CLAIMS

1. A vector comprising a DNA encoding a RNA molecule operably linked to a promoter suitable for expression of the DNA in a eukaryotic cell, wherein the RNA molecule encodes an infectious positive single stranded ((+)SS) RNA virus, ~~wherein the (+)SS RNA virus is selected from the family Astroviridae, Caliciviridae, Picornaviridae, Coronoviridae, Retroviridae, Togaviridae, or Flaviviridae, wherein the DNA comprises three to six introns, and wherein at least one intron is in the region encoding a non-structural protein and one intron is in a region encoding a structural protein.~~

~~2. The vector of claim~~wherein the DNA comprises at least three introns, wherein at least one intron is in a region encoding the capsid, wherein at least one intron is in a region encoding the envelope, wherein at least one intron is in a region encoding non-structural protein 1, wherein the (+)SS RNA virus comprises a flavivirus, ~~alphavirus, picornavirus, rubivirus, coronavirus, Norwalk virus, Hepatitis A virus, Hepatitis C virus, severe acute respiratory (SAR) virus,~~ wherein the DNA encoding the RNA molecule comprises SEQ ID NO:1; the DNA encoding the RNA molecule comprises at least 90%, 95%, 96%, 97%, 98%, or ~~lentivirus,~~ 99% sequence identity with SEQ ID NO:1, and the introns in SEQ ID NO: 1 are intact; ~~optionally~~the DNA comprises at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with a DNA encoding nucleic acid sequence of JEV SA14-14-2 strain and the introns are inserted immediately after nucleotide 414, nucleotide 2213, and nucleotide 3134 of the DNA encoding the nucleic acid sequence of JEV SA14-14-2 strain, or the DNA encodes nucleic acid sequence of JEV SA14-14-2 strain and the introns are inserted immediately after nucleotide 414, nucleotide 2213, and nucleotide 3134 of the DNA encoding JEV SA14-14-2 strain; and wherein the vector can be used to generate full-length viral nucleic acid or a homogenous population of virus.

2. The vector of claim 1, wherein the flavivirus comprises a ~~Japanese encephalitis virus (JEV), Dengue virus, Yellow Fever virus, West Nile virus, tick borne encephalitis virus, Hepatitis C virus, or Zika virus; or~~ JEV.

~~wherein the flavivirus is JEV.~~

3. The vector of claim 1 or 2, wherein the (+)SS RNA virus is encoded by a chimeric RNA molecule comprising the RNA of at least two different (+)SS RNA viruses,

optionally wherein the at least two different (+)SS RNA viruses comprise JEV and at least one other virus, Yellow fever virus and at least one other virus, or Dengue virus and at least one other virus,

further optionally wherein the at least one other virus is a Zika virus, a West Nile virus, a Yellow fever virus, or a Dengue virus; or

wherein the at least two different (+)SS RNA viruses are JEV and Zika virus.

4. The vector of any one of claims 1-3,

wherein the intron contains a stop codon or several stop codons~~.~~,

further optionally wherein the DNA comprises three introns, at least four introns, at least five introns, or at least six introns.

5. The vector of any one of claims 1-4, wherein the infectious (+) SS RNA virus ~~is~~comprises a nonpathogenic virus,

optionally wherein the nonpathogenic virus ~~is~~comprises an attenuated virus.

6. The vector of any one of claims 1-5, wherein the promoter comprises a CMV promoter, a RSV promoter, a SV40 promoter, a HSV promoter, a human Pol I promoter, a human Pol II promoter, or a human Pol III promoter.

7. A composition comprising the vector of any one of claims 1-6 and a carrier.

8. A ~~homogeneous~~homogenous population of clonally purified (+) SS RNA virus obtained from eukaryotic cells transfected with the vector of any one of claims 1-6.

9. A composition comprising the homogeneous population of clonally purified (+) SS RNA virus of claim 8 and a carrier.

10. A pharmaceutical composition comprising the vector of any one of claims 1-6~~,~~ or the ~~homogeneous~~homogenous population of clonally purified (+)SS RNA virus of claim 8, and a pharmaceutically acceptable carrier.

11. A vaccine comprising a therapeutically effective amount of a vector of any one of claims 1-6, the pharmaceutical composition of claim 10, or a homogeneous clonally purified live (+)SS RNA virus population of claim 8.

~~12.~~12. The vaccine of claim 11 for use in protecting a subject against a disease caused by an infectious (+)SS RNA virus,
optionally wherein the subject is a mammal,
further optionally wherein the mammal is a human or a veterinary animal.

13. A method of preparing a homogeneous clonally purified live (+)SS RNA virus population, wherein the method comprises transfecting the vector of any one of claims~~-~~ 1-6 into a eukaryotic cell and isolating (+)SS RNA viruses, thereby obtaining ~~the~~a homogeneous clonally purified live (+)SS RNA virus population, optionally wherein the eukaryotic cell is a Vero cell, a CHO cell, or a MDCK cell.

~~optionally wherein the eukaryotic cell is a Vero cell, a CHO cell, or a MDCK cell.~~

~~13.~~

14. A method of preparing a vaccine for protecting a subject against a disease caused by an infectious (+) SS RNA virus, wherein the method comprises transfecting the vector of any one of claims 1-6 into a eukaryotic cell and isolating the (+) SS RNA viruses, thereby obtaining a vaccine, optionally wherein the eukaryotic cell is a Vero cell, a CHO cell, or a MDCK cell.

~~optionally wherein the eukaryotic cell is a Vero cell, a CHO cell, or a MDCK cell.~~

~~14. The vaccine of claim 11 for use in protecting a subject against a disease caused by an infectious (+)SS RNA virus,~~

~~optionally wherein the subject is a mammal,~~

~~further optionally wherein the mammal is a human or a veterinary animal~~

15. A method of preparing a stable plasmid, wherein the method comprises ~~-~~:

obtaining a DNA encoding a genomic RNA of ~~an infectious (+)SS RNA virus; and~~ a flavivirus;

introducing at least three ~~to six~~ introns into the DNA to obtain a stable plasmid, wherein at least one intron is in ~~the~~a region encoding ~~a structural protein of the (+)SS RNA virus and~~ the capsid,

wherein at least one intron is in a region encoding the envelope,

wherein at least one intron is in ~~the~~a region encoding ~~a~~ non-structural protein ~~of the (+) SS RNA virus;~~1,

optionally wherein the intron contains a stop codon or several stop codons~~-~~,

further optionally wherein the DNA comprises three introns, at least four introns, at least five introns, or at least six introns,

wherein

the DNA encoding the RNA comprises SEQ ID NO:1;

the DNA encoding the RNA molecule comprises at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO:1, and the introns in SEQ ID NO:1 are intact;

the DNA comprises at least 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with a DNA encoding nucleic acid sequence of JEV SA14-14-2 strain and the introns are inserted immediately after nucleotide 414, nucleotide 2213, and nucleotide 3134 of the DNA encoding the nucleic acid sequence of JEV SA14-14-2 strain, or

the DNA encodes nucleic acid sequence of JEV SA14-14-2 strain and the introns are inserted immediately after nucleotide 414, nucleotide 2213, and nucleotide 3134 of the DNA encoding the nucleic acid sequence of JEV SA14-14-2 strain; and

wherein the vector can be used to generate full-length viral nucleic acid or a homogenous population of virus.

16. A method of preparing a vector, wherein the vector is in accordance with any one of claims 1-6, and wherein the method comprises transfecting the vector into a host cell and isolating the vector from the host cell.

17. An isolated cell transfected with a vector of any one of claims 1-6.