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VIRUS PURIFICATION

FIELD OF THE INVENTION

The disclosure relates to methods for the purification of viruses for use in vaccines.

BACKGROUND OF THE INVENTION

Regulatory agencies such as the World Health Organization establish standards and guidelines for the production of pharmaceutical compositions administered to humans, such as vaccines, that limit quantity and components of the compositions. Meeting these standards is particularly challenging with regard to production of vaccines containing biological agents, such as viruses, which must be propagated on cell substrates. Such vaccine preparations must be sterile (*i.e.*, free from independently replicating organisms) and may contain no more than 10 ng of host cell DNA per human dose, among other requirements. These standards are in place in order to ensure safety of the composition for human administration, but may introduce challenges in the development of processes used to produce such compositions.

Protamine was originally isolated from the sperm of salmon and other species of fish but is now produced primarily through recombinant biotechnology. It is a highly cationic peptide that binds to negatively charged molecules such as nucleic acids to form a stable ion pair. Its use in removing host cell nucleic acid is well document.

SUMMARY

During the course of routine virus purification, it was observed that addition of protamine sulfate to a virus harvest produced on a cell substrate removed not only contaminating DNA derived from host cells, as expected, but surprisingly also virtually eliminated immature and otherwise non-infectious virus particles from the preparation. This finding provides a streamlined, gentle, reproducible and broadly-applicable process for obtaining highly-purified infectious virus particles for applications such as vaccine preparation; furthermore, the process is not dependent on the charge of the virus particle.

Disclosed herein are downstream processes for purifying virus particles from a crude preparation. The downstream process can be applied to either a virus which has not adapted for propagation on a particular cell substrate or for a partial / fully cell substrate adapted virus particle.

Aspects of the invention provide processes for the purification of infectious virus particles comprising the steps of (a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate; (b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising a protamine salt, preferably a protamine sulphate, to obtain a virus preparation (b); and further purifying the virus preparation (b) by method or methods selecting for size of the virus particles, such as e.g. a sucrose density gradient centrifugation to obtain a virus preparation (c) comprising the infectious virus particles.

In some embodiments, the concentration of protamine sulphate in step (b) is about 1 to 10 mg/ml, more preferably about 1 to 5 mg/ml, more preferably about 1 to 2 mg/ml. In one embodiment, the concentration of protamine sulphate in step (b) is about 2 mg/mL. In one embodiment, the concentration of protamine sulphate is 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml. In a preferred embodiment, the concentration of protamine sulphate in step (b) is about 1.6 mg/ml or about 2 mg/ml.

In some embodiments, the residual host cell DNA of the virus preparation (c) is less than 1 µg/mL, especially less than 900, 800, 700, 600, 500, 400, 300 or 200 ng/mL, preferably less than 100 ng/mL. In a preferred embodiment, the residual host cell DNA of the virus preparation (c) is less than 10 ng/mL. In some embodiments, the residual host cell protein of the final virus preparation (c) is less than 10 µg/mL, especially less than 9, 8, 7, 6, 5, 4, 3 or 2 µg/mL, preferably less than 1 µg/mL. In a preferred embodiment, the residual host cell protein of the virus preparation (c) is less than 100 ng/mL. In some embodiments, the residual non-infectious virus particles of the final virus preparation (c) is less than 10 µg/mL, especially less than 9, 8, 7, 6, 5, 4, 3 or 2 µg/mL, preferably less than 1 µg/mL. In a preferred embodiment, the residual non-infectious virus particles of the virus preparation (c) is less than 100 ng/mL.

In some embodiments, the residual protamine is less than 1 µg/mL, especially less than 900, 800, 700, 600, 500, 400, 300 or 200 ng/mL, preferably less than 100 ng/mL, more preferably is below the detection limit of HPLC, in particular below the detection limit in the final drug substance. In some embodiments, the PS content is tested by HPLC or size exclusion chromatography (SEC). For example, HPLC is

validated for PS determination in JEV sucrose gradient pool samples as a routine release assay and is very sensitive (i.e., LOQ 3 µg/mL; LOD 1 µg/mL). In the current invention, PS content in virus DS samples was <LOD. In one embodiment, the HPLC assessment of PS content can be performed on a Superdex Peptide 10/300GL column (GE: 17-5176-01) using 30% Acetonitrile, 0,1% Trifluoroacetic acid as solvent with a flow rate of 0,6 ml/min at 25°C and detection at 214 nm. A more sensitive method of measurement for residual protamine in a purified virus preparation is mass spectrometry (MS). In some embodiments, the residual PS levels in a virus preparation are tested by MS or other such highly sensitive method, e.g., nuclear magnetic resonance (NMR). With this method, residual PS, as well as fragments and/or breakdown products of PS, can be detected at trace amounts, such as levels as low as, for example, 10^6 , 10^7 or 10^8 molecules per typical sample load. In some embodiments, the PS levels are tested in the sucrose gradient pool. In some embodiments, the PS levels are tested in the drug product. In some embodiments, the PS levels are tested in the drug substance.

In some embodiments, the crude harvest (a) comprising the virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b). In some embodiments, the one or more pre-purification step(s) comprises digesting host cell genomic DNA in the crude harvest (a) comprising the virus particles and impurities by enzymatic treatment. In some embodiments, the one or more pre-purification step(s) comprises filtration, ultrafiltration, concentration, buffer exchange and/or diafiltration. In some embodiments, the one or more pre-purification steps is filtration using a filter having a pore size equal to or less than 1 µm. In some embodiments, the filter has a pore size equal to or less than 0.2 µm. In a preferred embodiment, the filter has a pore size of 0.2 µm. In some embodiments, the concentration and/or ultra/diafiltration and/or buffer exchange is performed by tangential flow filtration (TFF). In some embodiments, ultra/diafiltration of the crude harvest (a) comprising the virus particles and impurities is performed using a hollow fiber membrane having a cut-off of equal to or less than 300 kDa. In a preferred embodiment, the hollow fiber membrane has a cut-off of 100 kDa.

In some embodiments, the virus particle is a live virus, a chimeric virus, an attenuated live virus, a modified live virus, or a recombinant live virus. In a further step, the virus particles of the invention may be optionally inactivated. In some embodiments, the virus particle is an attenuated form of the virus particle. For example, the virus may have reduced infectivity, virulence, and/or replication in a host, as compared to a wild-type virus. In some embodiments, the virus is a mutated or modified virus, for example the nucleic acid of the virus may contain at least one mutation relative to the wild-type virus. In some embodiments, the virus is a recombinant live virus, meaning a virus that is generated recombinantly and may contain nucleic acid from different sources.

In some embodiments, the virus particle is a live virus, an attenuated live virus, a modified live virus, or a recombinant live virus. In some embodiments, the virus belongs to a virus family selected from the group consisting of Paramyxoviridae, Orthomyxoviridae, Flaviviridae, Filoviridae, Arenaviridae, Rhabdoviridae, and Coronaviridae. In some embodiments, the virus belongs to a virus family selected from the group consisting of Togaviridae (being live or inactivated), such as alphaviruses, or Flaviviridae (being live or inactivated). In some embodiments, the virus is a virus of the family Flaviviridae, i.e. a flavivirus. In other embodiments, the virus is a Zika virus or Yellow Fever virus. In preferred embodiments, the virus is a Zika virus. In a most preferred embodiment, the Zika virus is a Zika virus from the Asian lineage.

In some embodiments, the relative reduction of impurity of the final virus preparation relative to the liquid medium (a) comprising the virus particles and impurities is in a range from 60 to 95%. In some embodiments, the residual impurity of the final virus preparation is less than 1%. We observed a decrease in the HCP peaks and the non-infectious aggregate peaks in the HPLC-SEC or SDS-PAGE. An exact quantification is difficult but one can measure the density of the SDS-PAGE bands and other methods.

In some embodiments, the filtration of step in (b)(ii) of claim 1 is performed using a filter having a pore size equal to or greater than 1 μm . In some embodiments, the filter has a pore size equal to or greater than 0.2 μm . In a preferred embodiment, the filter has a pore size of 0.2 μm .

In some embodiments, the virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero- α His cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line. In some embodiments, said cell line is a duck cell line. In some embodiments, said cell line is a diploid avian cell line. In some embodiments, said cell line is EB66 cell line. In a preferred embodiment, said cell line is a Vero cell line.

Aspects of the invention provide a use of any of the processes described herein for manufacturing a composition for immunization against a viral infection. In a preferred embodiment, the composition is a vaccine. In one embodiment, the composition or vaccine is directed against Chikungunya virus. In one embodiment, the composition or vaccine is directed against a flavivirus. In one embodiment, the

composition or vaccine is directed against Yellow Fever virus. In one embodiment, the composition or vaccine is directed against Zika virus such as e.g. a Zika virus of the Asian lineage.

Other aspects provide compositions comprising the virus particles obtainable by any of the processes described herein for treating and/or preventing a viral infection. In one embodiment, the viral infection is caused by Chikungunya virus. In one embodiment, the viral infection is caused by a flavivirus. In one embodiment, the viral infection is caused by Yellow Fever virus. In one embodiment, the viral infection is caused by Zika virus such as e.g. a Zika virus of the Asian lineage.

In some embodiments, the attenuated form of ChikV is derived from the LR2006-OPY1 ChikV infectious clone (La Reunion isolate). In some embodiments, the attenuated form of ChikV is the Δ 5nsP3 mutant as described by Hallengård et al. (Novel Attenuated Chikungunya Vaccine Candidates Elicit Protective Immunity in C57BL/6 mice (2014) Journal of Virology 88(5):2858–2866) or an immunogenic variant thereof. The immunogenic variant of the Δ 5nsP3 ChikV mutant is herein defined as having at least 80% sequence identity to the nucleotide sequence of the Δ 5nsP3 mutant sequence as provided by SEQ ID NO: 77, especially at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99% sequence identity to SEQ ID NO: 77.

In some embodiments, the Zika virus is derived from the Asian lineage. In some embodiments, the Zika virus is a Zika virus as described partially or fully in Sequence section of this application, i.e. any of sequences SEQ ID Nos 2 to 69 or 78, in particular all partly or fully described Zika viruses of the Asian lineages or an immunogenic variant thereof. The immunogenic variants of the Zika virus or Zika virus of the Asian lineages are herein defined as having at least 80% sequence identity to the nucleotide sequence of the sequences described in any of sequences SEQ ID Nos 2 to 69 or 78, especially at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least 99% sequence identity.

In some embodiments, the process of the invention results in an enrichment of infectious virus particles from the crude harvest comprising infectious virus particles and non-infectious virus particles and other virus products such that the enrichment of the infectious virus particles is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, preferably at least 80%, especially 85% relative to the total virus particle content of the crude harvest (a) comprising the virus particles and impurities.

In some embodiments, the residual impurity of the final virus preparation with respect to all impurities in the crude harvest is less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, preferably less than 5% as determined by SEC-HPLC (Size Exclusion Chromatography – HPLC).

In some embodiments, the filtration step of the virus preparation (b) after contact with the solid-phase matrix is performed using a filter having a pore size equal to or greater than 1 μm . In some embodiments, the filter has a pore size equal to or greater than 0.2 μm . In a preferred embodiment, the filter has a pore size of about 0.2 μm , such as 0.22 μm .

In some embodiments, the Zika virus, or Chikungunya virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero- α His cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line. In some embodiments, said cell line is a duck cell line. In some embodiments, said cell line is a diploid avian cell line. In some embodiments, said cell line is EB66 cell line. In a preferred embodiment, said cell line is a Vero cell line.

Aspects of the invention provide a use of any of the processes described herein for manufacturing a composition for immunization against a Zika virus, Yellow Fever, or Chikungunya virus infection. In a preferred embodiment, the composition is a vaccine. In preferred embodiments, the vaccine is administered to the subject once, twice or three or more times. In a preferred embodiment, the vaccine is administered once or twice. In a preferred embodiment, the vaccine is administered only once.

The herein disclosed in vivo data regarding immunogenicity of the inactivated Zika virus vaccine of the current invention indicates that the virus is surprisingly potently immunogenic and also highly cross-protective (very similar immunogenicity in African and Asian strains). Data indicate that immunogenicity was unexpectedly higher than the recently reported inactivated Zika virus vaccine candidate (Larocca, et. al, 2016, Nature doi:10.1038/nature18952.). Inactivated viruses are among the safest vaccines and especially preferred for delivery to populations where safety is especially concerning, such as pregnant women, children and immunocompromised individuals, which makes the herein disclosed inactivated Zika virus particularly suitable. Obtaining a high titer of inactivated virus is a challenge in the field. The herein disclosed process for purifying inactivated Zika virus results in not only a high yield, but also a very pure drug substance.

Other aspects provide compositions comprising the virus particles obtainable by any of the processes described herein for treating and/or preventing a Chikungunya virus infection.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are not intended to be drawn to scale. The figures are illustrative only and are not required for enablement of the disclosure. For purposes of clarity, not every component may be labeled in every drawing, alignments were performed with the multi alignment package Jalview (Waterhouse et al., 2009, Bioinformatics 25 (9) 1189-1191). In the drawings:

Figure 1: Average distance tree (by % identity, nt), complete genomes.

Figure 2: Neighbor joining tree (by % identity, nt), complete genomes.

Figure 3: Pairwise alignment-Jalview (% identity, nt), complete genomes.

Figure 4: Average distance tree (by % identity, aa), E-protein.

Figure 5: Neighbor joining tree (by % identity, aa), E-protein.

Figure 6: Pairwise alignment-Jalview (% identity, aa), E-protein.

Figure 7: Alignment (shading: % identity, aa), E-protein.

Figure 8: An example of virus particle maturation in the host cell. As observed in flaviviruses, full maturation of the particles requires proteolytic cleavage of the precursor membrane glycoprotein (prM) by the host protease furin. Not all prM molecules are cleaved, resulting in the release of mature, mosaic or immature-like conformations from the cells. Mosaic and immature forms are generally not infectious—only mature virions are infective and have hemagglutinin (HA) / TCID50 activity. (Figure adapted from Plevka, et al., Maturation of flaviviruses starts from one or more icosahedrally independent nucleation centres, EMBO reports (2011) 12, 602–606).

Figure 9: CHIKV schematic genome, including non-structural and structural proteins (labeled “CHIKV”) as well as a schematic representation of the Δ nsP3 attenuated Chikungunya virus used to exemplify the

purification process of the current invention (labeled “ $\Delta 5\text{nsP3}$ ”). The black triangle indicates the approximate location of the deletion in the nsP3 coding region. (Figure adapted from Hallengård et al. 2014, *supra*.)

Figure 10: Flow-chart showing an exemplary downstream $\Delta 5\text{nsP3}$ CHIK virus purification process from the crude harvest to formulation of the (vaccine) drug substance, a preferred embodiment of the process of the invention.

Figure 11: Absorbance at 214 nm, 260 nm and 280 nm of individual sucrose gradient centrifugation (SGC) fractions of a representative purification run of the process of the invention (A); the SEC-HPLC analysis of the final pooled fractions containing purified infectious attenuated $\Delta 5\text{nsP3}$ ChikV virus particles (B); and a silver-stained SDS-PAGE gel showing the protein content of the virus preparation following different steps of the process of the invention (defined in the table below the figure) (C). The SGC purified pool consisting of SGC fractions F7-F11 is shown in lane 12.

Figure 12: SEC area (mAU*min; right axis) and TCID_{50} results (log $\text{TCID}_{50}/\text{mL}$; left axis) of attenuated $\Delta 5\text{nsP3}$ ChikV production harvests before and after PS treatment. The grey portions of the bars indicate large losses in SEC area following PS treatment, but no corresponding change in the total number of infectious particles (indicated by black portions of the bars) (A); SEC profile of virus preparation before and after PS addition, showing a complete removal of large size virus aggregates by PS treatment as well as a reduction in host cell proteins (HCP) and LMW impurities (B).

Figure 13: Electron micrographs of attenuated $\Delta 5\text{nsP3}$ ChikV harvest before and after PS treatment.

Figure 14: Preparation of the sucrose gradient.

Figure 15: Comparison of four different sucrose gradient centrifugation experiments performed to empirically determine the optimal combination of sucrose layers for CHIKV purification. The CHIKV content in the gradient fractions was determined by SEC. The sucrose content in the gradient fractions was determined by refractometry (comparing the value of the refractive index of the sucrose solution to that of sucrose standard curve the concentration of sucrose solution can be determined with good accuracy, this is also referred to as "Brix" scale that is calibrated to give the percentage (w/w) of sucrose dissolved in water, i.e. “°Bx”). Protamine sulphate (PS) was determined by SEC. PS is separated within the sucrose gradient alongside host cell derived residual contaminants and was therefore used to assess

the quality of CHIKV separation from residual contaminants in the tested gradients. A: CHIKV load material containing 10 % sucrose was loaded on top of one 50 % (w/w) sucrose layer. Determination of sucrose content in the fractions showed the formation of a linear gradient. SEC showed concentration of CHIKV within a sucrose concentration range from 40 -30 % (w/w) sucrose. PS SEC showed insufficient separation of PS from CHIKV. B: CHIKV load material containing 10 % sucrose was loaded on top of a two layer system consisting of a 50 % (w/w) sucrose bottom layer and a second 35 % (w/w) sucrose layer. Determination of sucrose content in the fractions showed the formation of a linear gradient. SEC showed concentration of CHIKV within a sucrose concentration range from 40 -30 % (w/w) sucrose. PS SEC showed acceptable separation of PS from CHIKV, however a slight overlap is still present. C: CHIKV load material containing 10 % sucrose was loaded on top of a two layer system consisting of a 50 % (w/w) sucrose bottom layer and a second 25 % (w/w) sucrose layer. Determination of sucrose content in the fractions showed the formation of a linear gradient. SEC showed concentration of CHIKV within a sucrose concentration range from 40 -30 % (w/w) sucrose. PS SEC showed a good separation of PS from CHIKV. D: CHIKV load material containing 10 % sucrose was loaded on top of a three layer system consisting of a 50 % (w/w) sucrose bottom layer as well as a 35 % and a 15 % (w/w) sucrose layer. Determination of sucrose content in the fractions showed the formation of a linear gradient and SEC showed concentration of CHIKV within a sucrose concentration range from 40 -30 % (w/w) sucrose. PS SEC showed a very good separation of PS and residual contaminants from CHIKV. Of the four tested sucrose layer systems the combination of 3 layers (shown in Figure 16D) showed the best separation of the virus particles from residual contaminants and was therefore used for further DSP development.

Figure 16: Relative amounts of attenuated $\Delta 5nsP3$ ChikV particles and other components by SEC-HPLC analysis at the different steps of the process of the invention including, from top to bottom: crude harvest (a); 10X concentrated harvest; diafiltrated concentrated harvest; PS treated material; CC700-treated material and SGC purified pool.

Figure 17: An exemplary downstream virus purification process from the crude harvest to formulation of the drug substance (vaccine), a preferred embodiment of the process of the invention (A). A flow-chart of an exemplary virus inactivation process is shown in (B).

Figure 18: PS treatment results in selective removal of Zika virus aggregates and Vero HCP and LMW impurities (SEC-HPLC of 30x concentrated Zika Virus harvest day 5).

Figure 19: SEC-HPLC of individual 30x concentrated Zika harvest prior PS treatment at different time points.

