sequence coding for a transmembrane domain, and/or a nucleotide sequence coding for a cytoplasmic domain.
7. The method of claim 6, further comprising exchanging the nucleotide sequence encoding the signal peptide in the optimized nucleotide sequence with a nucleotide sequence encoding the signal peptide from a high titer rescued strain.
8. The method of claim 6 or 7, further comprising exchanging the nucleotide sequence encoding the transmembrane domain with a nucleotide sequence encoding the transmembrane domain from a high titer rescued strain.
9. The method of any one of claims 6-8, further comprising exchanging the nucleotide sequence encoding the cytoplasmic domain with a nucleotide sequence encoding the cytoplasmic domain from a high titer rescued strain.
10. The method of any one of claims 7-9, wherein the high titer rescued strain is A/PuertoRico/8/34 (PR8).
11. The method of any one of the preceding claims, where in the engineered influenza structural protein is an influenza type A hemagglutinin protein.
12. The method of claim 11, wherein the hemagglutinin protein is a subtype selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, and H17.

13. The method of any one of the preceding claims, wherein the structural protein-specific influenza codon usage preferences are set forth in Tables 1-10.

14. The method of any one of the preceding claims, wherein reverse translating the amino acid sequence to generate a first nucleotide sequence comprises use of codon usage preferences specific for influenza viruses.

15. The method of any one of the preceding claims, wherein the second nucleotide sequence encodes a wild type version of the influenza structural protein and is identified from a publicly available database comprising influenza nucleotide sequences.

16. The method of claim 3, wherein the 5' non-coding sequence comprises the nucleotide sequence of SEQ ID NO: 23 and/or the 3' non-coding sequence comprises the nucleotide sequence of SEQ ID NO: 24 or wherein the 5' non-coding sequence comprises the nucleotide sequence of SEQ ID NO: 103 and/or the 3' non-coding sequence comprises the nucleotide sequence of SEQ ID NO: 104.

17. The method of any one of the proceeding claims, wherein the engineered influenza structural protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, and SEQ ID NO: 102.

18. A method of expressing an optimized nucleotide sequence, the method comprising:
generating an optimized nucleotide sequence encoding an engineered influenza structural protein according to the method of any one of claims 1-17;

inserting the optimized nucleotide sequence into an expression plasmid; and
expressing the optimized nucleotide sequence to generate the engineered influenza structural protein.

19. A reverse genetics method for producing an infectious influenza virus, the method comprising:

(i) generating an optimized nucleotide sequence encoding an engineered influenza structural protein according to the method of any one of claims 1 to 17;

(ii) transfecting mammalian cells with one or more expression vectors, wherein the one or more expression vectors comprise: a) the optimized nucleotide sequence encoding the engineered influenza structural protein generated in step (i) and b) nucleotide sequences coding for influenza proteins from one or more donor viruses; and,

(iii) producing the infectious influenza virus.

20. The method of claim 19, wherein the one or more donor viruses are selected from the group consisting of A/Puerto Rico/8/34 (H1N1) (PR8), B/Lee/40, and B/Panama/45/90.

21. The method of claim 19 or 20, wherein the infectious influenza virus is an infectious reassortant influenza virus comprising the genetic material of one or more donor viruses.

22. The method of claim 21, wherein the infectious reassortant influenza virus is chimeric.

23. The method of any one of claims 19-22, further comprising:

harvesting the infectious influenza virus; and

infecting eggs or mammalian cells with the harvested influenza virus.

24. A method of preparing an influenza vaccine composition, the method comprising:

(i) generating an optimized nucleotide sequence encoding an engineered influenza structural protein according to the method of any one of claims 1 to 17;

(ii) generating a seed virus by transfecting mammalian cells with a set of expression vectors, one or more of which comprises the optimized nucleotide sequence encoding the engineered influenza structural protein generated in step (i);

(iii) harvesting the seed virus; and
producing infectious influenza virus by infecting eggs or mammalian cells with the seed virus;

(iv) harvesting the infectious influenza virus after multiplication in the eggs or mammalian cells;

(v) purifying the harvested infectious influenza virus;

(vi) optionally inactivating the purified virus; and

(vii) mixing the purified virus with a pharmaceutically acceptable carrier.

25. An influenza vaccine composition for use in inducing an immune response to one or more influenza polypeptides in a subject,

wherein the influenza vaccine composition comprises a purified infectious influenza virus that comprises an optimized nucleotide sequence encoding an engineered influenza structural protein that comprises at least one amino acid difference from an engineered influenza structural protein encoded by a non-optimized parental nucleotide sequence of the optimized nucleotide sequence;

wherein the engineered influenza structural protein encoded by the non-optimized parental nucleotide sequence shares at least 90% sequence identity with an influenza structural protein encoded by a second nucleotide sequence, which non-optimized parental nucleotide and second nucleotide sequences comprise one or more positions where codons are different from one another;

wherein the optimized nucleotide sequence comprises codons that match codons from the second nucleotide sequence at every position where the codons in the non-optimized parental nucleotide and second nucleotide sequences code for the same amino acid;

wherein the optimized nucleotide sequence comprises codons that match codons that are based on structural protein-specific influenza codon usage preferences at every position where the codons in the non-optimized parental nucleotide and second nucleotide sequences code for a different amino acid; and,

wherein translation of the optimized nucleotide sequence and expression of the encoded engineered influenza structural protein are improved for one or more expression systems relative to the non-optimized parental nucleotide sequence.