CLAIMS

1. A modified Flavivirus virus in which expression of viral proteins is reduced compared to a parent virus, in which the reduction in expression is the result of recoding the envelope (E) region, the nonstructural protein 3 (NS3) region, both the E and NS3 regions, or prM.

2. A modified Flavivirus virus according to Claim 1, in which one or both of the E protein-encoding sequence and the NS3 protein-encoding sequence are recoded by reducing the codon pair bias or codon usage bias of the protein-encoding sequence.

3. A modified Flavivirus virus according to Claim 2, in which reducing the codon-pair bias comprises identifying a codon pair in the parent protein-encoding sequence having a codon-pair score that can be reduced, and reducing the codon-pair bias by substituting the codon pair with a codon pair that has a lower codon-pair score.

4. A modified Flavivirus virus according to Claim 2, in which reducing the codon-pair bias comprises rearranging the codons of a parent protein-encoding sequence.

5. A modified Flavivirus virus according to Claim 1, in which one or both of the E protein-encoding sequence and the NS3 protein-encoding sequence are recoded by increasing the number of CpG or UpA di nucleotides compared to a parent virus.

6. A modified Flavivirus virus according to Claim 1, in which each of the recoded prM/E protein-encoding sequence and the recoded NS3 protein-encoding sequence have a codon pair bias less than, -0.05, −0.1, or less than −0.2, or less than −0.3, or less than −0.4.

7. A modified Flavivirus virus according to Claim 1, in which one or both of the E protein-encoding sequence and the NS3 protein-encoding sequence are recoded by replacing one or more codons with synonymous codons that are less frequent in the viral host.

8. A modified Flavivirus virus according to Claim 1, in which the parent virus is a Flavivirus selected from the group consisting of Zika virus, dengue fever virus, West Nile virus, yellow fever virus, Japanese encephalitis virus, Spondweni virus, Saint Louis encephalitis virus, and Powassan virus.

9. A modified Flavivirus virus according to Claim 1, in which the parent virus is a natural isolate.

10. A modified Flavivirus virus according to Claim 1, in which the parent virus is a mutant of a natural isolate.

11. A Flavivirus vaccine composition for inducing a protective immune response in a subject, which comprises a modified virus according to any preceding claim.

12. A modified virus according to any of Claims 1 to 10 for use in a method of eliciting a protective immune response in a subject, in which a prophylactically or therapeutically effective dose of a vaccine composition comprising the modified virus is to be administered to the subject.

13. A modified virus for use according to Claim 12, in which at least one adjuvant is to be further administered to the subject.

14. A modified virus for use according to Claim 12, in which the immune response is cross-protective against a heterologous Flavivirus virus.

15. A method of making a modified Flavivirus virus genome comprising:

obtaining the nucleotide sequence encoding the envelope protein of a Flavivirus virus and the nucleotide sequence encoding the nonstructural 3 proteins of a Flavivirus virus;

recoding the envelope encoding nucleotide sequence to reduce protein expression and recoding the nonstructural protein 3-encoding nucleotide sequence to reduce protein expression, and

substituting a nucleic acid having the recoded envelope-encoding nucleotide sequence and a nucleic acid having the recoded nonstructural protein 3-encoding nucleotide sequence into a parent Flavivirus virus genome to make a modified Flavivirus virus genome; whereby expression of the recoded envelope-encoding nucleotide sequence and expression of the recoded nonstructural protein 3-encoding nucleotide sequence is reduced compared to the parent virus.

16. A modified Flavivirus in which expression of viral proteins is reduced compared to a parent virus, wherein the reduction in expression is the result of recoding envelope (E) region, the nonstructural protein 3 (NS3) region, both the E and NS3 regions, or prM for use in a method of eliciting an immune response in a subject in need thereof, in which:

a prime dose of the modified Flavivirus, or an attenuated Flavivirus produced by a method other than codon-pair deoptimization, is to be administered to a subject in need thereof; and

one or more boost dose of the modified Flavivirus, or the attenuated Flavivirus by methods other than codon-pair deoptimization, is to be administered to the subject in need thereof;

in which at least the prime dose or the one or more boost dose is the modified virus.

17. A modified Flavivirus for use according to Claim 16, in which a first of the one or more boost dose is to be administered about 2 weeks after the prime dose.