Figure 20: SEC-HPLC of individual 30x concentrated Zika harvest post PS treatment at different time points.

Figure 21: Representative SDS-PAGE from the sucrose gradient harvest of a Zika purification is shown.

Figure 22: Correlation between JEV Antigen content in NIV analysed by ELISA and SEC-HPLC (Dionex Ultimate 3000, Superose 6 column).

Figure 23: Comparison of JEV and ZikaV harvest schedules/yields.

Figure 24: SEC-HPLC elution profile of ZikaV NIV. Data were processed on Dionex Ultimate 3000 / Superose 6 Increase column. Both panels are from the same chromatogram. The upper graph is the complete elution profile; the lower graph is an enlargement of the ZIKAV elution peak.

Figure 25: SEC-MALLS analysis of inactivated ZikaV.

Figure 26: Cumulative particle size distribution of Zika NIV.

Figure 27: Graphical representation of the neutralization of the Zika virus H/PF/2013 with pooled mouse sera. The number of plaques without serum was set to 100%. The EC50 was calculated with the 3-parameter method.

Figure 28: Graphical representation of the neutralization of the Zika virus MR766 with pooled mouse sera. The number of plaques without serum was set to 100%. The EC50 was calculated with the 3-parameter method.

Figure 29: Change in SEC profile of Yellow fever virus peak after PS addition according to the invention showing a complete removal of large size aggregates and LMW impurities.

DETAILED DESCRIPTION OF THE INVENTION

Disclosed herein are processes for the purification of infectious virus particles, i.e., mature, functional virus particles, e.g. flavivirus particles (Yellow Fever, Zika Virus, Japanese Encephalitis virus, Dengue virus) and/or alphavirus particles (Chikungunya virus). The processes disclosed are characterized by the removal of undesired by-products of virus production on host cells, such as non-infectious virus particles and aggregated and immature virus by-products. The processes provided herein allow the production of highly-purified virus preparations comprising mostly infectious virus particles. During the course of the invention, it was observed that protamine sulphate (PS), added to remove contaminating DNA during virus purification, resulted not only in removal of contaminating DNA, but also in the loss of a high percentage of total virus particles present in the preparation. Surprisingly, however, quantification of total infectious virus particles by TCID50 before and after PS treatment revealed that the absolute number of infectious virus particles did not change following this loss of total virus particles. This observation clearly shows that treatment with PS can facilitate selective removal of non-infectious, aggregated and immature viral by-products, leaving behind the infectious Chikungunya virus particles or other infectious virus particles. Because by-products produced during virus growth on host cells may have different (and undesirable) immunological properties or other unwanted side-effects or safety issues, a simple and robust way to remove these by-products is of high importance for the quality and safety of the final product.

Protamines are small arginine-rich nuclear proteins, present in high amounts in the sperm of fish, which have an important role in DNA packaging during spermatogenesis. Protamine sulfate (or “protamine” or “PS”) can form a stable ion pair with heparin and is thus commonly used during certain surgeries when the anti-coagulation effect of heparin is no longer needed. In large doses, protamine sulfate administered alone can also have a weak anticoagulant effect (“Protamine sulfate”. Wikipedia: The Free Encyclopedia. Wikimedia Foundation, Inc. 30 Sep 2015 Web. 26 Nov 2015 <https://en.wikipedia.org/wiki/Protamine_sulfate>). Protamine Sulphate is additionally routinely used in biotechnology applications such as DNA precipitation (e.g., removal of host cell DNA from cell culture processes), purification of DNA binding proteins and retroviral-mediated gene transfer.

Protamine is obtained from salmon sperm or produced recombinantly and is used as a sulphate salt. The four major peptides, which constitute almost the entire nitrogen-containing material in salmon protamine, have been fully characterized and found to be polypeptides of 30–32 amino acids in length, of which 21–22 residues are arginine. The average molecular mass is in the range of 4250 Da for the following sequence: PRRRRSSSRP VRRRRRPRVS RRRRRRGGR RR (SEQ ID NO: 1). Herein, protamine is also referred to as protamine salt, or preferably protamine sulphate.

The present invention relates to the use of protamine sulphate (PS) in a process of purification of a live virus, wherein the protamine sulphate facilitates the removal of impurities from a crude virus harvest, including non-infectious virus particles and aggregates. As seen in Fig. 8 using flaviviruses as an example, virus production in the host cell can result in the release of virus products which are not mature, and non-infectious particles, which can also be considered impurities according to the present invention. As such, the present invention also relates to the enrichment of infectious virus particles from a crude harvest containing a mixture of virus particles and other viral products in various stages of maturation.

The use of protamine sulphate can follow crude cell lysis or any further step after cell lysis (e.g. including after a pre-purification with filtration, chromatography etc) wherein the virus particles are further enriched or concentrated and/or other impurities are removed and/or buffer components are exchanged. The further steps may comprise filtration or concentration of the crude cell lysate.

The protamine sulphate may comprise the sequence PRRRRSSSRP VRRRRRPRVS RRRRRRGGRR RR (SEQ ID NO: 1) or a variant thereof wherein the amino acid sequence comprises from 28-35 amino acids, preferably 29-34, more preferably 30-33 amino acids, most preferably 31 or 32 amino acids. The protamine sulphate preferably comprises at least 19 arginine residues, more preferably at least 20 arginine residues, more preferably at least 21 arginine residues, even more preferably at least 22 residues, most preferably 20 or 21 arginine residues. Further, other protamine sulphate-like compounds or variants thereof may be used. Therefore, the use of the term "protamine salt" herein shall serve to encompass natural variations on SEQ ID NO: 1, preferably, but not limited to, the protamine sulphate forms.

The process according to the current invention may also comprise the use of a sucrose gradient, preferably an optimized sucrose gradient. The sucrose gradient is preferably optimized for the removal of protamine sulfate, also for the removal of immature viral particles or other viral particles which are non-infectious or host cell proteins or nucleic acids (DNA, RNA, mRNA, etc) or other host cell debris. In the current invention the optimized sucrose gradient comprises at least two, at least three, at least four layers of sucrose solutions with different densities. In one embodiment, the virus preparation to be purified is provided in a sucrose solution which has a density of about 8%, about 9%, about 10%, about 11%, about 12% sucrose (w/w), preferably about 10%. In one embodiment, one sucrose solution in the gradient has a density of about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55% sucrose (w/w), preferably about 50%. In one embodiment, one sucrose solution in the gradient has a density of about 30%, about 31%, about 32%, about 33%, about 34%, about

35%, about 36%, about 37%, about 38%, about 39%, about 40% sucrose (w/w), preferably about 35%. In one embodiment, one sucrose solution in the gradient has a density of about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20% sucrose (w/w), preferably about 15% sucrose. In a preferred embodiment, the sucrose gradient comprises three layers of sucrose solutions of about 50%, about 35% and about 15% (w/w) sucrose and the virus composition to be purified is contained in about 10% (w/w) sucrose. Because the invention provided for means to not only test for host cell DNA but also immature viral particles, the skilled person in the art is able to more precisely optimize the sucrose gradient for most efficient purification and include additional tools such as PRNT assay to monitor purification success.

The process comprising the use of protamine sulphate of the invention can be applied to purification of any virus for use in pharmaceutical compositions, for example, for a pharmaceutical composition such as a vaccine where it is important that the virus is in its infectious form. The virus to be purified may be any live virus, any live attenuated virus or any live chimeric virus, preferably a live wild type virus such as a Zika virus of the Asian lineage. In one embodiment, the virus particle is also be later inactivated. In a preferred embodiment, the virus is inactivated with formaldehyde.

In a preferred embodiment, the produced Zika virus is derived from the Asian lineage (which includes the strains found in South America and all strains derived from any Asian lineage). In some other embodiments, the produced Zika virus is a Zika virus as described in the Sequence section of this application (SEQ ID NO: 2 to 69 or 78).

In another preferred embodiment, the live attenuated Chikungunya virus is the protective ChikV-ICRES1- Δ 5nsP3 described by Hallengård *et al.* (Novel Attenuated Chikungunya Vaccine Candidates Elicit Protective Immunity in C57BL/6 mice (2014) J. Virology, 88(5):2858–2866). Briefly, the ChikV genome carries a positive-sense single-stranded RNA genome of 11 Kb containing two open reading frames encoding nonstructural proteins (nsP1 to nsP4) and structural proteins (C, E3, E2, 6K, and E1), respectively (see Fig. 9, top construct). The attenuated virus Δ 5nsP3, based on the La Reunion ChikV strain LR2006-OPY1, was obtained by the substitution of amino acid residues 1656 to 1717 of the P1234 polyprotein with a small linker (aa sequence AYRAAAG) in the hypervariable region of the nsP3 protein (see Fig. 9, bottom construct). The Δ 5nsP3 ChikV mutant was shown to be infectious, highly immunogenic and protective against challenge with wild type ChikV (Hallengård, *et al.*, *supra* and Hallengård, *et al.*, Prime-Boost Immunization Strategies against Chikungunya Virus (2014) J. Virology,

88(22):13333-13343). In one embodiment, the live attenuated Chikungunya virus may be a variant of the ChikV-ICRES1-Δ5nsP3 attenuated mutant virus.

A preferred embodiment of the process of the current invention is shown in Figure 10 (Chikungunya virus) and Figure 17A (Zika virus).

Table 1. Overview of process buffers and stock solutions

Buffer	Composition	Final pH	Final conductivity [mS/cm]
A	0.5 M NaOH		n.a.
B	0.1 M NaOH		n.a.
C	25 mM Tris, 150 mM NaCl	7.4±0.2	16.5
D	1 M Tris	7.4±0.2	n.a.
E	4.5 M NaCl	n.a.	n.a.
F	1 M NaCl	n.a.	n.a.
G	1 % SDS	n.a.	n.a.
H	50 % (w/w) Sucrose in 25 mM Tris, 150 mM NaCl	7.4±0.2	n.a.
I	35 % (w/w) Sucrose in 25 mM Tris, 150 mM NaCl	7.4±0.2	n.a.
J	15 % (w/w) Sucrose in 25 mM Tris, 150 mM NaCl	7.4±0.2	n.a.
K	10 x PBS	7.4±0.2	n.a.
L	50 mg/mL Protamine sulphate	7.4±0.2	n.a.
M	Drug substance formulation buffer (10mM Tris(hydroxymethyl)-aminomethan, 5 % Sucrose, 1% (10 mg/mL) rHSA)	7.4±0.2	1.3

Table 2. Abbreviations

°Bx	Degrees Brix = sugar content (w/w) of an aqueous solution
BSA	Bovine serum albumin
CC700	Capto™ Core 700
ChikV	Chikungunya virus
CPE	Cytopathic effect
EtOH	Ethanol
EU	Endotoxin units
DS	Drug Substance
DP	Drug Product
DSP	Downstream Process
HCP	Host cell protein
hcDNA	Host cell DNA
hpi	Hours post infection
HPLC	High Performance Liquid Chromatography
ID	Inner diameter
JEV	Japanese Encephalitis virus
LAL	Limulus amebocyte lysate
LDS buffer	Lithium dodecyl sulfate sample loading buffer
LOD	Limit of detection
LOQ	Limit of quantitation
MALLS	Multiangle light scattering
mAU	Milli absorbance units

MS	Mass spectroscopy
NIV	Neutralized inactivated virus
PBS	Phosphate buffered saline
PD	Process development
PFU	Plaque forming units
p.i.	Post-infection
PS	Protamine sulphate or protamine sulfate
rcf	Relative centrifugal force
rHSA	Recombinant human serum albumin
Rms radius	Root mean square radius
rMSB	Research master seed bank
RSD	Relative standard deviation
SEC	Size exclusion chromatography
SGC	Sucrose gradient centrifugation
SGP	Sucrose gradient purified
SDS	Sodium dodecyl sulphate
TBS	Tris buffered saline
TFF	Tangential flow filtration
TCID50	Tissue culture infectious dose 50%
UF/DF	Ultrafiltration/diafiltration
WFI	Water for injection
ZikaV	Zika virus

Brix:

Degrees Brix (°Bx) is the sugar content of an aqueous solution. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass. °Bx corresponds to the sucrose content in % (w/w), eg. 45 °Bx equals 45 % (w/w) sucrose.

Table A. Primers for Zika virus sequencing: lower case letters indicate bases not included in ZIKA but containing restriction sites for later cloning when needed (therefore, two Tms provided).

Primer Pair	Oligoname	Primer sequence (5'-3') restriction sites (lower case)	Tm (gene-specific)	Tm (entire primer)	Amplicon size [bp]
1	9320_Zika_PF_1F	SEQ ID NO: 80 ttaggatccGTTGTTGATCTGTGTGAAT	69.9	74.6	707
	9321_Zika_PF_1R	SEQ ID NO: 81 taactcgagCGTACACAACCCAAGTT	69.3	75.6	
2	9322_Zika_PF_2F	SEQ ID NO: 82 ttaggatccTCACTAGACGTGGGAGTG	70	73.9	704
	9323_Zika_PF_2R	SEQ ID NO: 83	69.8	73.7	

Primer Pair	Oligoname	Primer sequence (5'-3') restriction sites (lower case)	Tm (gene-specific)	Tm (entire primer)	Amplicon size [bp]
		taactcgagAAGCCATGTCYGATATTGAT			
3	9324_Zika_PF_3F	SEQ ID NO: 84 ttaggatccGCATACAGCATCAGGTG	72.3	74.5	712
	9325_Zika_PF_3R	SEQ ID NO: 85 taactcgagTGTGGAGTTCCGGTGTCT	72	76.4	
4	9326_Zika_PF_4F	SEQ ID NO: 86 ttaggatccGAATAGAGCGAARGTTGAGATA	70.9	74	712
	9327_Zika_PF_4R	SEQ ID NO: 87 taactcgAGTGGTGGGTGATCTTCTTCT	70.5	73.7	
5	9328_Zika_PF_5F	SEQ ID NO: 88 ttaggatcCAGTCACAGTGGAGGTACAGTAC	70.3	75	704
	9329_Zika_PF_5R	SEQ ID NO: 89 taactcgagCRCAGATACCATCTTCCC	71.5	77.3	
6	9330_Zika_PF_6F	SEQ ID NO: 90 ttaggatCCCTTATGTGCTTGGCCTTAG	70.7	72.7	698
	9331_Zika_PF_6R	SEQ ID NO: 91 taactcgagTCTTCAGCCTCCATGTG	70.4	76.9	
7	9332_Zika_PF_7F	SEQ ID NO: 92 ttaggatccAATGCCCACTCAAACATAGA	71.9	75	716
	9333_Zika_PF_7R	SEQ ID NO: 93 taactcgagTCATTCTTTCTTCAGCCCTT	71	74	
8	9334_Zika_PF_8F	SEQ ID NO: 94 ttaggatccAAGGGTGATCGAGGAAT	70.9	75.2	703
	9335_Zika_PF_8R	SEQ ID NO: 95 taactcgagTTCCCTTCAGAGAGAGGAGC	71.9	73.4	
9	9336_Zika_PF_9F	SEQ ID NO: 96 ttaggatccTCTTTTGCAAAGTGCATC	71.9	75	699

Primer Pair	Oligoname	Primer sequence (5'-3') restriction sites (lower case)	Tm (gene-specific)	Tm (entire primer)	Amplicon size [bp]
	9337_Zika_PF_9R	SEQ ID NO: 97 taactcgagTCCAGCTGCAAAGGGTAT	71	74.9	
10	9338_Zika_PF_10F	SEQ ID NO: 98 ttaggatccGTGTGGACATGTACATTGA	71.4	75.8	706
	9339_Zika_PF_10R	SEQ ID NO: 99 taactcgagCCCATTGCCATAAAGTC	70.4	75.8	
11	9340_Zika_PF_11F	SEQ ID NO: 100 ttaggatccTCATACTGTGGTCCATGGA	71.6	78.1	692
	9341_Zika_PF_11R	SEQ ID NO: 101 taactcgagGCCCATCTCAACCCTTG	74	78	
12	9342_Zika_PF_12F	SEQ ID NO: 102 ttaggatccTAGAGGGCTTCCAGTGC	70.9	74	707
	9343_Zika_PF_12R	SEQ ID NO: 103 taactcgAGATACTCATCTCCAGGTTTGTG	70.2	72.2	
13	9344_Zika_PF_13F	SEQ ID NO: 104 ttaggatccGAAAACAAAACATCAAGAGTG	70.6	75.4	726
	9345_Zika_PF_13R	SEQ ID NO: 105 taactcgagGAATCTCTGTGCATGTGCCT	71.9	75.6	
14	9346_Zika_PF_14F	SEQ ID NO: 106 ttaggatccTTGATGGCAGACCAAC	73.1	75.6	715
	9347_Zika_PF_14R	SEQ ID NO: 107 ttaggatccGTTGTTGATCTGTGTGAAT	70.8	77.9	
15	9348_Zika_PF_15F	SEQ ID NO: 108 taactcgagCAGGTCAATGTCCATTG	71.9	75.4	719
	9349_Zika_PF_15R	SEQ ID NO: 109 ttaggatccGTTGTGTTCTATTGCTGGT	73.9	77.2	
16	9350_Zika_PF_16F	SEQ ID NO: 110	72.3	75.4	703

Primer Pair	Oligoname	Primer sequence (5'-3') restriction sites (lower case)	Tm (gene-specific)	Tm (entire primer)	Amplicon size [bp]
		taactcgaGTGATCAGRCCCCAGC			
	9351_Zika_PF_16R	SEQ ID NO: 111 ttaggatccTGCTGCCCAGAAGAGAA	72	76.3	
17	9352_Zika_PF_17F	SEQ ID NO: 112 taactcgaGCACCAACAYGGGTCTT	73.6	76	705
	9353_Zika_PF_17R	SEQ ID NO: 113 ttaggatcCTCAAGGACGGTGTGGC	72	75.5	
18	9354_Zika_PF_18F	SEQ ID NO: 114 taactcgagCAATGATCTTCATGTTGGG	71.7	75.8	699
	9355_Zika_PF_18R	SEQ ID NO: 115 ttaggatccTATGGGGGAGGACTGGT	71	74.1	
19	9356_Zika_PF_19F	SEQ ID NO: 116 taactcGAGCCCAGAACCTTGGATC	73.3	75.5	711
	9357_Zika_PF_19R	SEQ ID NO: 117 ttaggatcCAGACCCCCAAGAAGGC	71.3	76.9	
20	9358_Zika_PF_20F	SEQ ID NO: 118 taactcgagCCCCTTGGTCTTGTCT	71.7	75	706
	9359_Zika_PF_20R	SEQ ID NO: 119 ttaggatccAGGAAGGATGTATGCAGATG	71.9	73.9	
21	9360_Zika_PF_21F	SEQ ID NO: 120 taactcgagACATTTGCGCATATGATTTTG	70.4	75.7	709
	9361_Zika_PF_21R	SEQ ID NO: 121 ttaggatccAGGAAGGACACACAAGAGT	71.8	75	
22	9362_Zika_PF_22F	SEQ ID NO: 122 taactcgagACAGGCTGCACAGCTTT	70	79.1	581
	9363_Zika_PF_22R	SEQ ID NO: 123 ttaggatccTCTCTCATAGGGCACAGAC	74.8	81.1	

SEQUENCES

SEQ ID NO: 1

A typical form of protamine

PRRRSSSRP VRRRRRPRVS RRRRRRGRR RR

Provided below are examples of nucleic acid sequences of the genomes of Zika viruses that may be used in the methods, compositions, and/or vaccines described herein.

