

SUMMARY

In one aspect, the present disclosure refers to a nucleic acid construct which comprises a cDNA molecule encoding a full length antigenomic (+) RNA strand of a non-segmented negative-sense single-stranded RNA virus, especially of a measles virus (MV), wherein the cDNA molecule further comprises inserted therein, at least a first additional transcription unit (ATU) and a second additional transcription unit (ATU), wherein the at least first and second additional transcription units (ATUs) are localized within a single intergenic region of the cDNA encoding the antigenomic (+) RNA strand as a single expression cassette, and wherein each ATU comprises a heterologous polynucleotide operably inserted within the ATU allowing the expression of a heterologous polypeptide encoded by the heterologous polynucleotide.

In another aspect, the present disclosure refers to a recombinant bacterial artificial chromosome (BAC) plasmid wherein the nucleic acid construct as disclosed herein is operably cloned.

In yet another aspect, the present disclosure refers to a recombinant infectious non-segmented negative-sense single-stranded RNA virus, especially a recombinant infectious Measles virus, wherein the genome of said recombinant infectious virus comprises or consists in a nucleic acid construct as disclosed herein.

In a further aspect, the present disclosure refers to A process for rescuing recombinant infectious non-segmented negative-sense single-stranded RNA virus, especially recombinant infectious measles virus expressing at least one heterologous polypeptide expressed from a heterologous nucleotide sequence inserted within an ATU localized within a cDNA molecule encoding a full length antigenomic (+) RNA strand of a measles virus (MV); comprising: (a) In cells, in particular HEK293 helper cells, stably expressing T7 RNA polymerase and measles virus N and P proteins and/or transfected with an expression vector encoding T7 RNA polymerase and/or transfected with an expression vector encoding measles virus N and P proteins, transfecting the nucleic acid construct as disclosed herein; (b) Maintaining the transfected cells of step (a) in conditions suitable for the production of recombinant infectious virus and/or virus like particles; (c) Infecting cells, in particular Vero cells, in conditions enabling the propagation of the recombinant infectious virus by co-cultivating these cells with the cells issued from

step (b); in particular at 32°C; (d) Harvesting the recombinant infectious virus and/or virus like particles, in particular at 32°C.

In yet another aspect, the present disclosure refers to a seed preparation or a stock preparation of a recombinant infectious non-segmented negative-sense single-stranded RNA virus, especially of a recombinant infectious measles virus that consists in a single virus clone or respectively an amplified single virus clone of a recombinant infectious virus rescued by reverse genetics from the nucleic acid as disclosed herein or from the recombinant BAC plasmid as disclosed herein.

In one aspect, the present disclosure refers to a vaccine which comprises a clonally selected and amplified recombinant infectious negative-sense single-stranded RNA virus, especially a clonally selected and amplified recombinant infectious measles virus obtainable from the rescue by reverse genetics of the nucleic acid construct as disclosed herein or from the recombinant BAC plasmid as disclosed herein.

In another aspect, the present disclosure refers to a combination vaccine which comprises a recombinant measles virus which encodes at least two, in particular three or more than three immunogenic polypeptides originating from or derived from distinct pathogens infecting human, in particular from at least two viruses selected from the group of Chikungunya virus, West Nile virus and Zika virus, wherein the immunogenic polypeptides are expressed as individual polypeptides by the recombinant virus.

In yet another aspect, the present disclosure refers to ribonucleoprotein of a recombinant measles virus strain which comprises a RNA genome assembled with the transcriptase complex proteins of the measles virus wherein the genome contains the recombinant full length antigenomic (+) RNA strand of the measles virus (MV) and inserted therein, sequences encoding at least a first additional transcription unit (ATU) and a second additional transcription unit (ATU), wherein the at least first and second additional transcription units (ATUs) are localized within a single intergenic region of the recombinant antigenomic (+) RNA strand as a single expression cassette, and wherein each ATU comprises a heterologous polynucleotide operably inserted within the ATU allowing the expression of a heterologous polypeptide encoded by the heterologous polynucleotide as disclosed herein.