18. A modified Flavivirus for use according to Claim 16 or 17, in which the Flavivirus is a Zika virus.

19. A modified Flavivirus for use according to Claim 16 or 17, in which the Flavivirus is selected from the group consisting of dengue fever virus, West Nile virus, yellow fever virus, Japanese encephalitis virus, Spondweni virus, Saint Louis encephalitis virus, and Powassan virus.

20. A modified Flavivirus for use according to any of Claims 16 to 19, in which one or both of the E protein-encoding sequence and the NS3 protein-encoding sequence are recoded by reducing the codon pair bias or codon usage bias of the protein-encoding sequence.

21. A modified Flavivirus for use according to any of Claims 16 to 19, in which one or both of the E protein-encoding sequence and the NS3 protein-encoding sequence are recoded by increasing the number of CpG or UpA di nucleotides compared to a parent virus.

22. A modified Flavivirus for use according to any of Claims 16 to 19, in which reducing the codon-pair bias comprises identifying a codon pair in the parent protein-encoding sequence having a codon-pair score that can be reduced, and reducing the codon-pair bias by substituting the codon pair with a codon pair that has a lower codon-pair score.

23. A modified Flavivirus for use according to any of Claims 16 to 19, in which reducing the codon-pair bias comprises rearranging the codons of a parent protein-encoding sequence.

24. A modified Flavivirus for use according to any of Claims 16 to 19, in which each of the recoded prM/E protein-encoding sequence and the recoded NS3 protein-encoding sequence

have a codon pair bias less than, -0.05, −0.1, or less than −0.2, or less than −0.3, or less than −0.4.

25. A modified Flavivirus for use according to any of Claims 16 to 19, in which one or both of the E protein-encoding sequence and the NS3 protein-encoding sequence are recoded by replacing one or more codons with synonymous codons that are less frequent in the viral host.

26. A modified Flavivirus for use according to any of Claims 1 to 10, further comprising recoding a NS5 region.

27. A modified Flavivirus according to any of Claims 1 to 10, in which the Flavivirus is a modified Zika virus in which expression of viral proteins is reduced compared to a parent virus, in which the reduction in expression is the result of recoding the envelope (E) region, the nonstructural protein 3 (NS3) region, both the E and NS3 regions, or prM.

28. A Zika vaccine composition for inducing a protective immune response in a subject, comprising a modified virus according to Claim 27.

29. A Zika vaccine composition according to Claim 28 for use in a method of eliciting a protective immune response in a subject, in which a prophylactically or therapeutically effective dose of the vaccine composition is to be administered to the subject.

30. A Zika vaccine composition for use according to Claim 29, in which at least one adjuvant is to be administered to the subject.

31. A Zika vaccine composition for use according to Claim 29, in which the immune response is cross-protective against a heterologous Zika virus.

32. A method of making a modified Flavivirus genome according to Claim 15 in which the Flavivirus is a Zika virus.

CLAIMS

1. A modified Zika virus in which expression of viral proteins is reduced compared to a parent virus, in which the reduction in expression is the result of recoding the envelope (E) region, the nonstructural protein 3 (NS3) region, both the E and NS3 regions, or prM, in which one or both of the E protein-encoding sequence and the NS3 protein-encoding sequence are recoded by reducing the codon pair bias, and in which the modified virus has a safety factor of at least 10^2, in which the safety factor is defined by the ratio of LD_{50}/PD_{50}.

2. A modified Zika virus according to Claim 1, in which reducing the codon-pair bias comprises identifying a codon pair in the parent protein-encoding sequence having a codon-pair score that can be reduced, and reducing the codon-pair bias by substituting the codon pair with a codon pair that has a lower codon-pair score.

3. A modified Zika virus according to Claim 1, in which reducing the codon-pair bias comprises rearranging the codons of a parent protein-encoding sequence.

4. A modified Zika virus according to Claim 1, in which one or both of the E protein-encoding sequence and the NS3 protein-encoding sequence are recoded by also increasing the number of CpG or UpA di nucleotides compared to a parent virus.

5. A modified Zika virus according to Claim 1, in which each of the recoded prM/E protein-encoding sequence and the recoded NS3 protein-encoding sequence have a codon pair bias less than, -0.05, −0.1, or less than −0.2, or less than −0.3, or less than −0.4.

6. A modified Zika virus according to Claim 1, in which the parent virus is a natural isolate.

7. A modified Zika virus according to Claim 1, in which the parent virus is a mutant of a natural isolate.

8. A Zika vaccine composition for inducing a protective immune response in a subject, which comprises a modified virus according to any preceding claim.