SEQ ID NO: 2

KU321639.1 Zika virus strain ZikaSPH2015, Brazil, complete genome (SEQ ID NO: 2)

GTTGTTACTGTTGCTGACTCAGACTGCGACAGTTCGAGTTTGAAGCGAAAGCTAGCAACAGTATCAACAGGTTTTATTGGATT
 TGGAAACGAGAGTTTCTGGTCATGAAAAACCAAAAAAGAAATCCGGAGGATTCCGGATTGTCAATATGCTAAAACGCGGAG
 TAGCCCGTGTGAGCCCTTTGGGGGCTTGAAGAGGCTGCCAGCCGACTTCTGCTGGGTCATGGGCCCATCAGGATGGTCTTG
 GCAATTCTAGCCTTTTGGAGATTACGGCAATCAAGCCATCACTGGGTCTCATCAATAGATGGGGTTCAGTGGGGAAAAAGA
 GGCTATGGAAATAATAAAGAAGTTCAAGAAAGATCTGGCTGCCATGCTGAGAATAATCAATGCTAGGAAGGAGAAGAAGAG
 ACGGGGCGCAGATACTAGTGTGGAATTGTTGGCTCCTGCTGACCACAGCTATGGCAGCGGAGGTCACTAGACGTGGGAGT
 GCATACTATATGTACTTGGACAGAAACGATGCTGGGGAGGCCATATCTTTCCAACCACATTGGGGATGAATAAGTGTATAT
 ACAGATCATGGATCTTGGACACATGTGTGATGCCACCATGAGCTATGAATGCCCTATGCTGGATGAGGGGGTGAACACAGAT
 GACGTCGATTGTTGGTGCAACACGACGTCAACTTGGGTGTGTACGGAACCTGCCATCACAAAAAGGTGAAGCACGGAGAT
 CTAGAAGAGCTGTGACGCTCCCCTCCATTCCACTAGGAAGCTGCAACGCGGTGCAAACTGTTGGAATCAAGAGAATAC
 ACAAAGCACTTGATTAGAGTCGAAAATTGGATATTCAGGAACCTGGCTTCGCGTTAGCAGCAGCTGCCATCGCTTGGCTTTTG
 GGAAGCTCAACGAGCCAAAAAGTCATATACTTGGTCATGATACTGCTGATTGCCCCGGCATACAGCATCAGGTGCATAGGAGT
 CAGCAATAGGGACTTTGTGGAAGGTATGTCAGGTGGGACTTGGGTGATATTGTCTTGAACATGGAGGTTGTGTACCGTA
 ATGGCACAGGACAAACCGACTGTCGACATAGAGCTGGTTACAACAACAGTCAGCAACATGGCGGAGGTGAAGATCCTACTGCT
 ATGAGGCATCAATATCAGACATGGCTTCGGACAGCCGCTGCCAACACAAGGTGAAGCCTACCTTGACAAGCAATCAGACACT
 CAATATGTCTGCAAAAGAAGCTTAGTGGACAGAGGCTGGGGAAATGGATGTGGACTTTTGGCAAAGGGAGTCTGGTGACAT
 GCGCTAAGTTTGCATGCTCCAAGAAAATGACCGGGAAGAGCATCCAGCCAGAGAATCTGGAGTACCGGATAATGCTGTCACT
 TCATGGCTCCCAGCACAGTGGGATGATCGTTAATGACACAGGACATGAAACTGATGAGAATAGAGCGAAGGTTGAGATAACG
 CCCAATTCACCAAGAGCCGAAGCCACCCTGGGGGGTTTGGAAAGCTAGGACTTGATTGTGAACCGAGGACAGGCCTTGACTT
 TTCAGATTTGTATTACTTGACTATGAATAACAAGCACTGGTTGGTTTACAAGGAGTGGTTCCACGACATTCCATTACCTTGGCAC
 GCTGGGGCAGACACCGGAACCTCCACACTGGAACAACAAAGAAGCACTGGTAGAGTTCAAGGACGCACATGCCAAAAGGCAA
 ACTGTCGTGGTTCTAGGGAGTCAAGAAGGAGCAGTTCACACGGCCCTTGTGGAGCTCTGGAGGCTGAGATGGATGGTGCAA
 AGGGAAGGCTGTCCTCTGGCCACTTGAAATGTCGCCTGAAAAATGGATAAATCTAGATTGAAGGGCGTGTCACTCCTTGTGT
 ACCGCAGCGTTCACATTACCAAGATCCCGGCTGAAACACTGCACGGGACAGTCACAGTGGAGGTACAGTACGCAGGGACAG
 ATGGACCTTGCAAGGTTCCAGCTCAGATGGCGGTGGACATGCAAACTCTGACCCAGTGGGAGGTTGATAACCGCTAACCCC
 GTAATCACTGAAAGCACTGAGAACTCTAAGATGATGCTGGAACCTTGATCCACCATTTGGGGACTCTTACATTGTATAGGAGTC
 GGGGAGAAGAAGATCACCCACCACTGGCACAGGAGTGGCAGCACCATTGGAAAAGCATTGGAAGCCACTGTGAGAGGTGCC
 AAGAGAATGGCAGTCTTGGGAGACACAGCCTGGGACTTTGGATCAGTTGGAGGCGCTCTCAACTATTGGGCAAGGGCATCC
 ATCAAATTTTTGGAGCAGCTTCAAATCATTGTTTGGAGGAATGTCTGGTTCTCAAAATCTCATTGGAACGTTGCTGATGTG
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 CTCTGCTGATGTGGGTGCTCGGTGGACTTCTAAAGAAGGAGACGAGATGCGGTACAGGGGTGTTCTGTCTATAACGACGTT
 GAAGCCTGGAGGGACAGGTACAAGTACCATCCTGACTCCCCCGTAGATTGGCAGCAGCAGTCAAGCAAGCCTGGGAAGATG
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 GAGAATGGAGTTCAACTGACGGTCTGTGTGGGATCTGTAAAAACCCCATGTGGAGAGGTCCACAGAGATTGCCCGTGCCTG
 TGAACGAGCTGCCCCAGGCTGGAAGGCTTGGGGGAAATCGCACTTCGTGAGAGCAGCAAAGACAAATAACAGCTTTGTCGT
 GGATGGTGACACTGAAGGAATGCCCACTCAACATAGAGCATGGAACAGCTTTCTGTGGAGGATCATGGGTTCCGGGTA

TTTCACACTAGTGTCTGGCTCAAGGTTAGAGAAGATTATTCATTAGAGTGTGATCCAGCCGTTATTGGAACAGCTGTTAAGGGA
AAGGAGGCTGTACACAGTGAATCTAGGCTACTGGATTGAGAGTGAGAAGAATGACACATGGAGGCTGAAGAGGGCCCATCTG
ATCGAGATGAAAACATGTGAATGGCCAAAGTCCACACATTGTGGACAGATGGAATAGAAGAGAGTGTATCTGATCATACCCA
AGTCTTTAGCTGGGCCACTCAGCCATCACAATACCAGAGAGGGGTACAGGACCCAAATGAAAGGGCCATGGCACAGTGAAGA
GCTTGAAATTCGGTTTGAGGAATGCCAGGCACTAAGGTCCACGTGGAGGAAACATGTGGAACAAGAGGACCATCTCTGAGA
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CCAACCAAGGTTGTGCTGCTGAAATGGAGGAAGCCCTTAGAGGGCTTCCAGTGCGTTATATGACAACAGCAGTCAATGTAC
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AGGACAACCAAATGGCAATCATCATGTTAGCAGTAGGTCTTCTGGGCTTGATTACCGCCAATGAAGCTGGATGGTTGGAG
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CGGCCAGCCTCAGCTTGGGCCATCTATGCTGCCTTGACAACCTTCACTACCCAGCCGTCCAACATGCAGTGACCACTTCATACA
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ACCCTGTTGTGGATGGAATAGTGGTGA CTGACATTGACACAATGACAATTGACCCCCAAGTGGAGAAAAAGATGGGACAGGT
GCTACTCATGGCAGTAGCCGTCTCCAGCGCCATACTGTGCGGACCGCCTGGGGGTGGGGGGAGGCTGGGGCCCTGATCACA
GCCGCAACTTCCACTTTGTGGGAAGGCTCTCCGAACAAGTACTGGAACCTCTACAGCCACTTCACTGTGTAACATTTTTAGG
GGAAGTTACTTGGCTGGAGCTTCTAATCTACACAGTAACAAGAAACGCTGGCTTGGTCAAGAGACGTGGGGGTGGAACAG
GAGAGACCTGGGAGAGAAATGGAAGGCCCGCTTGAACCAGATGTCGGCCCTGGAGTTCTACTCTACAAAAAGTCAGGCAT
CACCGAGGTGTGCAGAGAAGAGGCCCGCCGCGCCCTCAAGGACGGTGTGGCAACGGGAGGCCATGCTGTGTCCCGAGGAAG
TGCAAAGCTGAGATGGTTGGTGGAGCGGGGATACCTGCAGCCCTATGGAAAGGTCATTGATCTTGGATGTGGCAGAGGGGG
CTGGAGTTACTACGCCGCCACCATCCGCAAAGTTCAAGAAGTGAAAGGATACAAAAAGGAGGCCCTGGTCATGAAGAACCC
GTGTTGGTGCAAAGCTATGGGTGGAACATAGTCCGTCTTAAGAGTGGGGTGGACGTCTTTCATATGGCGGCTGAGCCGTGTG
ACACGTTGCTGTGTGACATAGGTGAGTCATCATCTAGTCTGAAGTGGAAAGCACGGACGCTCAGAGTCTCTCCATGGTG
GGGGATTGGCTTAAAAAAGACCAGGAGCCTTTGTATAAAAGTGTGTGCCCATACACCAGCACTATGATGGAAACCTTGGGA
GCGACTGCAGCGTAGGTATGGGGAGGACTGGTCAGAGTGCCACTCTCCGCAACTCTACACATGAGATGTACTGGGTCTCT
GGAGCGAAAAAGCAACACCATAAAAAAGTGTGTCCACCACGAGCCAGCTCCTTGGGGCGCATGGACGGGCCTAGGAGGCCA
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SEQ ID NO: 3

KU497555.1 Zika virus isolate Brazil-ZKV2015, Brazil, complete genome

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SEQ ID NO: 4

KU501215.1 Zika virus strain PRVABC59, Puerto Rico, complete genome

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SEQ ID NO: 5

KU509998.1 Zika virus strain Haiti/1225/2014, Haiti, complete genome

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SEQ ID NO: 6

KU527068.1 Zika virus strain Natal RGN, Brazil: Rio Grande do Norte, Natal, complete genome

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SEQ ID NO: 7

KU681081.3 Zika virus isolate Zika virus/H.sapiens-tc/THA/2014/SV0127- 14, Thailand, complete genome

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SEQ ID NO: 8

KU681082.3 Zika virus isolate Zika virus/H.sapiens-tc/PHL/2012/CPC-0740, Philippines, complete genome

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SEQ ID NO: 9

KU707826.1 Zika virus isolate SSABR1, Brazil, complete genome

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KU744693.1 Zika virus isolate VE_Ganxian, China, complete genome

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SEQ ID NO: 11

LC002520.1 Zika virus genomic RNA, strain: MR766-NIID, Uganda, complete genome

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SEQ ID NO: 12

AY632535.2 NC_012532.1 Zika virus strain MR 766, Uganda, complete genome

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SEQ ID NO: 13

KJ776791.1, Zika virus strain H/PF/2013 polyprotein gene, complete cds

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TCCAACCTGGGAGAACTACCTGGTCAATCCATGGAAGGGGAGAATGGATGACCACTGAAGACATGCTTGTGGTGTGGAACAGA
GTGTGGATTGAGGAGAACGACCACATGGAAGACAAGACCCAGTTACGAAATGGACAGACATTCCTATTTGGGAAAAAGGG
AAGACTTGTGGTGTGGATCTCTCATAGGGCACAGACCGCGCACCACTGGGCTGAGAACATTAAAAACACAGTCAACATGGT
GCGCAGGATCATAGGTGATGAAGAAAAGTACATGGACTACCTATCCACCAAGTTCGCTACTTGGGTGAAGAAGGGTCTACA
CCTGGAGTGTGTAAGCAACATCTTAGTGTTCAGGCCTGCTAGTCAGCCACAGCTTGGGGAAGCTGTGCAGCCTGTGAC
CCCCCAGGAGAAGCTGGGAAACCAAGCCTATAGTCAGGCCGAGAACGCCATGGCACGGAAGAAGCCATGCTGCCTGTGAGC
CCCTCAGAGGACACTGAGTCAAAAAACCCACGCGCTTGGAGGCGCAGGATGGGAAAAGAAGGTGGCGACCTTCCCCACCT
TCAATCTGGGGCCTGAAGTGGAGATCAGCTGTGGATCTCCAGAAGAGGGACTAGTGGTTAGAGGAG

In some embodiments, the Zika virus has a RNA genome corresponding to the DNA sequence provided by the nucleic acid sequence of any one of SEQ ID NOs: 2-13 or 73. In some embodiments, the Zika virus has a variant genome that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to any one of SEQ ID NOs: 2-13 or 78.

Provided below are amino acid sequences of the E-proteins of Zika strains that may be used in the methods, compositions, and/or vaccines described herein.

SEQ ID NO: 14

isol-ARB15076.AHF49784.1.Central_African_Republic/291-788 Flavivirus envelope glycoprotein E.

IRCIQVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTNSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWNGCGLFGKGLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSQMIVNDENRAKVEVTPNSPRA
EATLGGFGSLGLDCEPTGLDFSDLYLTMMNKHVWLHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGS
QEGAVHTALAGALEAEMDGAKGRFLSGHLKCRKMDKRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQM
AVDMQTLTPVGRILITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKITHHWHRSSTIGKAFAETVRGAKRMAVLGDTAWDF
GSVGGVFNSLKGHIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

SEQ ID NO: 15

isol-ibH_30656.AEN75265.1.Nigeria/291-788 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F T C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D E N R A K V E V T P N S P R A
E A T L G G F G S L G L D C E P R T G L D F S D L Y L T M N N K H W L V H K E W F H D I P L P W H S G A D T E T P H W N N K E A L V E F K D A H A K R Q T V V V L G S
Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L K C R L K M D K L R L K G V S Y S L C T A A F T F T K V P A E T L H G T V T V E V Q Y A G R D G P C K V P A Q M
A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G D K K I T H H W H R S G S I I G K A F E A T V R G A K R M A V L G D T A W D F
G S V G G V F N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L V W L G L N T K N G S I S L T C L A L G G V M I F L S T A V S A

SEQ ID NO: 16

ArB1362.AHL43500.1.-/291-794 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F T C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D X X X X X X N R A E V E V T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L F S G H L K C R L K M D K L R L K G V S Y S L C T A A F T F T K V P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G D K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G
D T A W D F G S V G G V F N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L V W L G L N T K N G S I S L T C L A L G G V M I F L S T A V S A

SEQ ID NO: 17

ArD128000.AHL43502.1.-/291-794 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F T C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M X X X X X G H E T D E N R A K V E V T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y L T M N N K H R L V R K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L F S G H L K C R L K M D K L R L K G V S Y S L C T A A F T F T K V P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G D K K I T H H W L K K G S S I G K A F E A T V R G A K R M A V L G D
T A W D F G S V G G V F N S L G K G V H Q I F G A A F K S L F G G M S W F S Q I L I G T L L V W L G L N T K N G S I S L T C L A L G G V M I F L S T A V S A

SEQ ID NO: 18

ArD158095.AHL43505.1.-/291-794 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F T C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D I G H E T D E N R A K V E V T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L F S G H L K C R L K M D K L R L K G V S Y S L C T A A F T F T K V P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G D K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G
D T A W D F G S V G G V F N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L V W L G L N T K N G S I S L T C L A L G G V M I F L S T A V S A

SEQ ID NO: 19

ArD158084.AHL43504.1.-/291-794 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F T C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D I G H E T D E N R A K V E V T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L F S G H L K C R L K M D K L R L K G V S Y S L C T A A F T F T K V P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G D K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G
D T A W D F G S V G G V F N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L V W L G L N T K N G S I S L T C L A L G G V M I F L S T A V S A

SEQ ID NO: 20

isol-ARB13565.AHF49783.1.Central_African_Republic/291-794 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F T C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D I G H E T D E N R A K V E V T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L F S G H L K C R L K M D K L R L K G V S Y S L C T A A F T F T K V P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G D K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G
D T A W D F G S V G G V F N S L G K G V H Q I F G A A F K S L F G G M S W F S Q I L I G T L L V W L G L N T K N G S I S L T C L A L G G V M I F L S T A V S A

SEQ ID NO: 21

isol-ARB7701.AHF49785.1.Central_African_Republic/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPHWAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLFSGLKCRKMDKRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLG
DTAWDFGSGVGGVFNSLKGIVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

SEQ ID NO: 22

isol-ArD_41519.AEN75266.1.Senegal/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPHWAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLFSGLKCRKMDKRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLG
DTAWDFGSGVGGVFNSLKGIVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

SEQ ID NO: 23

MR766-NIID.BAP47441.1.Uganda/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMTVNDIGYETDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPHWAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLFSGLKCRKMDKRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPCK
IPVQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGVGGVFNSLKGIVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

SEQ ID NO: 24

LC002520.1/326-829 Zika virus genomic RNA, strain: MR766-NIID, Uganda, Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMTVNDIGYETDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPHWAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLFSGLKCRKMDKRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPCK
IPVQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGVGGVFNSLKGIVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

SEQ ID NO: 25

isol-MR_766.AEN75263.1.Uganda/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGYETDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPHWAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLFSGLKCRKMDKRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPCK
IPVQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGVGGVFNSLKGIVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

SEQ ID NO: 26

ArD7117.AHL43501.1.-/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPHWAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLFSGLKCRKMDKRLKGVSYSLCTAVCTAAKVPAETLHGTVTVEVQYAGTDGPCK

KVPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSRGSTIGKAFEATVRGAKRMAVL
GDTAWDFGSGVGFNSLKGKIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

SEQ ID NO: 27

AY632535.2/326-825 NC_012532.1 Zika virus strain MR 766, Uganda, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIGYETDEDRAKVEVTPNSPR
AEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNKEALVEFKDAHAKRQTVVVL
GSQEGAVHTALAGALEAEMDGAKGRFLSGHLKCRCLKMDKLRKGVSYSLCTAAFTFTKVP AETLHGTVTVEVQYAGTDGPCKIPVQ
MAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGDTAW
DFGSGVGFNSLKGKIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

SEQ ID NO: 28

MR_766.AAV34151.1.Uganda/291-790 Flavivirus envelope glycoprotein E. |Q32ZE1|Q32ZE1_9FL
IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIGYETDEDRAKVEVTPNSPR
AEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNKEALVEFKDAHAKRQTVVVL
GSQEGAVHTALAGALEAEMDGAKGRFLSGHLKCRCLKMDKLRKGVSYSLCTAAFTFTKVP AETLHGTVTVEVQYAGTDGPCKIPVQ
MAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGDTAW
DFGSGVGFNSLKGKIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

SEQ ID NO: 29

MR_766.YP_009227198.1.Uganda/1-500 envelope protein E [Zika virus]
IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIGYETDEDRAKVEVTPNSPR
AEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNKEALVEFKDAHAKRQTVVVL
GSQEGAVHTALAGALEAEMDGAKGRFLSGHLKCRCLKMDKLRKGVSYSLCTAAFTFTKVP AETLHGTVTVEVQYAGTDGPCKIPVQ
MAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGDTAW
DFGSGVGFNSLKGKIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

SEQ ID NO: 30

KU681081.3/308-811 Zika virus isolate Zika virus/H.sapiens-tc/THA/2014/SV0127- 14, Thailand, Flavivirus envelope
glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHTDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHTGADTGTPHWNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAKGRFLSSGHLKCRCLKMDKLRKGVSYSLCTAAFTFTKIP AETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITEGTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLG
DTAWDFGSGVGFNSLKGKIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 31

isol-Zika_virus%H.sapiens-tc%THA%2014%SV0127-_14.AMD61710.1.Thailand/291-794 Flavivirus envelope
glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHTDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHTGADTGTPHWNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAKGRFLSSGHLKCRCLKMDKLRKGVSYSLCTAAFTFTKIP AETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITEGTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLG
DTAWDFGSGVGFNSLKGKIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 32

CK-ISL_2014.AIC06934.1.Cook_Islands/1-504 Flavivirus envelope glycoprotein E. (Fragment) OS=Zika virus GN=E
PE=4 SV=1

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKRLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLAGGVLIPLSTAVSA

SEQ ID NO: 33

Natal_RGN.AMB18850.1.Brazil: _Rio_Grande_do_Norte,_ Natal/291-794 Flavivirus envelope glycoprotein E.]

