POLYNUCLEOTIDES FOR MODIFYING ORGANISMS

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CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This international patent application claims benefit of U.S. provisional patent application No. 63/379,056, filed October 11, 2022 and incorporated herein by reference in its entirety, and of U.S. provisional patent application No. 63/266,967, filed January 20, 2022 and incorporated herein by reference in its entirety.

INCORPORATION OF SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML file format and is hereby incorporated by reference in its entirety. The XML file, created on January 13, 2023, is named P13757WO00.xml and is 727,335 bytes in size. The sequence listing named P13757US01.xml which is 603,608 bytes in size for U.S. provisional patent application No. 63/379,056, filed October 11, 2022, is incorporated herein by reference in its entirety. The sequence listing named VL70006_ST25 which is 441,582 bytes in size for U.S. provisional patent application No. 63/266,967, filed January 20, 2022, is incorporated herein by reference in its entirety.

BACKGROUND

[0003] There is need in the art for modifying polynucleotides for improving phenotypes and genotypes of organisms; in particular, for agricultural applications to improve plants such as crop plants.

[0004] Partitiviruses (PV) are dsRNA viruses having a bipartite genome which is separately encapsidated. One (PV) genome comprises a dsRNA encoding an RNA-dependent RNA polymerase and the other PV genome comprises a dsRNA encoding a capsid protein (Roossinck, M. doi/10.1111/nph.15364).

SUMMARY

[0004a] In a first aspect, the present disclosure provides a method of manufacturing a synthetic partitiviral satellite particle, comprising combining a recombinant single-stranded RNA (ssRNA) molecule with a viral capsid protein, wherein the recombinant ssRNA molecule comprises from 5’ terminus to 3’ terminus:

(a) a 5’ RNA replication element recognized by a partitiviral RNA-dependent RNA polymerase (RDRP), wherein the 5’ RNA replication element comprises at least a segment of the 5’ untranslated

region (UTR) of the partitiviral genome or a variant thereof having one or more nucleotide substitutions, insertions, and/or deletions;

(b) a cargo RNA molecule;

(c) a 3’ RNA replication element recognized by the RDRP, wherein the 3’ RNA replication element comprises at least a segment of the 3’ untranslated region (UTR) of the partitiviral genome or a variant thereof having one or more nucleotide substitutions, insertions, and/or deletions;

wherein the 5’ RNA replication element, the cargo RNA molecule, and the 3’ RNA replication element are operably linked and wherein the cargo RNA molecule is heterologous to the 5’ RNA replication element and the 3’ RNA replication element; and an encapsidation recognition element (ERE), and wherein the ERE provides for encapsidation of the ssRNA by the viral capsid protein.

[0005] Recombinant DNA molecules comprising a first promoter which is operably linked to DNA encoding an RNA molecule, wherein the RNA molecule comprises from 5’ terminus to 3’ terminus: (a) a 5’ RNA replication element recognized by a partitiviral RNA-dependent RNA polymerase (RDRP); (b) a cargo RNA molecule; and (c) a 3’ RNA replication element recognized by the RDRP; wherein the 5’ RNA replication element, the cargo RNA molecule, and the 3’ RNA replication element are operably linked, wherein the promoter and the cargo RNA molecule are heterologous to the 5’ RNA replication element and the 3’ RNA replication element are provided. Cells, vectors, and agricultural formulations