9. A modified Zika virus according to any of Claims 1 to 7 for use in a method of eliciting a protective immune response in a subject, in which a prophylactically or therapeutically

effective dose of a vaccine composition comprising the modified virus is to be administered to the subject.

10. A modified Zika virus for use according to Claim 9, in which at least one adjuvant is to be further administered to the subject.

11. A modified Zika virus for use according to Claim 9, in which the immune response is cross-protective against a heterologous Zika virus.

12. A method of making a modified Zika virus genome comprising:

obtaining the nucleotide sequence encoding the envelope protein of a Zika virus and the nucleotide sequence encoding the nonstructural 3 proteins of a Zika virus;

recoding the envelope (E) protein encoding nucleotide sequence to reduce protein expression and recoding the nonstructural protein 3-encoding nucleotide sequence to reduce protein expression, and

substituting a nucleic acid having the recoded E protein-encoding nucleotide sequence and a nucleic acid having the recoded nonstructural protein 3-encoding nucleotide sequence into a parent Zika virus genome to make a modified Zika virus genome; whereby expression of the recoded E protein-encoding nucleotide sequence and expression of the recoded nonstructural protein 3-encoding nucleotide sequence is reduced compared to the parent virus, in which one or both of the E protein-encoding sequence and the NS3 protein-encoding sequence are recoded by reducing the codon pair bias, and in which the modified Zika virus has a safety factor of at least 10^2, in which the safety factor is defined by the ratio of LD_{50}/PD_{50}.

13. A modified Zika virus in which expression of viral proteins is reduced compared to a parent virus, in which the reduction in expression is the result of recoding envelope (E) region, the nonstructural protein 3 (NS3) region, both the E and NS3 regions, or prM, in which one or both of the E protein-encoding sequence and the NS3 protein-encoding sequence are recoded by reducing the codon pair bias, and in which the modified virus has a safety factor of at least 10^2, in which the safety factor is defined by the ratio of LD_{50}/PD_{50}, for use in a method of eliciting an immune response in a subject in need thereof, in which:

a prime dose of the modified Zika virus, or an attenuated Zika produced by a method other than codon-pair deoptimization, is to be administered to a subject in need thereof; and

one or more boost dose of the modified Zika virus, or the attenuated Zika by methods other than codon-pair deoptimization, is to be administered to the subject in need thereof;

in which at least the prime dose or the one or more boost dose is the modified Zika virus.

14. A modified Zika virus for use according to Claim 13, in which a first of the one or more boost dose is to be administered about 2 weeks after the prime dose.

15. A modified Zika virus for use according to Claim 13 or 14, in which one or both of the E protein-encoding sequence and the NS3 protein-encoding sequence are recoded by increasing the number of CpG or UpA di nucleotides compared to a parent virus.

16. A modified Zika virus for use according to Claim 13 or 14, in which reducing the codon-pair bias comprises identifying a codon pair in the parent protein-encoding sequence having a codon-pair score that can be reduced, and reducing the codon-pair bias by substituting the codon pair with a codon pair that has a lower codon-pair score.

17. A modified Zika virus for use according to Claim 13 or 14, in which reducing the codon-pair bias comprises rearranging the codons of a parent protein-encoding sequence.

18. A modified Zika virus for use according to Claim 13 or 14, in which each of the recoded prM/E protein-encoding sequence and the recoded NS3 protein-encoding sequence have a codon pair bias less than, -0.05, −0.1, or less than −0.2, or less than −0.3, or less than −0.4.

19. A modified Zika virus according to any of Claims 1 to 7, in which a NS5 region is recoded.

20. A modified Zika virus according to any of Claims 1 to 7, in which the Zika is a modified Zika virus in which expression of viral proteins is reduced compared to a parent virus, in which the reduction in expression is the result of recoding the envelope (E) region, the nonstructural protein 3 (NS3) region, both the E and NS3 regions, or prM.

21. A Zika vaccine composition for inducing a protective immune response in a subject, comprising a modified virus according to Claim 20.

22. A Zika vaccine composition according to Claim 21 for use in a method of eliciting a protective immune response in a subject, in which a prophylactically or therapeutically effective dose of the vaccine composition is to be administered to the subject.

23. A Zika vaccine composition for use according to Claim 22, in which at least one adjuvant is to be administered to the subject.

24. A Zika vaccine composition for use according to Claim 22, in which the immune response is cross-protective against a heterologous Zika virus.