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKRLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLAGGVLIPLSTAVSA

SEQ ID NO: 34

isol-Si323.AMC37200.1.Colombia/1-504 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKRLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLAGGVLIPLSTAVSA

SEQ ID NO: 35

KU707826.1/317-820 Zika virus isolate SSABR1, Brazil, Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKRLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLAGGVLIPLSTAVSA

SEQ ID NO: 36

KU509998.1/326-829 Zika virus strain Haiti/1225/2014, Haiti, Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKRLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLAGGVLIPLSTAVSA

SEQ ID NO: 37

isol-GDZ16001.AML82110.1.China/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKRLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLAGGVLIPLSTAVSA

SEQ ID NO: 38

BeH819015.AMA12085.1.Brazil/291-794 Flavivirus envelope glycoprotein E.]

IRCI GVS NRDFVEGMSGGTWVDV VLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L K C R L K M D K L R L K G V S Y S L C T A A F T F T K I P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G E K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G D
T A W D F G S V G G A L N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L M W L G L N T K N G S I S L M C L A L G G V L I F L S T A V S A

SEQ ID NO: 39

MRS_OPY_Martinique_PaRi_2015.AMC33116.1.Martinique/291-794 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDV VLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L K C R L K M D K L R L K G V S Y S L C T A A F T F T K I P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G E K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G D
T A W D F G S V G G A L N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L M W L G L N T K N G S I S L M C L A L G G V L I F L S T A V S A

SEQ ID NO: 40

KU501215.1/308-811 Zika virus strain PRVABC59, Puerto Rico, Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDV VLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L K C R L K M D K L R L K G V S Y S L C T A A F T F T K I P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G E K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G D
T A W D F G S V G G A L N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L M W L G L N T K N G S I S L M C L A L G G V L I F L S T A V S A

SEQ ID NO: 41

Haiti%1225%2014.AMB37295.1.Haiti/291-794 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDV VLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L K C R L K M D K L R L K G V S Y S L C T A A F T F T K I P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G E K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G D
T A W D F G S V G G A L N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L M W L G L N T K N G S I S L M C L A L G G V L I F L S T A V S A

SEQ ID NO: 42

KU527068.1/308-811 Zika virus strain Natal RGN, Brazil: Rio Grande do Norte, Natal, Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDV VLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L K C R L K M D K L R L K G V S Y S L C T A A F T F T K I P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G E K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G D
T A W D F G S V G G A L N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L M W L G L N T K N G S I S L M C L A L G G V L I F L S T A V S A

SEQ ID NO: 43

isol-Z1106027.ALX35662.1.Suriname/5-508 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDV VLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L K C R L K M D K L R L K G V S Y S L C T A A F T F T K I P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G E K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G D
T A W D F G S V G G A L N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L M W L G L N T K N G S I S L M C L A L G G V L I F L S T A V S A

SEQ ID NO: 44

isol-FLR.AMM39804.1.Colombia:_Barranquilla/291-794 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L C R L K M D K L R L K G V S Y S L C T A A F T F T K I P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G E K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G D
T A W D F G S V G G A L N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L M W L G L N T K N G S I S L M C L A L G G V L I F L S T A V S A

SEQ ID NO: 45

PLCal_ZV_isol-From_Vero_E6_cells.AHL37808.1.Canada/254-757 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L C R L K M D K L R L K G V S Y S L C T A A F T F T K I P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G E K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G D
T A W D F G S V G G A L N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L M W L G L N T K N G S I S L M C L A L G G V L I F L S T A V S A

SEQ ID NO: 46

BeH818995.AMA12084.1.Brazil/291-794 Flavivirus envelope glycoprotein E. [Zika virus].

IRCI GVS NRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L C R L K M D K L R L K G V S Y S L C T A A F T F T K I P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G E K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G D
T A W D F G S V G G A L N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L M W L G L N T K N G S I S L M C L A L G G V L I F L S T A V S A

SEQ ID NO: 47

H/PF/2013.AHZ13508.1.French_Polynesia/291-794 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L C R L K M D K L R L K G V S Y S L C T A A F T F T K I P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G E K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G D
T A W D F G S V G G A L N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L M W L G L N T K N G S I S L M C L A L G G V L I F L S T A V S A

SEQ ID NO: 48

PRVABC59.AMC13911.1.Puerto_Rico/291-794 Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L C R L K M D K L R L K G V S Y S L C T A A F T F T K I P A E T L H G T V T V E V Q Y A G T D G P C K
V P A Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E L D P P F G D S Y I V I G V G E K K I T H H W H R S G S T I G K A F E A T V R G A K R M A V L G D
T A W D F G S V G G A L N S L G K G I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L M W L G L N T K N G S I S L M C L A L G G V L I F L S T A V S A

SEQ ID NO: 49

KU321639.1/326-829 Zika virus strain ZikaSPH2015, Brazil, Flavivirus envelope glycoprotein E.

IRCI GVS NRDFVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T
P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T
V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L C R L K M D K L R L K G V S Y S L C T A A F T F T K I P A E T L H G T V T V E V Q Y A G T D G P C K

VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 50

ZikaSPH2015.ALU33341.1.Brazil/291-794 Flavivirus envelope glycoprotein E.

IRCIQVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 51

103344.AMC13912.1.Guatemala/291-794 polyprotein [Zika virus]. 103344.AMC13912.1.Guatemala Flavivirus envelope glycoprotein E.

IRCIQVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEIRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 52

isol-Brazil-ZKV2015.AMD16557.1.Brazil/291-794 Flavivirus envelope glycoprotein E.

IRCIQVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGTQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 53

KU497555.1/308-811 Zika virus isolate Brazil-ZKV2015, Flavivirus envelope glycoprotein E.

IRCIQVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGTQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 54

isol-ZJ03.AMM39806.1.China/291-794 Flavivirus envelope glycoprotein E.

IRCIQVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGARRMAVLG
DTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 55

isol-FSS13025.AFD30972.1.Cambodia/291-794 Flavivirus envelope glycoprotein E.

IRCIQVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT

PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRLLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGGALNSLGKGIHQIFGAAGKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 56

isol-Z1106032.ALX35660.1.Suriname/291-794 Flavivirus envelope glycoprotein E. [Zika virus]

IRCIQVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTNSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRLLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGGALNSLGKGIHQIFGAAGKSLFGGMSWFSQILIGTLLMWLGLNNAKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 57

isol-Z1106033.ALX35659.1.Suriname/291-794 Flavivirus envelope glycoprotein E. [Zika virus]

IRCIQVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTNSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRLLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGGALNSLGKGIHQIFGAAGKSLFGGMSWFSQILIGTLLMWLGLNNAKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 58

isol-BeH828305.AMK49165.1.Brazil/291-794 Flavivirus envelope glycoprotein E.

IRCIQVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTNSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRLLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGGALNSLGKGIHQIFGAAGKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 59

isol-GD01.AMK79468.1.China/291-794 Flavivirus envelope glycoprotein E.

IRCIQVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTNSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNGTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRLLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGGALNSLGKGIHQIFGAAGKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 60

isol-Z1106031.ALX35661.1.Suriname/291-794 Flavivirus envelope glycoprotein E.

IRCIQVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTNSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMMNNKHVLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRLLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VLAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGGALNSLGKGIHQIFGAAGKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 61

ACD75819.1.Micronesia/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPAVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKLRKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVLIFLSTAVSA

SEQ ID NO: 62

KU681082.3/308-811 Zika virus isolate Zika virus/H.sapiens-tc/PHL/2012/CPC-0740, Philippines, Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKLRKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVLIFLSTAVSA

SEQ ID NO: 63

isol-Zika_virus%H.sapiens-tc%PHL%2012%CPC-0740.AMD61711.1.Philippines/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKLRKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVLIFLSTAVSA

SEQ ID NO: 64

isol-BeH823339.AMK49164.2.Brazil/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEATISDIASDSRCPTQGEAYLDKQS
DTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITP
NSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
AVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKLRKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

SEQ ID NO: 65

isol-P6-740.AEN75264.1.Malaysia/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDXGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKLRKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGDKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLG
DTAWDFGSGVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVLIFLSTAVSA

SEQ ID NO: 66

KU744693.1/326-829 Zika virus isolate VE_Ganxian, China, Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLAHKEWFHDIPLPWHAGAATGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKLRKGVSYSLCTAAFTFTKIPAETVDGTVTVEGQYGGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGD
TAWDFGSGVGGALNSLGKGIHQIIGAFAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSG

SEQ ID NO: 67

isol-VE_Ganxian.AMK79469.1.China/291-794 Flavivirus envelope glycoprotein E.

IRCIQVSNRDFVEGMSGGTWVDVVLEHGGCVTAMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSQMLVNDTGHETDENRAKVEIT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLAHKEWFHDIPLPWHAGAATGTPHWNNKEALVEFKDAHAKRQT
VVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKRLKMDKRLKGVSYSLCTAAFTFTKIPAETVDGTVTVVEGQYGGTDGPCK
VPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGDKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGD
TAWDFGVSVGALNSLGKGIHQIIGAFAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSG

SEQ ID NO: 68

ArD157995.AHL43503.1.-/291-794 Flavivirus envelope glycoprotein E.

ISCIGVSNRDLVEGMSGGTWVDVVLEHGGCVTEMAQDKPTVDIELVTMTVSNMAEVRSYCYEASLSDMASASRCPTQGEPSLDK
QSDTQSVCKRTLVDGRWGNGCGIFGKGSVLTCSKFTCKKMPGKSIQPENLEYRIMLPVHGSQHSQGMIVNDIGHETDENRAKVEV
TPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTTPHWNNKEALVEFKDAHAKRQ
TVVVLGSQEGAVHTALAGALEAEMDGAAGRLFSGHLKRLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQSAGTDGPC
KVPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGDKKITHHWHRSRGSTIGKAFEATVRGAKRMAVL
GDTAWDFGVSVGVFNSLGKGIHQIFGAFAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

SEQ ID NO: 69

MR_766.ABI54475.1.Uganda/291-788 Flavivirus envelope glycoprotein E.

IRCIQVSNRDFVEGMSGGTWVDVVLEHGGCVTMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ
SDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSQGMIVNDENRAKVEVTPNSPRA
EATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTTPHWNNKEALVEFKDAHAKRQTVVVLGS
QEGAVHTALAGALEAEMDGAAGRLFSGHLKRLKMDKRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQM
AVDMQTLTPVGRITANPVITESTENSKMMLELDPFGDSYIVIGVGDKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGDTAWDF
GVSQGVFNSLGKGIHQIFGAFAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

SEQ ID NO: 70

5'-(dIdC)₁₃-3'

dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC

SEQ ID NO: 71

KLK peptide

KLKLLLLLKLK

Provided below are examples of nucleic acid sequences of the genomes of Chikungunya, Japanese Encephalitis and yellow fever viruses that may be used in the methods, compositions, and/or vaccines described herein.

SEQ ID NO: 72

Chikungunya virus strain LR2006_OPY1, complete genome ACCESSION: DQ443544

ATGGCTGCGTGAGACACACGTAGCCTACCACTTTCTTACTGCTCTACTCTGCAAAGCAAGAGATTAATAACCCATCATGGATC
CTGTGTACGTGGACATAGACGCTGACAGCGCCTTTTGAAGGCCCTGCAACGTGCGTACCCCATGTTTGAGGTGGAACCAA
GGCAGGTACACCGAATGACCATGCTAATGCTAGAGCGTTCTCGCATCTAGCTATAAACTAATAGAGCAGGAAATTGACCC
CGACTCAACCATCTGGATATCGGCAGTGCGCCAGCAAGGAGGATGATGTCGGACAGGAAGTACCACTGCGTCTGCCCAGT
GCGCAGTGCGGAAGATCCCAGAGACTCGCCAATTATGCGAGAAAGCTAGCATCTGCCGACAGGAAAAGTCTGGACAGAA
ACATCTCTGGAAAGATCGGGGACTTACAAGCAGTAATGGCCGTGCCAGACACGGAGACGCCAATTCTGCTTACACACAG
ACGTCTCATGTAGACAGAGAGCAGACGTCGCTATATACCAAGACGTCTATGCTGTACACGACCCACGTCGCTATACACCA
GGCGATTAAAGGGGTCCGAGTGGCGTACTGGGTTGGGTTGACACAACCCCGTTCATGTACAATGCCATGGCGGGTGCCTA
CCCCTCACTCTGACAACTGGGCAGATGAGCAGGTACTGAAGGCTAAGAACATAGGATTATGTTCAACAGACCTGACGGA

AGGTAGACGAGGCAAGTTGTCTATTATGAGAGGGAAAAAGCTAAAACCGTGCGACCGTGTGCTGTTCTCAGTAGGGTCAAC
GCTCTACCCGGAAGCCGCAAGCTACTTAAGAGCTGGCACCTGCCATCGGTGTTCCATTTAAAGGGCAAACCTCAGCTTCACA
TGCCGCTGTGATACAGTGTTTCGTGTGAGGGCTACGTCGTTAAGAGAATAACGATGAGCCCAGGCCCTTTATGGAAAAACC
ACAGGGTATGCGGTAACCCACCACGCAGACGGATTCTGATGTGCAAGACTACCGACACGGTTGACGGCGAAAGARTGTCA
TTCTCGGTGTGCACATACGTGCCGGCGACCATTTGTGATCAAATGACCGGCATCCTTGCTACAGAAGTCACGCCGGAGGATG
CACAGAAGCTGTTGGTGGGGCTGAACCAGAGAATAGTGTTAACGGCAGAACGCAACGGAATACGAACACCATGAAAAAT
TATCTGCTTCCCGTGGTCGCCAAGCCTTCAGTAAGTGGGCAAAGGAGTGCCGGAAAGACATGGAAGATGAAAACTCCTG
GGGGTCAGAGAAAGAACTGACCTGCTGCTGTCTATGGGCATTCAAGAAGCAGAAAACACACACGGTCTACAAGAGGCCT
GATACCCAGTCAATTGAGAAGGTTGAGGCCGAGTTTGACAGCTTTGTGGTACCGAGTCTGTGGTCTCGGGGTTGTCAATCC
CTTTGAGGACTAGAATCAAATGTTGTTAAGCAAGGTGCCAAAAACCGACCTGATCCCATACAGCGGAGACGCCCCGAGAAG
CCCGGGACGCAGAAAAAGAAGCAGAGGAAGAAGCAGAGAAGCAGAACTGACTCGCGAAGCCCTACCACCTCTACAGGCAGCA
CAGGAAGATGTTGAGTCTGAAATCGACGTGGAACAGCTTGAGGACAGAGCGGGCGCAGGAATAATAGAGACTCCGAGAG
GAGCTATCAAAGTTACTGCCAACCAACAGACCACGTCGTGGGAGAGTACCTGGTACTCTCCCCGAGACCGTACTACGTAG
CCAGAAGCTCAGTCTGATTACGCTTTGGCGGAGCAAGTGAAGACGTGCACGCACAACGGACGAGCAGGGAGGTATGCGG
TCGAAGCGTACGACGGCCGAGTCTAGTGCCCTCAGGCTATGCAATCTCGCCTGAAGACTCCAGAGTCTAAGCGAAAGCG
CAACGATGGTGTATAACGAAAGAGAGTTCGTAACAGAAAGCTACACCATATTGCGATGCACGGACGACCTGAACACCG
ACGAAGAGTCGTATGAGCTGGTGAGGGCAGAGAGGACAGAACACGAGTACGTCTACGACGTGGATCAGAGAAGATGCTG
TAAGAAGGAAGAAGCCGAGGACTGGTACTGGTGGGCGACTTGACTAATCCGCCCTACCACGAATTCGCATATGAAGGGCT
AAAAATCCGCCCTGCCTGCCATACAAATTCAGTCATAGGAGTCTTCGGAGTACCGGGATCTGGCAAGTCAGTATTATC
AAGAACCTAGTTACCAGGCAGGACCTGGTGACTAGCGGAAAGAAAGAAAAGTCCAAGAAATCACCACCGACGTGATGAG
ACAGAGAGGTCTAGAGATATCTGCACGTACGGTTGACTCGCTGCTCTTGAATGGATGCAACAGACCAAGTCGACGTGTTGA
CGTAGACGAGGGCTTTGCGTGCCACTCTGGAACGCTACTTGCTTTGATCGCCTTGGTGAGACCAAGGCAGAAAGTTGACTT
TGTGGTGACCCGAAGCAGTGCGGCTTCTCAATATGATGCAGATGAAAGTCAACTATAATCACAACATCTGCACCCAAGTGT
ACCACAAAAGTATCTCCAGGCGGTGTACACTGCCTGTGACCGCCATTGTGTCATCGTTGCATTACGAAGGCAAAATGCGCAC
TACGAATGAGTACAACAAGCCGATTGTAGTGGAACACTACAGGCTCAACAAAACCTGACCCTGGAGACCTCGTGTTAACGTG
CTTCAGAGGGTGGGTTAAACAACCTGCAAATTCGATGATGAGGTCATGACAGCAGCCGCATCCCAAGGGTTAAC
CAGAAAAGGAGTTTACGCAGTTAGACAAAAGTTAATGAAAACCGCTCTATGCATCAACGTGAGAGCAGTCAACGTACTC
CTAACGCGTACGGAAGGTAACTGGTATGGAAGACACTTTCCGGCGACCCGTGGATAAAGACGCTGCAGAACCCACCGAAA
GGAAACTTCAAAGCAACTATTAAGGAGTGGGAGGTGGAGCATGCATCAATAATGGCGGGCATCTGCAGTCACCAATGAC
CTTCGATACATTCCAAAATAAAGCCAACGTTTGTGGGCTAAGAGCTTGGTCCCTATCCTCGAAACAGCGGGGATAAACTA
AATGATAGGCAGTGGTCTCAGATAATTCAAGCCTTCAAAGAAGACAAAGCATACTCACCTGAAGTAGCCCTGAATGAAATAT
GTACGCGCATGTATGGGGTGGATCTAGACAGCGGGCTATTTTCTAAACCGTTGGTGTCTGTGATTACGCGGATAACCACTG
GGATAATAGGCCTGGAGGGAAAAATGTTCCGATTAAACCCGAGGCAGCATCCATTCTAGAAAGAAAGTATCCATTACAAAA
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GGCCAACAGGAGACTACCACACTCATTAGTGCCGAACACCGCCAGTAAAGGGGAAAGAATGGAATGGCTGGTTAACA
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SEQ ID NO: 73

