Claims

[Claim 1]

An alphavirus replicon particle (ARP), which comprises

(1-i) Venezuelan Equine Encephalitis Virus (VEEV) structural proteins comprising capsid and envelope, and

(1-ii) a VEEV replicon comprising a polynucleotide encoding VEEV non-structural proteins nsp1, nsp2, nsp3 and nsp4 and at least one gene of interest,

wherein the VEEV capsid protein comprises one or more alteration(s) in the Nuclear Localization Signal (NLS), or wherein the VEEV structural proteins comprise one or alteration(s) at the furin site in VEEV E3 protein, or,

(2-i) Chikungunya Virus (CHIKV) structural proteins comprising capsid and/or envelope, and

(2-ii) a VEEV replicon comprising a polynucleotide encoding VEEV non-structural proteins nsp1, nsp2, nsp3 and nsp4 and at least one gene of interest.

[Claim 2]

The ARP of claim 1, wherein the ARP comprises:

(1-i) VEEV structural proteins comprising capsid and envelope, and

(1-ii) a VEEV replicon comprising a polynucleotide encoding VEEV non-structural proteins nsp1, nsp2, nsp3 and nsp4 and at least one gene of interest.

[Claim 3]

The ARP of claim 1 or claim 2, wherein one or more of lysine or arginine in the VEEV capsid protein Nuclear Localization Signal is substituted with asparagine or alanine.

[Claim 4]

The ARP of claim 1, wherein the ARP comprises:

(2-i) CHIKV structural proteins comprising capsid and/or envelope, and

(2-ii) a VEEV replicon comprising a polynucleotide encoding VEEV non-structural proteins nsp1, nsp2, nsp3 and nsp4 and at least one gene of interest.

[Claim 5]
The ARP of any one of claims 1-4, wherein the CHIKV structural proteins comprise one or more alterations in the CHIKV E2 protein.

[Claim 6]
The ARP of any one of claim 1-5, wherein the CHIKV structural proteins comprise one or more alterations at the furin site in the CHIKV E3 protein.

[Claim 7]
The ARP of any one of claims 1-6, wherein the CHIKV is CHIKV strain 37997 or strain OPY-1.

[Claim 8]
The ARP of any one of claims 1-7, wherein the VEEV is VEEV strain TC-83.

[Claim 9]
The ARP of any of claims 1-8, wherein the gene of interest encodes an antigen.

[Claim 10]
A method for preparing alphavirus replicon particles, comprising the steps of co-transfecting cells with:
(1-i) a vector comprising a polynucleotide encoding VEEV non-structural protein nsp1, nsp2, nsp3 and nsp4, and at least one gene of interest,
(1-ii) a vector comprising a polynucleotide encoding a VEEV capsid protein, and
(1-iii) a vector comprising a polynucleotide encoding a VEEV E3-E2-6K-E1,
wherein the capsid protein comprises one or more amino acid alteration(s) in the NLS, or
(2-i) a vector comprising a polynucleotide encoding VEEV non-structural protein nsp1, nsp2, nsp3 and nsp4, and at least one gene of interest,
(2-ii) a vector comprising a polynucleotide encoding a CHIKV capsid protein, and
(2-ii) a vector comprising a polynucleotide encoding a CHIKV E3-E2-6K-E1,
culturing the transfected cells, and
purifying the ARPs from the cell culture.

[Claim 11]
The method of claim 10, wherein the cells are co-transfected with:
(1-i) a vector comprising a polynucleotide encoding VEEV non-structural protein nsp1, nsp2, nsp3 and nsp4, and at least one gene of interest,
(1-ii) a vector comprising a polynucleotide encoding a VEEV capsid protein, wherein the capsid protein comprises one or more amino acid alteration(s) in the NLS, and
(1-iii) a vector comprising a polynucleotide encoding a VEEV E3-E2-6K-E1.

[Claim 12]
The method of claim 10 or claim 11, wherein one or more of lysine or arginine in the VEEV capsid protein Nuclear Localization Signal is substituted with asparagine or alanine.

[Claim 13]
The method of claim 10, wherein the cells are co-transfected with:
(2-i) a vector comprising a polynucleotide encoding VEEV non-structural protein nsp1, nsp2, nsp3 and nsp4, and at least one gene of interest,
(2-ii) a vector comprising a polynucleotide encoding a CHIKV capsid protein, and
(2-ii) a vector comprising a polynucleotide encoding a CHIKV E3-E2-6K-E1.

[Claim 14]
The method of any one of claims 10-13, wherein the CHIKV structural proteins comprise one or more alternation(s) in the CHIKV E2 protein.

[Claim 15]
The method of any one of claims 10-14, wherein the CHIKV structural proteins comprise one or more alteration(s) at the furin site in the CHIKV E3 protein.

[Claim 16]
The method of any one of claims 10-15, wherein the CHIKV is CHIKV strain 37997 or strain OPY-1.

[Claim 17]
The method of any one of claims 10-16, wherein the VEEV is VEEV strain TC-83.

[Claim 18]

The method of any of claims 10-17, wherein the gene of interest encodes an antigen.