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 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, or lentivirus,

optionally 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

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.

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

optionally wherein the nonpathogenic virus is 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 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 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. 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 homogeneous clonally purified live (+)SS RNA virus population,

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

13. 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.

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

introducing three to six introns into the DNA, wherein at least one intron is in the region encoding a structural protein of the (+)SS RNA virus and at least one intron is in the region encoding a non-structural protein of the (+) SS RNA virus;

optionally wherein the intron contains a stop codon or several stop codons.

16. A method of preparing a vector, wherein the vector is in accordance with any one of claims 1-6, 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.