Japanese encephalitis virus strain SA14-14-2, complete genome, ACCESSION: KC517497

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SEQ ID NO: 75

Japanese encephalitis virus strain SA14-14-2, complete genome, ACCESSION: AF315119

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SEQ ID NO: 76

>gi|564014614|gb|KF769015.1| Yellow fever virus strain 17D-204, complete genome

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Attenuated Chikungunya "Delta5nsP3" sequence

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SEQ ID NO: 78

ZIKV Sequence H/PF/2013 as sequenced

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GACTGCAGTGGAAGCTGTGAACGATCCAAGTTCTGGGCTCTAGTGGAACAAGGAAAGAGAGACCACCTGAGAGGAGAGTG
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SEQ ID NO: 79

AHZ13508.1, Zika virus polyprotein from Polynesian outbreak (H/PF/2013)

MKNPKKSGGFRIVNMLKRGVARVSPFGLKRLPAGLLGHGPIRMVLAILAFLRFTAIPSLGLINRWGSGVKKKEAMEIHKFKKDL
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 AAAIAWLLGSSTSQKVIYLVILLIAPAYSIRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTNSMAE
 VRSYCYEASIDMASDSRCPQTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQENLEYRIML
 SVHGSQHSQMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGLDCEPRTGLDFSDLYLTMNNKHVLVHKEWFHDIPLPHW
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 AFTFTKIPAEHLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKIT
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 NIMWRSVEGELNAILEENGVLTVVVGSVKNPMWRGPQRLPVPVNLPHGWKAWGKSYFVRAAKTNNFVVDGDTLKECPLKH
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 EALGFLNEDHWMGRENSGGGVEGLGLQRLGYVLEMSRIPGGRMYADDTAGWDTRISRFDLNEALITNQMEKGRALALAIKY
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RVSPGAGWSIRETACLAKSYAQMWQLLYFHRRDLRLMANAICSSVPVDWVPTGRTTWSIHGKGEWMTTEDMLVVWNRVWIEE
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SEQ ID NO: 81
9321_Zika_PF_1R
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SEQ ID NO: 82
9322_Zika_PF_2F
ttaggatccTCACTAGACGTGGGAGTG

SEQ ID NO: 83
9323_Zika_PF_2R
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SEQ ID NO: 84
9324_Zika_PF_3F
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SEQ ID NO: 85
9325_Zika_PF_3R
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SEQ ID NO: 86
9326_Zika_PF_4F
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SEQ ID NO: 87
9327_Zika_PF_4R
taactcgAGTGGTGGGTGATCTTCTTCT

SEQ ID NO: 88
9328_Zika_PF_5F
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SEQ ID NO: 89
9329_Zika_PF_5R
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SEQ ID NO: 90
9330_Zika_PF_6F
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SEQ ID NO: 91
9331_Zika_PF_6R
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9332_Zika_PF_7F

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9333_Zika_PF_7R

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SEQ ID NO: 94

9334_Zika_PF_8F

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SEQ ID NO: 95

9335_Zika_PF_8R

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SEQ ID NO: 96

9336_Zika_PF_9F

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SEQ ID NO: 97

9337_Zika_PF_9R

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SEQ ID NO: 98

9338_Zika_PF_10F

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SEQ ID NO: 99

9339_Zika_PF_10R

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SEQ ID NO: 100

9340_Zika_PF_11F

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9341_Zika_PF_11R

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SEQ ID NO: 102

9342_Zika_PF_12F

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SEQ ID NO: 103

9343_Zika_PF_12R

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SEQ ID NO: 104

9344_Zika_PF_13F

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SEQ ID NO: 105

9345_Zika_PF_13R

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SEQ ID NO: 106
9346_Zika_PF_14F
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SEQ ID NO: 107
9347_Zika_PF_14R
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SEQ ID NO: 108
9348_Zika_PF_15F
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SEQ ID NO: 109
9349_Zika_PF_15R
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SEQ ID NO: 110
9350_Zika_PF_16F
taactcgaGTGATCAGRGCCCCAGC

SEQ ID NO: 111
9351_Zika_PF_16R
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SEQ ID NO: 112
9352_Zika_PF_17F
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SEQ ID NO: 113
9353_Zika_PF_17R
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SEQ ID NO: 114
9354_Zika_PF_18F
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SEQ ID NO: 115
9355_Zika_PF_18R
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SEQ ID NO: 116
9356_Zika_PF_19F
taactcGAGCCCAGAACCTTGGATC

SEQ ID NO: 117
9357_Zika_PF_19R
ttaggatcCAGACCCCAAGAAGGC

SEQ ID NO: 118
9358_Zika_PF_20F
taactcgagCCCCTTTGGTCTGTCT

SEQ ID NO: 119
9359_Zika_PF_20R
ttaggatccAGGAAGGATGTATGCAGATG

SEQ ID NO: 120
9360_Zika_PF_21F
taactcgagACATTGCGCATATGATTTTG

SEQ ID NO: 121
9361_Zika_PF_21R
ttaggatccAGGAAGGACACACAAGAGT

SEQ ID NO: 122
9362_Zika_PF_22F
taactcgagACAGGCTGCACAGCTTT

SEQ ID NO: 123
9363_Zika_PF_22R
ttaggatccTCTCTCATAGGGCACAGAC

In some embodiments, the Zika virus has a polyprotein, including an envelope (E) protein, with an amino acid sequence provided by any one of SEQ ID NO: 14-69 or 78. In some embodiments, the polyprotein or E protein sequence is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to any one of SEQ ID NOs: 2-69 or 78.

The terms "identical" or percent "identity" in the context of two or more nucleic acids or amino acid sequences refer to two or more sequences or subsequences that are the same. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (*e.g.*, at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) over a specified region or over the entire sequence, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length. In some embodiments, the identity exists over the length of a protein, such as the E protein.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. Methods of alignment of sequences for comparison are well known in the art. See, *e.g.*, by the local homology algorithm of Smith and Waterman (1970) Adv. Appl. Math. 2:482c, by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443, 1970, by the search for similarity method of Pearson and Lipman. Proc. Natl. Acad. Sci. USA 85:2444, 1988, by computerized

implementations of these algorithms (GAP, BESTFIT, FASTA, Jalview and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), by multi sequence alignment implementation using e.g. CLUSTALW (Larkin et al., (2007). *Bioinformatics*, 23, 2947-2948.) or MAFFT (Kato & Toh 2008 *Briefings in Bioinformatics* 9:286-298), or by manual alignment and visual inspection (see. *e.g.*, Brent et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (Ringbou ed., 2003)). Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., *Nuc. Acids Res.* 25:3389-3402, 1997 and Altschul et al., *J. Mol. Biol.* 215:403-410, 1990, respectively.

EXAMPLES

Example 1: Development of a purification process for live attenuated Chikungunya virus vaccine produced in Vero cells

A downstream process was developed for the purification of infectious Chikungunya virus particles whereby non-infectious virus particles and aggregates are removed by the addition of protamine sulphate. The unexpected and novel purification properties of protamine sulphate (PS) were evaluated in purification processes for Chikungunya Virus (ChikV) as follows:

A downstream purification process for the attenuated Chikungunya virus mutant “ $\Delta 5nsP3$ ” (as described by Hallengård et al., 2014, *supra* and provided by SEQ ID NO: 77) produced under standard cell culture conditions in Vero cells was developed. The attenuated $\Delta 5nsP3$ Chikungunya virus was derived from the strain LR2006-OPY1, the complete genome of which is provided herein as SEQ ID NO: 72. Briefly, the downstream process consists of crude harvest filtration followed by concentration and diafiltration on a tangential flow filtration (TFF) system. Host cell DNA and host cell proteins were reduced by precipitation with protamine sulphate and by batch adsorption, respectively. Sucrose density gradient centrifugation was done as a final polishing step. Out of 16 roller bottles 1×10^{12} total PFU were purified with an overall DSP process yield of 10-15 % (~1 log₁₀ TCID₅₀ loss). Sucrose gradient pool samples were characterized with regard to product-related impurities, such as hcDNA, HCP and endotoxins and met safety criteria.

Harvest of Vero cell culture medium containing ChikV $\Delta 5nsP3$

ChikV $\Delta 5nsP3$ was grown on Vero cells in roller bottles. A first harvest was performed after 24 hours post infection (hpi; day 1 harvest) and stored at 2-8°C until further processing. After the first harvest, fresh medium was added and the roller bottles were returned to the incubator. A second harvest was done after 48 hours post infection (day 2 harvest) and stored at 2-8°C.

Filtration of crude cell culture harvest

At both harvest timepoints, the crude harvest was immediately filtered using a 0.2 μm filter capsule (GE ULTA™ CG, 2 inch). The filtered harvest after 48 hpi was pooled together with the 24 hpi harvest and the pooled filtered harvest material was immediately further processed by ultrafiltration.

Purification of ChikV $\Delta 5nsP3$ by tangential flow filtration (TFF)

The pooled filtered harvest material was further processed by tangential flow filtration (TFF) in order to concentrate the harvest, reduce host cell proteins and replace the depleted cell culture medium with a defined buffer system (buffer exchange). A Millipore TFF system (Millipore Pellicon II mini membrane holder) equipped with a 100 kDa cutoff PES membrane module (Pellicon2 Biomax, 1000 cm²) was used for concentration and buffer exchange. A Pellicon2 Biomax membrane module was mounted on the Pellicon II mini filter holder and the device was connected to a peristaltic pump. The system was first rinsed with ultra-pure water and then sanitized by recirculation of 0.1 M NaOH for 60 min. In case the system was not used immediately, it was stored in 0.1 M NaOH until use. Prior to use the system was rinsed with 1 L of RO-water followed by buffer A until the permeate pH value was constant at pH 7.4 ± 0.2.

Adjustment of the ChikV Δ5nsP3 harvest (pH, salt)

The pooled filtered harvest material was adjusted to a final concentration of 25 mM Tris and 150 mM NaCl using stock solutions of both components (see Table 1). This adjustment was done to increase buffering capacity and to reduce unspecific adsorption to the membrane. The necessary volumes of stock solutions D (1 M Tris, pH 7.4) and E (4.5 M NaCl) were calculated as follows:

Volume of stock solution D (1 M Tris, pH 7.4) added to pooled harvest = Volume of pooled filtered harvest / 40

Volume of stock solution E (4.5 M NaCl) added to pooled harvest = Volume of pooled filtered harvest / 30

Example: 4 L harvest obtained from 20 RB (850cm²) would require addition of 100 mL stock solution D (1 M Tris, pH 7.4) and 133 mL stock solution E (4.5 M NaCl).

The calculated volumes of stock solution D and Buffer E were added to the pooled filtered harvest under gentle stirring. The adjusted harvest was then stirred using a magnetic stirrer for 5 minutes at room temperature.

Concentration and diafiltration of the ChikV Δ5nsP3 harvest by TFF

In a first step, the adjusted harvest material was concentrated approximately 10 fold. The feed flowrate was approximately 220 mL/min. The transmembrane flux at a transmembrane pressure of approximately 0.6 bar was in the range of 90 ± 5 mL/min per 1000 cm² membrane. After concentration, the cell culture medium was exchanged against 25 mM Tris, 150 mM NaCl, pH 7.5, by continuous diafiltration with 6 volume exchanges. The diafiltration buffer was supplied to the feed vessel from a measuring cylinder by a second peristaltic pump set to a flowrate of approximately 90 mL/min. Minor flowrate adjustments of the second peristaltic pump in the range of ± 10 mL/min were done manually to ensure a constant volume of

harvest in the feed vessel. After 6 volume exchanges, diafiltration was stopped. The liquid remaining in the membrane module was recovered by pumping the module empty with air.

Sucrose addition to diafiltrated ChikV Δ5nsP3 material

After diafiltration, sucrose stock solution H (50% (w/w) sucrose solution) was added to the diafiltrated material to achieve a final sucrose concentration of 10% (w/w). The volume of buffer H was calculated as follows:

Volume of stock solution H added (mL) = Volume (mL) of diafiltrated ChikV material x 0.25
(dilution factor = 1:4) (i.e., final sucrose concentration is 10%)

Example: 400 mL diafiltrated ChikV solution would require addition of 100 mL stock solution H (50% sucrose).

The calculated volume of solution H was added to the diafiltrated ChikV Δ5nsP3 material under gentle stirring and the solution was then stirred using a magnetic stirrer for a further 5 minutes at room temperature. (At this stage of the process the material can be either immediately further processed or stored frozen (< -65°C, hold step).)

DNA reduction by protamine sulphate precipitation

A DNA precipitation step using protamine sulphate (PS) was performed to reduce hcDNA. Protamine sulphate stock solution L (50 mg/mL PS in PBS) was added to the diafiltrated ChikV Δ5nsP3 material to a final nominal concentration of ~1.6 mg/mL. The necessary volume of stock solution L was calculated as follows:

Volume of stock solution L (50 mg/mL PS) added = Volume of diafiltrated ChikV Δ5nsP3 material in 10% sucrose / 31

Example: 500 mL diafiltrated ChikV Δ5nsP3 solution in 10% sucrose would require addition of 16 mL stock solution L (50 mg/mL PS in PBS).

The protamine sulphate stock solution was added while stirring the ChikV Δ5nsP3 material using a magnetic stirrer followed by incubation at 2-8°C for 30 minutes. After incubation, the precipitate was not removed. The material was immediately further processed by batch adsorption with Capto™ Core 700 chromatography media.

Batch adsorption with Capto™ Core 700

To reduce HCPs, a batch adsorption step with Capto™ Core 700 (CC700) chromatography medium was performed after DNA precipitation. CC700 slurry (50% slurry in buffer A) was added directly to the protamine sulphate treated material. The required slurry volume was determined based on the volume of Δ5nsP3 ChikV harvest material (d1+d2) and was calculated as follows:

Volume of CC700 slurry added to PS-treated concentrated harvest (mL) = Volume of $\Delta 5\text{nsP3}$ ChikV harvest material (mL) $\times$ 0.02 (dilution factor = 1:50) (i.e., final concentration of CC700 is 1%)

After slurry addition, the material was incubated at 4°C for 15 minutes under constant agitation using a magnetic stirrer. After incubation, the CC700 solid matter was allowed to settle by gravity for 10 minutes. The $\Delta 5\text{nsP3}$ ChikV material was then removed from the top of the solution in order to avoid blocking of the filter by the CaptoCore particles. The remaining CaptoCore particles and the DNA precipitate were then removed from the solution by filtration using a 0.2 μm Mini Kleenpak EKV filter capsule (Pall). The resulting filtrate was further processed by sucrose density gradient centrifugation.

Sucrose density gradient centrifugation

Sucrose density gradient centrifugation (SGC) was used for final concentration and polishing of the $\Delta 5\text{nsP3}$ ChikV material. The $\Delta 5\text{nsP3}$ ChikV material was loaded on top of a solution consisting of three layers of sucrose with different densities. The three sucrose layers were selected based on a preliminary study which showed the formation of a linear sucrose gradient and good separation of the virus particles from residual contaminants. The optimal volumes of the sucrose solutions were determined empirically. The volumes of individual layers for a centrifugation at 500 mL scale are shown in Table 3.

Table 3. Sucrose concentrations and volumes (500 mL scale).

Solution	Volume (mL)
Harvest with 10% sucrose	360
15% sucrose	40
35% sucrose	40
50% sucrose	60
Total volume	500

Preparation of the sucrose gradient

The sucrose gradient bottles (500 mL) were prepared by underlaying the individual sucrose layers. A 3.5 mm ID plastic tube was attached to 60 cm of peristaltic pump tubing. The plastic tube was mounted on a laboratory stand using a clamp and placed into the centrifuge bottle. The nozzle of the plastic tube was placed at the bottom of the bottle. Using a peristaltic pump set to a flow rate of 25 mL per minute, the $\Delta 5\text{nsP3}$ ChikV material and the sucrose solutions were pumped into the cylinder. A measuring cylinder was used as a feed vessel. The first solution pumped was the $\Delta 5\text{nsP3}$ ChikV material as it had the lowest density (10% sucrose (w/w)). Following the addition of the $\Delta 5\text{nsP3}$ ChikV material, the sucrose solutions were pumped in ascending order starting with the lowest (15%), followed by the 35% sucrose solution and finishing with the highest density sucrose solution (50%). After all sucrose solutions were transferred,

the plastic tubing was carefully removed in order not to disturb the layers. An illustration of a completed gradient is shown in Figure 14.

Centrifugation

Prior to centrifugation a Beckman Avanti JXN-26 centrifuge equipped with rotor Beckman 10.500 was pre-cooled to 4°C. The prepared SG bottles were carefully transferred into the pre-cooled (4°C) rotor so as to not to disturb the sucrose layers. The bottles were centrifuged at 10,000 rpm (~18,500 rcf) at 4°C for 17-20 hours. (In case a different centrifuge system with a different rotor would be used, the necessary speed and centrifugation times would need to be calculated based on the k-factor in order to achieve comparable centrifugation efficiency.)

Sucrose gradient harvest

Harvesting of the sucrose gradient following centrifugation was done manually using a peristaltic pump. A 3.5 mm ID plastic tube attached to 60 cm of peristaltic pump tubing was used for harvesting the sucrose gradient. The 500 mL bottle containing the centrifuged gradient was mounted onto a laboratory stand in a tilted position (~12°) using a clamp. The plastic tubing was then placed into the bottle touching the bottom edge of the bottle and was fastened in position using a clamp. This resulted in a small gap of 1-2 mm between the tubing inlet and the bottom of the bottle (see Fig. 14). Using a peristaltic pump set to a flow rate of 60 mL per minute, the gradient was harvested and manually split into 5 mL fractions. A third of the bottle volume was harvested and the rest was discarded. The fractions were immediately tested by measuring UV absorbance in a plate reader as described below.

Analysis of fractions by UV absorbance and SEC-HPLC

UV absorbance measurement was used as primary method for analysis of the sucrose gradient fractions. Absorbance at 214, 280 and 260 nm was tested immediately after fractionation was completed. Briefly, a 100 µL sample of each fraction was transferred into a 96 well plate and absorbance at 214, 260 and 280 nm was measured using a plate reader. The absorbance values were plotted against the fraction number. A representative profile is shown in Fig. 11A. The Δ5nsP3 ChikV containing fractions were indicated by a peak in all three measured wavelengths (Fig. 11A, grey shaded area). The presence of impurities was indicated by an increase of the UV214 signal after the main peak. The fractions comprising the main peak were pooled from the peak start to the valley of the 214 nm curve. This method can be used as single method for pooling Δ5nsP3 ChikV fractions.

