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 one or more of lysine or arginine in the VEEV capsid protein Nuclear Localization Signal (NLS) is substituted with asparagine or alanine, or

wherein the VEEV structural proteins comprise an alteration/mutation to the amino acid sequence at the furin site (Arg-X-X-Arg) 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, 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 4]

The ARP of any one of claims 1-3, wherein the CHIKV structural proteins comprise one or more alterations in the CHIKV E2 protein.

[Claim 5]
The ARP of any one of claims 1-4, wherein the CHIKV structural proteins comprise one or more alterations at the furin site in the CHIKV E3 protein.

[Claim 6]
The ARP of any one of claims 1-5, wherein the CHIKV is CHIKV strain 37997 or strain OPY-1.

[Claim 7]
The ARP of any one of claims 1-6, wherein the VEEV is VEEV strain TC-83.

[Claim 8]
The ARP of any one of claims 1-7, wherein the gene of interest encodes an antigen.

[Claim 9]
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 one or more of lysine or arginine in the VEEV capsid protein NLS is substituted with asparagine or alanine, or
wherein the VEEV E3-E2-6K-E1 comprises an alteration/mutation to the amino acid sequence at the furin site (Arg-X-X-Arg) in VEEV E3 protein, 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-iii) 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 10]

The method of claim 9, 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, and

(1-iii) a vector comprising a polynucleotide encoding a VEEV E3-E2-6K-E1.

[Claim 11]

The method of claim 9, 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-iii) a vector comprising a polynucleotide encoding a CHIKV E3-E2-6K-E1.

[Claim 12]

The method of any one of claims 9-11, wherein the CHIKV structural proteins comprise one or more alternation(s) in the CHIKV E2 protein.

[Claim 13]

The method of any one of claims 9-12, wherein the CHIKV structural proteins comprise one or more alteration(s) at the furin site in the CHIKV E3 protein.

[Claim 14]

The method of any one of claims 9-13, wherein the CHIKV is CHIKV strain 37997 or strain OPY-1.

[Claim 15]

The method of any one of claims 9-14, wherein the VEEV is VEEV strain TC-83.

[Claim 16]

The method of any one of claims 9-15, wherein the gene of interest encodes an antigen.