After identification of the virus containing fractions, the respective fractions were pooled. Pooling criteria for SGC fractions were based on UV 260 nm data, e.g. start of pooling at ~10% of peak maximum, end of pooling at ~30% of peak maximum. (Final pooling criteria at a manufacturing scale may need to be determined empirically.) The sucrose gradient pool was either stored at < -65 °C or immediately further formulated to drug substance (DS).

Size exclusion chromatography

The final pooled SGC fractions containing purified infectious $\Delta 5\text{nsP3}$ ChikV particles were analyzed for purity by SEC-HPLC. In brief, SEC was performed as follows: a Superose 6 10/300 Increase column (GE Healthcare) equilibrated with PBS + 250 mM NaCl, pH 7.4 at 1 ml/min and 25°C, was used to detect ChikV particles at 214 nm detection wavelength in the pooled samples. SEC-HPLC is a semi-quantitative (relative yield) and qualitative (purity) method that separates intact virus particles from virus aggregates and host cell proteins (HCPs). The method cannot distinguish between infectious and non-infectious virus particles due to their identical retention time.

As shown in Fig. 11B, there were two defined peaks identified by SEC: the $\Delta 5\text{nsP3}$ ChikV peak and a peak corresponding to buffer components. The SGC step yield based on SEC-HPLC data for pooled fractions F6-F11 was estimated at ~70 %. The final purity of the $\Delta 5\text{nsP3}$ ChikV SGC pool, based on SEC-HPLC analysis, was estimated at >95%.

SDS-PAGE and silver stain

SDS-PAGE silver stain was performed in order to qualitatively assess sample purity throughout the purification process from the first crude harvest through SGC. Briefly, ChikV process samples analyzed by SDS-PAGE/silver stain were diluted 1:1.33 with LDS buffer and were heated to 70°C for 5 minutes. The samples were loaded onto 4-12 % Bis-Tris Gels (NuPAGE). Silver staining was done using the Silver Express staining kit (Invitrogen).

A silver-stained gel of a representative ChikV $\Delta 5\text{nsP3}$ purification is shown in Fig. 11C. The viral proteins E1, E2 and C are marked on the right-hand side of the gel. The final SGC pool (fraction 7- fraction 11) is shown in lane 12. Note that a defined HCP band migrating between ChikV protein E2 and C still appears after CaptoCore700 treatment that has been identified as a single band in SDS-PAGE. This impurity is removed by sucrose gradient centrifugation, but can still be seen in fractions 13 and 14 (corresponding to lanes 14 and 15 of Fig. 11C).

Enrichment of infectious Δ5nsP3 ChikV particles by PS treatment

Although generally used as a method of removing contaminating hcDNA, it was observed in the course of the present invention that PS treatment also removes virus aggregates and HCPs. Size exclusion chromatography (SEC-HPLC, as described above) was used throughout the purification process to determine the purity of the ChikV virus relative to impurities which also generate UV absorption.

As can be seen in Fig. 12B, treatment with PS reduces not only host cell proteins and low molecular weight contaminants of the Δ5nsP3 ChikV preparation, but also reduces the SEC area corresponding to virus products, including aggregates as indicated. A surprising finding, however, was that even a reduction of the total SEC area by 86% (in a representative experiment shown in Fig. 12A, grey portion of bars) did not result in a concomitant reduction in infectious virus particles as measured by TCID₅₀ (Fig. 12A, left axis). Instead, even though a large percentage of virus particles were removed by PS treatment, the majority of infectious particles remained. This observation indicates that PS treatment selectively enriches infectious virus particles from a larger pool of total virus particles present in the crude harvest.

TCID₅₀ was performed to quantify infectious virus particles during the course of the purification process and to assign an active virus titer to final drug substance and drug product samples. Briefly, Vero cells were seeded at 2×10^4 cells per well in 100 μL medium (EMEM with 2 mM L-Glutamine + 5 % FBS + 1 % antibiotic/antimycotic) in 96-well TC-treated flat-bottom plates and incubated overnight at 35°C / 5% CO₂. On day two, Vero cell monolayers were infected by adding 100 μL of 1:10 serial dilutions of test samples to each of quintuplicate wells seeded with Vero cells and incubated at 35°C / 5% CO₂. On day seven, plaques were counted by visualization under a microscope. The TCID₅₀ was calculated according to the Reed & Munch endpoint calculation method (Reed, L.J.; Muench, H. (1938) A simple method of estimating fifty percent endpoints, The American Journal of Hygiene 27: 493–497).

Furthermore, electron microscopy of Δ5nsP3 ChikV samples before and after PS treatment showed that not only large aggregates but also smaller non-infectious virus-like particles (essentially not fully assembled particles lacking the RNA genome) were effectively removed by PS (Fig. 13).

This enrichment of infectious virus particles was also observed when analyzing day one and day two crude harvests separately. As presented in Table 4, the SEC area (total virus particles) of the day 1 harvest remains roughly the same after PS treatment; whereas a large decrease in virus peak area is seen for the day 2 harvest after PS treatment. This observation was confirmed by MALLS analysis of the virus preparation, wherein it was seen that a higher percentage of virus particles were of the correct size

following PS treatment. Similarly to the results shown in Fig. 12, day 1 and day 2 harvests showed no reduction in infectious particles as measured by TCID₅₀ following PS treatment, indicating that mainly non-infectious, immature and/or aggregated virus particles are removed during the PS treatment and infectious particles are enriched in the preparation.

The PS-treated samples were further purified by sucrose gradient centrifugation (see Fig. 14 for a schematic preparation of an optimized sucrose gradient). An optimal sucrose gradient was determined experimentally as shown in Fig. 15. Results of the further purification of PS-treated ChikV on the optimized sucrose gradient of the invention are shown in Fig. 15D.

Table 4. Overview of the process of $\Delta 5\text{nsP3}$ ChikV purification as described in Example 1. SEC-MALLS analysis of harvests before and after PS treatment shows the removal of larger virus particles (aggregates), an effect that is particularly pronounced for day 2 harvests.

	SEC	MALLS		Infectious particles
	Area [mAU*min]	Total particles/mL	% correct size (20-40 nm)	TCID ₅₀ log 10
Harvest 1 (H1)	57	1.17E+11	49%	10.2
H1 + protamine sulphate	53	1.33E+11	81%	10.0
Harvest 2 (H2)	36	4.60E+09	3%	7.9
H2 + protamine sulphate	2	8.80E+09	59%	7.9
Combined Harvests (C)	67	2.60E+10	14%	9.9
C + protamine sulphate	24	8.00E+10	72%	10.1

Finally, an overview of the relative amounts of $\Delta 5\text{nsP3}$ ChikV particles and other components as measured by SEC-HPLC at various steps throughout the entire virus purification process from crude harvest (a) to the final SGC purified pool is presented in Fig. 16. In sum, not only are the vast majority of contaminants and undesired products removed by the process, infectious ChikV particles are highly purified. As shown by the previously presented data, the final preparation is a highly enriched preparation of infectious ChikV particles.

Drug Substance (DS) Formulation

The pooled SGC fractions are diluted with DS formulation buffer M (10 mM Tris, 5% Sucrose (w/w), 1% (10 mg/mL) rHSA, pH 7.4 $\pm$ 0.2). The final target volume of DS should be in the range of approximately 2 L. Based on current data the estimated range of the dilution factor might be 1:20 to 1:50.

Final DS sterile filtration

The final DS was filtered under aseptic conditions in a laminar flow hood using a sterility grade 0.2 µm syringe filter (e.g. 0.2 µm Mini Kleenpak EKV filter capsule with 220 cm² filter surface, Pall).

Quantification of host cell DNA (hcDNA) host cell protein (HCP) and endotoxin

The residual host cell DNA content of the sucrose gradient pool samples was determined by using the qPCR based assay. The DNA content in SGC pool was determined to be ≤ 0.002 ng/mL. The presence of residual host cell protein (HCP) from Vero cells was determined by ELISA. Residual host cell proteins present in the sucrose gradient pool samples were quantified using the Vero Cell HCP ELISA kit (Cygnus, F500). The residual host cell protein content in SGC pool was determined to be ≤ 200 ng/mL.

Endotoxin content of the SGC pool and DS was measured by Endosafe[®]-PTS[™] system (Charles River). The system uses Limulus Amebocyte Lysate (LAL) reagents by a kinetic chromogenic methodology to measure color intensity directly related to the endotoxin content in a sample. Each cartridge contains precise amounts of a licensed LAL reagent, chromogenic substrate and an endotoxin control standard. Samples were diluted 1:100 in WFI. The SGC Pool F7-F11 was determined to be < 5.00 EU/mL; likewise, the Drug Substance was also determined to have < 5.00 EU/mL.

The following specifications for impurities in final Drug product were proposed: hcDNA < 10 ng/dose; Endotoxins < 50 EU/dose; HCP < 200 ng/dose. These residual specifications would already be met in the highly concentrated SGC pool (~ 10 log TCID₅₀/mL), which provides a high margin of safety considering the high dilution factor of SGC pool to final DP of $> 1:1000$.

Example 2: Production of a Zika drug substance suitable for application as a vaccine in humans and animals

Materials and Methods:

For the production of ZikaV the JEV process platform (Srivastava et al., Vaccine 19 (2001) 4557-4565; US 6,309,650B1) was used as a basis. Small changes of certain process steps were adapted to ZikaV properties and to improve purity. A short summary of the process steps is outlined below (see also Figure 17A and B). Briefly, the unexpected and novel purification properties of protamine sulphate (PS) were evaluated in purification processes for Zika Virus similarly as found above. Again non-infectious virus particle aggregates, HCP and other LMW impurities were removed by PS precipitation as shown by

removal of aggregate shoulder in SEC-HPLC and no loss of infectious virus titer by PS treatment (Figure 18). Further optimization of the Zika purification protocol is provided below.

Upstream:

- Roller Bottle based Vero cell expansion (25x850cm² CellBind):
- 5% CO₂, 35°C, MEM+2mM L-Glutamine + 10% FBS
- Infection with ZikaV research Master Seed Bank (rMSB) at MOI 0.01
- Virus Production without serum
- 5% CO₂, 35°C, MEM+2mM L-Glutamine
- Multiple harvests (days 2, 3, 5 and 7) with re-feed
- Sterile filtration of harvests and storage at 2-8°C until further processing

Downstream:

- Pooling of harvests and concentration by ultrafiltration (100kDa)
- Stabilization of concentrated harvest (Tris/10% sucrose) for storage if required (-80°C)
- Removal of hcDNA by Protamine Sulphate (2 mg/mL)
- Sucrose Gradient Purification (optimized three layered gradient)
- Formaldehyde Inactivation (0.02%, 22°C, 10 days), neutralization with Na-metabisulfite
- Dilution to DS antigen target content and formulation with Aluminium hydroxide (0.5mg Al/mL)

Zika Virus Strain H/PF/2013 was originally isolated from a 51-year-old woman (accession number KJ776791.1, also SEQ ID NO: 13 herein) from French Polynesia. A sample was obtained from the European Virus Archive (EVAg; Ref-SKU: 001v-EVA1545). Based on this material, a research master seed bank (rMSB) was prepared on Vero cells as the cell substrate and the genomic sequence was checked by sequencing. Because the genomic sequence at the 5' and 3' flanking sequences of Zika virus strain H/PF/2013 was unknown, primers for sequencing were designed in those regions based on other Zika virus strains whereas the internal primers were designed from the published sequence (SEQ ID NOs: 80 to 123, see also Table A). The sequence obtained from the rMSB by use of these primers is provided by SEQ ID NO: 78. There was 100% overlap of the sequence with the published sequence of Zika Virus Strain H/PF/2013 (SEQ ID NO: 13). However, we sequenced additional regions 5' (an additional 40 bp) and 3' (an additional 160 bp) represented in SEQ ID NO: 78. In a preferred embodiment, the Zika virus of the invention comprises SEQ ID NO: 78. The genomic RNA is somewhat longer than the sequence according to SEQ ID NO: 78 (perhaps an additional 200 bp). Additionally, a Zika virus adapted to a host cell such as e.g. Vero cells may be expected to contain one or more mutations. For these reasons, the Zika virus of the current invention comprises the sequence of SEQ ID NO: 78 or, preferably, a sequence with

at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 78. Furthermore, because the viral genome is likely to contain even further flanking regions to SEQ ID NO: 78; in one embodiment, the Zika virus of the invention contains the sequence of SEQ ID NO: 78 and optionally further comprises extensions at the 5' and/or 3' ends of at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120 or at least 130 nucleotides. In a preferred embodiment, the Zika virus comprises at least the coding sequence for the entire polyprotein of Zika Virus Strain H/PF/2013 of the invention i.e. the amino acid sequence of SEQ ID NO: 79 or a polyprotein with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 79. Furthermore, the Zika virus comprises at least the coding sequence for the E-protein of Zika Virus Strain H/PF/2013 of the invention SEQ ID NO: 47 or an E-protein thereof with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 47.

Virus growth on Vero Cells

Vero cells were grown in Eagle's minimal essential medium (EMEM) containing 10% fetal bovine serum (FBS). Roller bottle cultures of Vero cell monolayers were infected with Zika Virus Strain H/PF/2013 at a multiplicity of infection (moi) of 0.01 plaque forming units (pfu) per cell. After 2 hours of virus adsorption, the cultures were washed 3 times with PBS and fed with EMEM without FBS and incubated at +35°C with 5% CO₂. Infected Vero cell cultures were incubated until the virus titer reaches a desired level.

The culture medium was harvested at days 2, 3, 5 and 7 and were pooled from those harvest days and then centrifuged in a standard centrifuge. The supernatants were then filtered. Virus culture supernatants were concentrated by TFF ultrafiltration to remove cell culture media components and to reduce batch volume.

Evaluation of harvest Procedure

The current JEV harvest process has scheduled harvests on days 3, 5, 7 and 9 post infection. To mimic the JEV process roller bottles were infected with ZIKV bank P4-FBS at an MOI of 0.01 in infection medium (MEM with 2% FBS + 2mM L-glutamine) for 2 hours. After removing the inoculum the cells were washed twice with PBS and 200 mL production medium (MEM+ 2mM L-glutamine) was added.

After taking a sample on day 2 the first virus harvest was conducted on day 3 after infection. At this point significantly higher CPE could be observed compared to cells where virus was removed on day 2. Plaque assay analysis showed that the viral titers on day 2 were in the same range as for the standard harvesting

schedule. However, starting with the day 3 harvest, the observed titers were significantly lower correlating with the increased CPE observed compared to the standard harvest schedule. On day 5 post infection no more living cells could be observed at all and the experiment was terminated with a final day 5 harvest.

Table 5: The calculated titers per plaque assay are summarized in the list below.

	Log 10 PFU/mL
sample day2	7.02
harvest day 3	6.66
harvest day 5	6.26

This finding led to an optimized harvest schedule to better control of CPE and allow additional harvest day 5 and 7, see Figure 23. For both harvest days the optimized ZikaV protocol yield significant higher virus titers compared to the modified protocol showing that the time of the first harvest is crucial for production yields. Additionally first harvesting at day 3 results in maximum 2 harvest points whereas first harvesting at day 2 allows for 4 harvest points further increasing the yield gain.

Downstream Purification of Zika virus

The purification process was carried out at room temperature (18-22°C) unless stated otherwise. Virus purification started with concentration of filtered combined harvest using 100 kDa cut-off TFF ultrafiltration modules to remove cell culture media components and reduce batch volume. After concentration, the pooled filtered harvest material was adjusted to a final concentration of 25 mM Tris pH 7.5 and 10% sucrose (w/w) using stock solution of both components (see Figure 19 for SEC-HPLC of different harvests prior to PS treatment). This allowed for freezing the concentrated harvest at <-65°C if required.

Host cell DNA and protein reduction as well reduction of non-infectious virus aggregates in the concentrated material was achieved by precipitation with protamine sulphate (2 mg/mL) followed by sucrose density centrifugation (2-8°C) as final polishing step (see Figure 20 for SEC-HPLC of different harvests post PS treatment). The purification process was designed to be completed within 2 working days with SGC starting on end of day 1 followed by fractionation and SDS-PAGE analysis on day 2. The sucrose gradient fractions were stored at 2-8 °C during the SDS-PAGE analysis (Silver staining) to identify the pure fractions containing ZikaV (see Figure 21). After pooling the relevant fractions, the pool

was diluted and inactivated by Formalin. After pooling the relevant fractions of sucrose gradient centrifugation, the pool was diluted 1:3 in PBS and inactivated by Formalin (0.02% v/v, 200ppm). Fractions were subjected to analysis by SDS-PAGE.

Effect of PS treatment on virus recovery

Samples of individual 30x concentrated harvests days 2, 3, 5 and 7 were analysed before (Figure 19) and after PS (Figure 20) treatment by SEC-HPLC and plaque assay. SEC-HPLC was used for determination of relative total ZikaV content (active + inactive) expressed as peak area, whereas the rel. ZikaV peak purity is given as relative content of virus monomer population to total virus peak. Plaque assay states the content of total active virus particles in each sample. Experimental results are summarized in Table 1. The virus peak recovery by SEC-HPLC was only between 12 to 36% with peak purity after PS treatment in the range of >90% (no virus aggregates detected). The recovery of active virus particles by plaque assay was all > 100% (130-700%, range within the variability of the assay) showing that no active virus particles were lost during PS treatment. These results show that during PS treatment only non-infective (immature and/or aggregated virus) particles were removed.

Table 6: ZikaV recovery by SEC-HPLC and plaque assay before and after PS treatment.

SEC-HPLC

	Peak area mAU*min			
Harvest day	30x conc	30x + PS	SEC Recovery (%)	rel. virus monomer content after PS (%)
Day 2	101.36	18.63	18	89%
Day 3	144.51	17.48	12	90%
Day 5	19.97	5.92	30	96%
Day 7	68.80	24.43	36	99%

Plaque Assay

	PFU/mL		
Harvest day	30x conc	30x + PS	Plaque Recovery (%)
Day 2	3E+08	5E+08	179
Day 3	2E+08	4E+08	193
Day 5	1E+08	9E+08	700
Day 7	3E+08	4E+08	132

Sucrose gradient centrifugation

The PS treated harvest was split in two parts and loaded on two centrifuge bottles.

Sucrose density gradient centrifugation (SGC) was used for final concentration and polishing of the ZikaV material. The ZikaV PS treated concentrated harvest was loaded on top of a solution consisting of three layers of sucrose with different densities. The three sucrose layers were selected based on a preliminary study which showed the formation of a linear sucrose gradient and complete separation of the virus particles from residual contaminants as demonstrated for ChikV (Fig. 15D). The optimal volumes of the sucrose solutions were determined empirically. The volumes of individual layers for a centrifugation in 100 mL bottle scale are shown in Table 2.

Table 7: Individual layers/volumes for a centrifugation in bottle.

Solution	Volume (mL)
PS treated harvest in 10% sucrose (L)	40
15% sucrose (J)	15
35% sucrose (I)	15
50% sucrose (H)	20
Total volume	90

The sucrose gradient bottles were prepared by stratifying the individual sucrose layers. A plastic tube was attached to peristaltic pump tubing. The plastic tube was mounted on a laboratory stand using a clamp and placed into the centrifuge bottle. The nozzle of the plastic tube was touching the bottom of the bottle. Using a peristaltic pump the ZikaV material and the sucrose solutions were pumped into the cylinder. A measuring cylinder was used as feed vessel. The first solution pumped was the ZikaV material as it represented the solution of lowest density (10 % sucrose (w/w)). After the ZikaV material the sucrose solutions were pumped in ascending order starting with the 15 % (w/w) solution J, followed by 35 % sucrose solution I and finishing with the highest density sucrose solution H (50 % (w/w)). The described setup is shown in Figure 14. After all sucrose solutions were transferred the plastic tubing was carefully removed in order not to disturb the layers.

Prior to centrifugation the centrifuge was pre-cooled to 4°C. The prepared SG bottles were carefully transferred into the pre-cooled rotor. (Note: Sudden movement of the bottles during transfer to the rotor must be avoided in order not to disturb the sucrose layers.) The bottles were centrifuged at ~11.000 RCF max at 4 °C for at least 20 hours, no brake/deceleration activated. In case a different centrifuge system

with a different rotor is used the necessary speed and centrifugation times need to be calculated based on the k-factor in order to achieve comparable centrifugation efficiency.

Harvesting of the sucrose gradient was done manually using a peristaltic pump. A plastic tube attached to peristaltic pump tubing was used for harvesting the sucrose gradient. The bottle containing the gradient was mounted onto a laboratory stand in a tilted position ($\sim 12^\circ$) using a clamp. The plastic tubing was then placed into the bottle touching the bottom edge of the bottle and was fastened in position using a clamp. This resulted in a small gap of 1-2 mm between the tubing inlet and the bottom of the bottle (see Figure 14).

Using a peristaltic pump set to a flow rate of 30 mL per minute the gradient was harvested and manually split into 2 mL fractions. A total number of 32 fractions per bottle were harvested (~ 64 mL) and the remaining volume was discarded. The fractions were immediately tested by SDS-PAGE / silver stain to identify the virus containing fractions with sufficient high purity. Representative SDS-PAGE is shown in Figure 21. Fraction 10-14 were pooled and further processed.

The purified viral solution was inactivated by incubation with 0.02% formaldehyde over a period of ten days in a 22°C controlled-temperature incubator. The formaldehyde is neutralized by addition of sodium metabisulphite on the tenth day.

The sucrose gradient pool (~ 17 mL after sampling) was further diluted 3-fold with PBS to a final volume of 51 mL in a PETG container. A volume of 1% formaldehyde (10,000 ppm) solution equivalent to 1/50 of the final volume of the pre-formaldehyde pool was added to this pool resulting in an effective concentration of 200 ppm. The formaldehyde-treated solution was mixed on a magnetic stirrer for 10 minutes. After sampling, the formaldehyde-treated viral solution was placed within a cooled incubator at $22^\circ\text{C} \pm 2^\circ\text{C}$. On Day 5 post addition of formaldehyde, the formaldehyde-treated viral solution was filtered through a $0.2 \mu\text{m}$ filter and then placed in the incubator at $22^\circ\text{C} \pm 2^\circ\text{C}$ again. On Day 10, after removing the 10-Day inactivation final sample, a volume of 1 % (of the weight of the final formaldehyde-treated viral solution) of 200 mM-sodium metabisulphite solution (2mM final concentration) was aseptically transferred into the PETG container containing the formaldehyde-treated viral solution. After mixing for 5 minutes on a magnetic stirrer, the neutralized inactivated viral solution is held at room temperature (20 to 25°C) for a minimum of 30 minutes. After sampling, the neutralized inactivated viral solution is stored at $5^\circ\text{C} \pm 3^\circ\text{C}$ until further processing.

Inactivation by formaldehyde

Critical parameters for this step are final formalin concentration, temperature, mixing and transfer into a new container. A preliminary acceptance criterion for maximum pfu/mL (determined by plaque assay) has been set on the diluted pool pre formaldehyde treatment.

The quality of the neutralized inactivated viral solution was monitored by the following parameters: Plaque assay on Day 10, SEC-HPLC, SDS-PAGE/Western Blot.

Interestingly, SEC-HPLC analysis of samples taken during the inactivation period followed by neutralization with bisulfite showed more or less constant peak area throughout the inactivation period. This is in contrast to JEV where losses of viral particles up to 60% are observed using the process disclosed by Srivastava et al. Vaccine 19 (2001) 4557-4565. In a scale-down model the viral losses were even much higher due to surface/area ratio at smaller scale and high losses due to unspecific adsorption. Differences of the ZikaV inactivation experiment and JEV inactivation were noticed as follows:

A) Much higher purity of ZikaV SGP pool with regard to residual PS (<2µg/mL) compared to JEV. The 3-fold ZikaV inactivated sample contained therefore <<1µg/mL of residual PS. Commercial JEV SGP pool contains on average ~120µg/mL (up to 152 µg/mL possible). The average dilution to inactivation solution of ~14-fold results in a residual PS content up to ~11 µg/mL. It may be that higher amount of residual PS could cause virus precipitation due to cross-linking / reaction with formalin.

B) ZikaV inactivation sample contained ~10% sucrose (3-fold dilution of SGP pool containing ~30-35% sucrose). Sucrose might have stabilizing effect of viral ZikaV particles during treatment with formalin.

Dilution to DS and Formulation with Aluminium hydroxide (DP)

For preparation of ZikaV drug substance used in mouse potency assay an antigen content (expressed as total viral particles or SEC peak area) of 5 times higher compared to Ixiaro was targeted. The basis for determination of antigen content was SEC-HPLC. Briefly, a Superose 6 10/300 Increase column (GE Healthcare) equilibrated with PBS + 250 mM NaCl, pH 7.4 at 1 ml/min and 25°C, was used to detect ZikaV at 214 nm detection wavelength in harvest samples and throughout the downstream process. In the current JEV process the antigen content in NIV is determined by a specific ELISA. A good correlation was observed between antigen content determined by ELISA and SEC-HPLC. On average, the antigen content in commercial NIV samples is in the range of 33 AU/mL corresponding to ~5.2mAU JEV peak area, see Figure 22.

ZikaV NIV day10 (Zika peak ~36 mAU, analysed on Waters HPLC/Superose6 Increase column) was diluted with PBS to a target of 6.3 (~5.7x dilution). Aluminium hydroxide was added to a final concentration of 0.5 mg/mL Aluminium (1/20 v/v Alum 2% stock solution added) to prepare ZikaV Drug Product (DP). The DP was gently mixed for 5 min. An aliquot of the DP was removed, Alum sedimented by centrifugation and the clear supernatant analysed by SEC-HPLC. No ZikaV peak was detected in the supernatant indicating complete adsorption (estimated as >95%) of viral particles on the mineral adjuvant. Formulated ZikaV DP was stored at 2-8°C.

The impurity profile of the inactivated Zika virus DS is comparable to the profile of JEV DS with the exception of a lower PS content (Table 8).

Table 8: Determination of impurity profile in Zika and JEV DS samples:

	Specification (JEV DS)	JEV	Zika
HCP (ng/mL)	<100 LOQ 12 ng/mL	<LOQ	<LOQ
DNA (pg/mL)	<200 LOQ 40 pg/mL	<40	<40
Aggregates by SEC-MALLS (%)	Not specified, part of characterization LOQ 5%	<LOQ	<LOQ
PS (µg/mL)	Specification only at SGP pool to demonstrate consistent process performance (19-152 µg/mL), *PS content in DS calculated based on PS content in SGP pool (~100 µg/mL) and average dilution factor (~28x) to DS; LOQ 2 µg/mL	~4*	<<LOQ

*Typical PS impurity in a JEV sample produced in accordance with protocol disclosed in Srivastava et al. Vaccine 19 (2001) 4557-4565.

SEC-MALLS Results

A representative SEC-HPLC elution profile of ZikaV NIV at 214 nm detection wave length is shown in Figure 24. Note that BSA (50 µg/mL) was added to the sample to minimize losses in HPLC glass vial due to unspecific surface adsorption. ZikaV monomer content was estimated as ~98% with a multimer content of ~2%.

SEC-MALLS analysis (Figure 25) of the sample confirmed the radius R_z of the monomer ZikaV population peak 1 as 21.6 nm and ~49 nm for the multimer peak 2. Cumulative particle size distribution showed that 89% of all viral particles are within a radius range between 18 to 25nm (Figure 26).

Results confirm purity and homogeneity of ZikaV NIV.

*Viral titer by plaque assay***Table 9:** Active ZikaV pfus were quantified by plaque assay throughout the process.

Sample	Pfu/mL
Harvest day 2 (filtered)	6.4×10^7
Harvest day 3 (filtered)	1.0×10^8
Harvest day 5 (filtered)	1.5×10^8
Harvest day 7 (filtered)	1.1×10^8
PS treated harvest 300x concentrate (=SGP load)	9.0×10^8
SGP pool	8.9×10^8
Inactivation start (SGP pool 1:3 diluted)	3.4×10^8
Inactivation day 5	<LOD
Inactivation day 10	<LOD

Comparison of PS and Benzonase on process performance

A direct comparison of DNA removal method of concentrated ZikaV harvest pool was done. One aliquot was treated with PS (2 mg/mL, 15min at room temperature), the other aliquot was treated with Benzonase (50 U/mL, 2 mM MgCl₂, 4h RT, 48h 2-8°C). Both samples were further purified by sucrose gradient as described in this report. Interestingly, the Benzonase treated samples did not yield any pure fractions after sucrose gradient centrifugation of the treated ZikaV harvest. In those fractions where the specific virus bands were detected, a high amount of host cell protein was detected throughout the collected fractions. The PS treated material resulted in pure ZikaV containing fractions as expected. This finding may suggest that PS is not only effective for DNA removal by precipitation; in addition it improves the recovery of virus particles in the gradient by disrupting interaction of DNA (fragments) and virus particles. Benzonase treatment does not remove DNA, it only results in its fragmentation. Residual DNA fragments might still interact with virus particles and residual HCPs resulting in cross-contamination and co-purification in the sucrose gradient. Pooled SGP fractions were also analysed by SEC-HPLC. Although a large peak was detected, SDS-PAGE confirmed that this sample was highly contaminated with HCPs. A large peak might be detected at UV214 and 280nm after SEC-HPLC analysis due to possible interaction of HCPs with large virus particles, changing the UV absorbance.

Immunogenicity of Vero grown Zika virus*Immunization of mice*

Prior to immunization, groups of ten 6-week-old female CD1 mice were bled via vena facialis and pre-immune sera were prepared. One intraperitoneal immunizations of 200 µL were administered. A dose

titration (12 µg, 3 µg, 1 µg, 0.33 µg, 0.11 µg, 0.037 µg and 0.012 µg, equivalent to the protein amount in IXIARO) of inactivated Zika virus formulated with aluminium hydroxide (Al(OH)₃) at a final concentration of 0.7%. Three weeks after immunization, blood was collected and immune sera were prepared. All animal experiments were conducted in accordance with Austrian law (BGB1 Nr. 501/1989) and approved by “Magistratsabteilung 58”.

Plaque reduction neutralization test (PRNT)

Twelve well plates were used for PRNT. Each well was seeded with 1 mL medium containing 4×10^5 Vero cells and incubated 35°C with 5%CO₂ overnight. Pools of heat inactivated sera from each dose group were tested in triplicate. The target viruses (H/PF/2013 (SEQ ID NO: 13) or MR766 (SEQ ID NO: 11)) were diluted to 100 pfu/165 µL. Equal volumes of target virus and serum dilution were incubated at 35°C with 5% CO₂ for 1 hour. The cell culture medium was aspirated from the Vero cells and 330 µL of the mixture target virus/serum dilution were added to each well and the plates were rocked back and forth 5 times before incubating for 2 hours at 35°C with 5% CO₂. To each well 1 mL of a 2% methylcellulose solution containing EMEM and nutrients was added, the plates were then incubated for 5 days at 35°C with 5% CO₂ before staining the cells for 1 hour with crystal violet/5% formaldehyde and subsequently washed 3 times with deionized water. The plates were air dried and the numbers of plaques in each well were manually counted.

Results

Neutralization was observed with serum pools from mice immunized with inactivated Zika virus vaccine (H/PF/2013) down to 37 ng (dosing equivalent to the amount protein in IXIARO®) against Zika viruses of both the Asian (H/PF/2013) and African (MR766) lineages (Figures 27 and 28, respectively). Complete inhibition was seen at the 1:20 serum dilution with an immunization dose down to 110 ng (dosing equivalent to the amount protein in IXIARO®). The neutralization of both the Asian (H/PF/2013) and African (MR766) lineages of the Zika virus was equivalent, which indicates high cross-neutralization between different Zika virus strains of the inactivated Zika virus vaccine (H/PF/2013).

Another neutralization assay was performed using the microneutralization assay as described by Larocca, et al. (2016, Nature doi:10.1038/nature18952). It was found that the inactivated Zika virus of the current invention had an MN50 (microneutralization) titer of 90 at 1 µg of inactivated purified virus.

Further methods: The immunogenicity of inactivated Zika virus preparations is assessed using a mouse model of Zika infection. Groups of adult mice are immunized subcutaneously (s.c.) with 500, 50, or 5 ng of inactivated Zika virus with adjuvant (*e.g.* aluminium hydroxide with or without IC31®), or without adjuvant. An additional group of mice receive PBS as a negative control. Each group is administered the indicated inoculum at t=0 and in some cases also at three to four weeks later (t=3/4). Beginning approximately three weeks after administration of the last immunization, serum samples are obtained from each of the mice at regular intervals. The serum samples are tested for the presence of neutralizing antibodies using PRNT.

The *in vivo* protective efficacy of the inactivated Zika virus preparations is also assessed using a mouse model of Zika infection, *i.e.* IFN-alpha/beta receptor knock-out mice (A129) (see *e.g.* Dowall et al., 4. March 2016, <http://dx.doi.org/10.1101/042358>) or blocking of the IFN-alpha/beta receptor by administration of anti-IFN-alpha/beta receptor monoclonal antibodies to C57BL/6 or BALB/c mice (see *e.g.* Pinto et al., 7. December 2011, DOI: 10.1371/journal.ppat.1002407). For protection assays, groups of 10 three- to eight-weeks-old A129, C57BL/6 or BALB/c mice are inoculated subcutaneously in the hindquarters with inactivated Zika virus with adjuvant (aluminium hydroxide) or without adjuvant at t=0. Age-matched controls are inoculated with PBS or non-specific antigens in alum. Mice are optionally boosted with a second administration of the indicated inoculation three to four weeks later. The mice are then challenged subcutaneously at three to eight weeks post immunization by inoculation with a deadly dose of live Zika virus. One day prior to challenge of C57BL/6 and BALB/c mice, they are passively administered (intraperitoneally) anti-IFN-alpha/beta receptor monoclonal antibodies. Challenged mice are monitored daily for morbidity and mortality for up to twenty-one days. Another alternative is to challenge intracranially adult vaccinated/non-vaccinated adult mice and observe protection.

It is expected that the Zika virus produced by the process of the invention will provide very similar functional read-outs in *in vitro*, *in vivo* and finally human trials as the currently licensed JEV vaccine in the EU and US and elsewhere, IXIARO®. The dosage may alter but due to the very similar impurity profile and almost identical manufacture, a very similar efficacy and safety result will be expected as was determined for the currently licensed JEV vaccine (licensed in the EU and US and elsewhere).

Discussion & Conclusion

The existing manufacturing platform for production of inactivated JEV vaccine IXIARO® was used as a basis for a manufacturing feasibility study of inactivated ZikaV vaccine candidate (Asian strain H/PF/2013). The virus was produced on Vero cells cultivated in roller bottles. The virus was purified by PS treatment followed by an optimized sucrose gradient. Inactivation was done by formalin treat (0.02 %, 103

10 days at 22 °C). For exploratory immunization studies in mice, a DP formulated with Alum was prepared with an estimated 5-fold higher virus particle content compared to IXIARO®, the commercial JEV Vaccine. The impurity profile of the DS met all criteria as defined in the specification for IXIARO®, the commercial JEV vaccine. The neutralization of both the Asian (H/PF/2013) and African (MR766) lineages of the Zika virus was equivalent, which indicates high cross-neutralization between different Zika virus strains of the inactivated Zika virus vaccine (H/PF/2013).

The in vivo data regarding immunogenicity of the inactivated Zika virus vaccine of the current invention indicates that the virus is surprisingly potently immunogenic and also highly cross-protective (very similar immunogenicity in African and Asian strains). Data indicate that immunogenicity was higher than the recently reported inactivated Zika virus vaccine candidate (Larocca, et. al, 2016, supra.). Inactivated viruses are among the safest vaccines and especially preferred for deliver to populations where safety is especially concerning, such as pregnant women, children and immunocompromised individuals, which makes the herein disclosed inactivated Zika virus particularly suitable. Obtaining a high titer of inactivated virus is a challenge in the field. The herein disclosed process for purifying inactivated Zika virus results in not only a high yield, but also a very pure drug substance.

Example 3: Development of a purification process for Yellow Fever virus vaccine produced in Vero cells

A downstream process was developed for the purification of infectious yellow fever virus particles whereby host cell nucleic acids, non-infectious virus particles and aggregates are removed by the addition of protamine sulphate as described in Examples 1 and 2. The unexpected and novel purification properties of protamine sulphate (PS) were evaluated in purification processes for yellow fever (YF) as follows:

As before the treatment of YF-harvest with PS significantly reduces the amount of aggregates as seen with SEC for two vaccine strains currently in development (Figure 29).

Further more detailed aspects of the invention:

A1. A process of purification of infectious alphavirus particles, preferably Chikungunya virus particles, comprising the steps of:

- a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;
- b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising a protamine salt, preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b);
- c) contacting the virus preparation (b) with (i) a solid-phase matrix packed in a column comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cut-off of the pores can enter the ligand-activated core and collecting the virus particles to obtain a virus preparation (d), or (ii) a solid-phase matrix comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cut-off of the pores can enter the ligand-activated core and separating the solid-phase matrix from the virus particles by filtration to produce a virus preparation (c); and
- d) further purifying the virus preparation (c) by sucrose density gradient centrifugation to obtain a virus preparation (d) comprising the infectious virus particles, wherein the residual host cell DNA of the virus preparation (d) is less than 100 ng/mL and the residual host cell protein of the final virus preparation (d) is less than 1 µg/mL.

A2. The process of A1, wherein the residual host cell DNA of the virus preparation (d) is less than 10 ng/mL and the residual host cell protein of the final virus preparation (d) is less than 100 ng/mL.

A3. The process of A1 or A2, wherein the crude harvest (a) comprising virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b).

A4. The process of any one of A1 to A3, wherein the one or more pre-purification step(s) comprises

- a) filtration using a filter having a pore size equal to or less than 0.2 µm; and/or
- b) digestion of host cell genomic DNA by enzymatic treatment; and/or

- c) ultra/diafiltration using a hollow fiber membrane having a pore size equal to or greater than 300 kDa, preferably equal to or greater than 100 kDa.

A5. The process of any one of A1 to A4, wherein the concentration of protamine sulphate is 1 to 2 mg/ml, more preferably 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml, most preferably 1.6 mg/ml.

A6. The process of any one of A1 to A5, wherein the molecule entering the core of the solid-phase matrix has a molecular weight less than 700 kDa.

A7. The process of any one of A1 to A6, wherein the ligand of the ligand-activated core of the solid-phase matrix is capable of binding the molecule that enters the ligand-activated core via cationic-, anionic-, hydrophobic- or mixed interactions.

A8. The process of any one of A1 to A7, wherein the ligand of the ligand-activated core of the solid-phase matrix is octylamine.

A9. The process of any one of A1 to A8, wherein the solid-phase matrix is used as a slurry and at a final concentration between 0.5% (v/v) and 10% (v/v), preferably 0.6%, 0.7%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, most preferably 1%.

A10. The process of any one of A1 to A9, wherein the solid-phase matrix is incubated with the protamine-treated virus preparation (b) at refrigerated temperatures (2°C to 8°C) with a stirring for at least 10 minutes, preferably 15 minutes, 30 minutes or 1 hour, most preferably 15 minutes.

A11. The process of any one of A1 to A10, wherein the enrichment of infectious virus particles in the final virus preparation relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

A12. The process of any one A1 to A11, wherein the filtration of step (c) of preferred aspect 1 is performed using a filter having a pore size equal to or less than 1 µm, preferably 0.2 µm.

A13. The process of any one of A1 to A12, wherein the residual impurity of the final virus preparation is less than 10%.

A14. The process of any one of A1 to A13, wherein the virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero- α His cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line.

A15. The process of A14, wherein said cell line is a Vero cell line.

A16. The process of any one of A1 to A15, wherein the Chikungunya virus is a live virus, an attenuated live virus, a chimeric virus, a modified live virus, or a recombinant live virus.

A17. The process of any one of A1 to A16, wherein the Chikungunya virus is the Δ 5nsP3 attenuated mutant or an immunogenic variant thereof.

A18. The process of any one of A1 to A17, wherein said process resulting in final virus preparation (d) is followed by an inactivation step, wherein the virus is inactivated preferably by formaldehyde.

A19. Use of the process according to any one of A1 to A18 for manufacturing a composition for immunization against a Chikungunya virus infection.

A20. The use according to A19, wherein the composition for immunization against a Chikungunya virus infection is a vaccine.

A21. A composition comprising the virus particles obtainable by the process of any one of A1 to A18 for treating and/or preventing a Chikungunya virus infection.

N1. A process of purification of infectious alphavirus particles, preferably Chikungunya virus particles, comprising the steps of:

- (a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;
- (b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising a protamine salt, preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b);
- (c) contacting the virus preparation (b) with (i) a solid-phase matrix packed in a column comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cut-off of the pores can enter the ligand-activated core and collecting the virus particles to obtain a virus preparation (d), or (ii) a solid-phase matrix comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cut-off of the pores can enter the ligand-activated core and separating the solid-phase matrix from the virus particles by filtration to produce a virus preparation (c); and
- (d) further purifying the virus preparation (c) by sucrose density gradient centrifugation to obtain a virus preparation (d) comprising the infectious virus particles, wherein the residual host cell DNA of the virus preparation (d) is less than 100 ng/mL and the residual host cell protein of the final virus preparation (d) is less than 1 µg/mL.

N2. The process of N1, wherein the residual host cell DNA of the virus preparation (d) is less than 10 ng/mL and the residual host cell protein of the final virus preparation (d) is less than 100 ng/mL.

N3. The process of N1 or 2, wherein the crude harvest (a) comprising virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b).

N4. The process of any one of N1 to 3, wherein the one or more pre-purification step(s) comprises

- (a) filtration using a filter having a pore size equal to or less than 0.2 μm ; and/or
- (b) digestion of host cell genomic DNA by enzymatic treatment; and/or
- (c) ultra/diafiltration using a hollow fiber membrane having a pore size equal to or greater than 300 kDa, preferably equal to or greater than 100 kDa.

N5. The process of any one of N1 to 4, wherein the concentration of protamine sulphate is 1 to 2 mg/ml, more preferably 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml, most preferably 1.6 mg/ml.

N6. The process of any one of N1 to 5, wherein the molecule entering the core of the solid-phase matrix has a molecular weight less than 700 kDa.

N7. The process of any one of N1 to 6, wherein the ligand of the ligand-activated core of the solid-phase matrix is capable of binding the molecule that enters the ligand-activated core via cationic-, anionic-, hydrophobic- or mixed interactions.

N8. The process of any one of N1 to 7, wherein the ligand of the ligand-activated core of the solid-phase matrix is octylamine.

N9. The process of any one of N1 to 8, wherein the solid-phase matrix is used as a slurry and at a final concentration between 0.5% (v/v) and 10% (v/v), preferably 0.6%, 0.7%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, most preferably 1%.

N10. The process of any one of N1 to 9, wherein the solid-phase matrix is incubated with the protamine-treated virus preparation (b) at refrigerated temperatures (2°C to 8°C) with a stirring for at least 10 minutes, preferably 15 minutes, 30 minutes or 1 hour, most preferably 15 minutes.

N11. The process of any one of N1 to 10, wherein the enrichment of infectious virus particles in the final virus preparation relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

N12. The process of any one of N1 to 11, wherein the filtration of step (c) of N1 is performed using a filter having a pore size equal to or less than 1 μm , preferably 0.2 μm .

N13. The process of any one of N1 to 12, wherein the residual impurity of the final virus preparation is less than 10%.

N14. The process of any one of N1 to 13, wherein the virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero- α His cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line.

N15. The process of N14, wherein said cell line is a Vero cell line.

N16. The process of any one of N1 to 15, wherein the Chikungunya virus is a live virus, an attenuated live virus, a chimeric virus, a modified live virus, or a recombinant live virus.

N17. The process of any one of N1 to 16, wherein the Chikungunya virus is the Δ 5nsP3 attenuated mutant or an immunogenic variant thereof.

N18. The process of any one of N1 to 17, wherein said process resulting in final virus preparation (d) is followed by an inactivation step, wherein the virus is inactivated preferably by formaldehyde.

N19. Use of the process according to any one of N1 to 18 for manufacturing a composition for immunization against a Chikungunya virus infection.

N20. The use according to N19, wherein the composition for immunization against a Chikungunya virus infection is a vaccine.

N21. A composition comprising the virus particles obtainable by the process of any one of N1 to 18 for treating and/or preventing a Chikungunya virus infection.

P1. A Zika virus vaccine comprising an optimally inactivated Zika virus particle, wherein the Zika virus particle is able to seroconvert a subject that is administered the Zika virus vaccine with at least a 70% probability.

P2. The Zika virus vaccine of P1, wherein the Zika virus particle is able to seroconvert the subject that is administered the Zika virus vaccine with at least a 80%, 85%, 90%, or 95% probability, preferably a 80% probability.

P3. The vaccine of P1 or 2, wherein the Zika virus particle has a RNA genome corresponding to the DNA sequence provided by any one of the nucleic acid sequences of SEQ ID NOs: 2-13, or a variant nucleic acid sequence that is at least 88% identical to any one of SEQ ID NOs: 2-13 and able to pack a virulent Zika virus.

P4. The vaccine of any one of P1-3, wherein the Zika virus particle has an E protein selected from the amino acid sequences provided by any one of SEQ ID NOs: 14-69, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14-69 and able to pack a virulent Zika virus.

P5. The vaccine of any one of P1-4, wherein the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

P6. The vaccine of P5, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent for longer than is required to completely inactivate the Zika virus as measured by plaque assay.

P7. The vaccine of P6, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde.

P8. The vaccine of P7, wherein the formaldehyde inactivation comprises contacting the Zika virus with formaldehyde for between 2-10 days.

P9. The vaccine of any one of P5-8, wherein the chemical activation is performed at about +4°C or about +22°C.

P10. The vaccine of any one of P1-9, further comprising an adjuvant.

P11. The vaccine of P10, wherein the adjuvant is an aluminum salt adjuvant.

P12. The vaccine of P11, wherein the aluminum salt adjuvant is aluminium hydroxide or aluminium phosphate salt.

P13. The vaccine of any one of P10-12, wherein the vaccine comprises or further comprises an adjuvant comprising a peptide and a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN).

P14. The vaccine of P13, wherein the peptide comprises the sequence KLKL5KLK (SEQ ID NO: 71) and the I-ODN comprises oligo-d(IC)13 (SEQ ID NO: 70).

P15. The vaccine of any one of P1-14, further comprising one or more pharmaceutically acceptable excipient.

Q1. A process of purification of infectious virus particles, comprising the steps of:

- (a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;
- (b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b);
- (c) further purifying the virus preparation (b) by an optimized sucrose density gradient centrifugation, wherein the optimized sucrose gradient is provided such that the protamine can be completely or almost completely separated from the virus fraction; and wherein the protamine concentration is reduced by this step to the extent that the protamine concentration in the final drug substance is below 1 µg/ml, preferably below 0.5 µg/mL, more preferably below 0.1 µg/mL, most preferably below 0.05µg/mL.

Q2. The process of Q2, wherein the virus particles are selected from the group consisting of flaviviruses, e.g. yellow fever virus or Zika virus and alphaviruses, e.g. Chikungunya.

Q3. The process of Q1 or Q2, additionally comprising the step of:
(d) a solid-phase matrix packed in a column comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cutoff of the pores can enter the ligand-activated core and collecting the virus particles.

Q4. The process of any of Q1 to 3, wherein the residual host cell DNA of the virus preparation (c) is less than 10 ng/mL and the residual host cell protein of the final virus preparation (c) is less than 100 ng/mL.

Q5. The process of any of Q1 to 4, wherein the crude harvest (a) comprising virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b).

Q6. The process of Q5, wherein the one or more pre-purification step(s) comprises
(a) filtration using a filter having a pore size equal to or less than 0.2 μm ; and/or
(b) digestion of host cell genomic DNA by enzymatic treatment; and/or
(c) ultra/diafiltration using a hollow fiber membrane having a pore size equal to or greater than 300 kDa, preferably equal to or greater than 100 kDa.

Q7. The process of any one of Q1 to 6, wherein the concentration of protamine sulphate is 0.5 to 3 mg/ml, more preferably 1 to 2 mg/ml, more preferably 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml, most preferably 1.6 mg/ml or 2 mg/ml.

Q8. The process of any one of Q1 to 7, wherein the enrichment of infectious virus particles in the virus preparation (c) or any final virus preparation relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

Q9. The process of any one of Q5 to 8, wherein the one or more pre-purification step(s) prior to step (b) of any of Q5 to 8 is performed using a filter having a pore size equal to or less than 1 μm , preferably 0.2 μm .

Q10. The process of any one of Q1 to 9, wherein the residual impurity of the virus preparation (c) is less than 10%.

Q11. The process of any one of Q1 to 10, wherein the virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero- α His cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line.

Q12. The process of Q11, wherein said cell line is a Vero cell line.

Q13. The process of any one of Q1 to 12, wherein the infectious virus particles is an infectious Zika virus particle that is a live virus, an attenuated live virus, a chimeric virus, a modified live virus, or a recombinant live virus.

Q14. The process of any one of Q1 to 13, wherein the Zika virus is a Zika virus strain of the Asian lineage or an immunogenic variant thereof.

Q15. The process of any one of Q1 to 14, wherein said process resulting in final virus preparation (c) or (d) is followed by an inactivation step, wherein the virus is inactivated preferably by formaldehyde.

Q16. Use of the process according to any one of Q1 to 15 for manufacturing a composition for immunization against a virus infection.

Q17. The use according to Q16, wherein the composition for immunization against a virus infection is an infection caused by a group of viruses consisting of yellow fever virus, Chikungunya virus and Zika virus.

Q18. A composition comprising the virus particles obtainable or obtained by the process of any one of Q1 to 17 for treating and/or preventing an infection, such as e.g. a Zika virus infection.

Q19. A Zika virus vaccine comprising an inactivated Zika virus particle grown on vero cells, wherein the Zika virus particle is able to seroconvert a subject that is administered the Zika virus vaccine with at least a 70% probability and comprises minor amounts of protamine sulphate, preferably below the detection limit.

Q20. The Zika virus vaccine of Q19, wherein the Zika virus particle is able to seroconvert the subject that is administered the Zika virus vaccine with at least a 80%, 85%, 90%, or 95% probability, preferably a 80% probability.

Q21. The vaccine of Q19 or 20, wherein the Zika virus particle has a RNA genome corresponding to the DNA sequence provided by any one of the nucleic acid sequences of SEQ ID NOs: 2-13, or a variant nucleic acid sequence that is at least 88% identical to any one of SEQ ID NOs: 2-13 and able to pack a virulent Zika virus.

Q22. The vaccine of any one of Q19, 20 and 21, wherein the Zika virus particle has an E protein selected from the amino acid sequences provided by any one of SEQ ID NOs: 14-69, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14-69 and able to pack a virulent Zika virus.

Q23. The vaccine of any one of Q19, 20 to 22, wherein the Zika virus obtained by culturing on Vero cells is purified by protamine sulfate precipitation and sucrose gradient centrifugation.

Q24. The vaccine of Q23, wherein the sucrose gradient centrifugation is an optimized sucrose gradient centrifugation.

Q25. The vaccine of Q24, wherein the optimized sucrose gradient centrifugation comprises a virus comprising fraction in a 10% (w/w) sucrose solution and three layers of sucrose with different densities, i.e. a first sucrose solution with 15% (w/w) sucrose solution, a second sucrose solution with 35% (w/w) sucrose solution, and a third sucrose solution with a 50% (w/w) sucrose solution.

Q26. The vaccine of any one of Q19, 20 to 25, wherein the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

Q27. The vaccine of Q26, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent for longer than is required to completely inactivate the Zika virus as measured by plaque assay.

Q28. The vaccine of Q27, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde.

Q29. The vaccine of Q28, wherein the formaldehyde inactivation comprises contacting the Zika virus with formaldehyde for between 2-10 days.

Q30. The vaccine of any one of Q27-29, wherein the chemical activation is performed at about +4°C or about +22°C.

Q31. The vaccine of any one of Q19 to 30, further comprising an adjuvant.

Q32. The vaccine of Q31, wherein the adjuvant is an aluminum salt adjuvant.

Q33. The vaccine of Q32, wherein the aluminum salt adjuvant is aluminium hydroxide or aluminium phosphate salt.

Q34. The vaccine of Q32, wherein the aluminum salt adjuvant is aluminium hydroxide with less than 1.25ppb Cu based on the final pharmaceutical composition comprising the Zika virus, preferably the inactivated Zika virus.

Q35. The vaccine of any one of Q19 to 34, further comprising one or more pharmaceutically acceptable excipient.

R1. Use of protamine, preferably a protamine salt, to separate infectious and non-infectious virus particles, host cell proteins and/or undefined low molecular weight materials.

R2. A process of purification of infectious virus particles, comprising the steps of:

- (a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;
- (b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b), wherein the enrichment of infectious virus particles in the virus preparation (b) relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

R3. The use of R1 or the process of R2, wherein the virus particles are selected from the group consisting of flaviviruses, e.g. yellow fever virus or Zika virus and alphaviruses, e.g. Chikungunya.

R4. A process of purification of infectious virus particles, comprising the steps of:

- (a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;
- (b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b);
- (c) further purifying the virus preparation (b) by one or more size exclusion methods such as (i) a sucrose density gradient centrifugation, (ii) a solid-phase matrix packed in a column

comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cutoff of the pores can enter the ligand-activated core and collecting the virus particles, and/or (iii) size exclusion chromatography to obtain a virus preparation (c) comprising the infectious virus particles, wherein the residual host cell DNA of the virus preparation (c) is less than 100 ng/mL and the residual host cell protein and the residual aggregates of infectious virus particles of the final virus preparation (c) is less than 1 µg/mL.

R5. The process of R4, wherein the residual host cell DNA of the virus preparation (c) is less than 10 ng/mL and the residual host cell protein of the final virus preparation (c) is less than 100 ng/mL.

R6. The process of any of R2 to 5, wherein the crude harvest (a) comprising virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b).

R7. The process of R6, wherein the one or more pre-purification step(s) comprises

- (a) filtration using a filter having a pore size equal to or less than 0.2 µm; and/or
- (b) digestion of host cell genomic DNA by enzymatic treatment; and/or
- (c) ultra/diafiltration using a hollow fiber membrane having a pore size equal to or greater than 300 kDa, preferably equal to or greater than 100 kDa.

R8. The process of any one of R2 to 7, wherein the concentration of protamine sulphate is 0.5 to 3 mg/ml, more preferably 1 to 2 mg/ml, more preferably 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml, most preferably 1.6 mg/ml.

R9. The process of any one of R2 to 8, wherein the enrichment of infectious virus particles in the virus preparation (c) or any final virus preparation relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

R10. The process of any one of R6 to 9, wherein the one or more pre-purification step(s) prior to step (b) of any of R6 to 9 is performed using a filter having a pore size equal to or less than 1 µm, preferably 0.2 µm.

R11. The process of any one of R2 to 10, wherein the residual impurity of the virus preparation (c) is less than 10%.

R12. The process of any one of R2 to 11, wherein the virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero- α His cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line.

R13. The process of R12, wherein said cell line is a Vero cell line.

R14. The process of any one of R2 to 13, wherein the Zika virus is a live virus, an attenuated live virus, a chimeric virus, a modified live virus, or a recombinant live virus.

R15. The process of any one of R2 to 14, wherein the Zika virus is a Zika virus strain of the Asian lineage or an immunogenic variant thereof.

R16. The process of any one of R2 to 15, wherein said process resulting in final virus preparation (c) is followed by an inactivation step, wherein the virus is inactivated preferably by formaldehyde.

R17. Use of the process according to any one of R1 to 16 for manufacturing a composition for immunization against a virus infection.

R18. The use according to R17, wherein the composition for immunization against a virus infection is an infection caused by a group of viruses consisting of yellow fever virus, Chikungunya virus and Zika virus.

R19. A composition comprising the virus particles obtainable or obtained by the process of any one of R2 to 16 for treating and/or preventing an infection.

What is claimed is:

CLAIMS

1. Use of protamine, preferably a protamine salt, to separate infectious virus particles from non-infectious virus particles.
2. The use according to claim 1, wherein the protamine salt also facilitates the separation of infectious virus particles from host cell proteins and/or low molecular weight materials.
3. A process of purification of infectious virus particles, comprising the steps of:
 - a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;
 - b) reducing impurities from said crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b), wherein the enrichment of infectious virus particles in the virus preparation (b) relative to total virus products in the crude harvest (a) is in the range of at least 50% to 95%, preferably at least 80%.
4. The use of claim 1 or 2 or the process of claim 3, wherein the virus particles are selected from the group consisting of flaviviruses, e.g. yellow fever virus, Dengue virus, Japanese encephalitis virus or Zika virus, and alphaviruses, e.g. Chikungunya virus.
5. A process of purification of infectious virus particles, comprising the steps of:
 - a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;
 - b) reducing impurities from said crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b);
 - c) further purifying said virus preparation (b) by one or more size exclusion methods such as (i) a sucrose density gradient centrifugation, (ii) a solid-phase matrix packed in a column comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cutoff of the pores

can enter the ligand-activated core and collecting the virus particles, and/or (iii) size exclusion chromatography to obtain a virus preparation (c) comprising the infectious virus particles, wherein the residual host cell DNA of the virus preparation (c) is less than 100 ng/mL and the residual host cell protein and the residual aggregates of infectious virus particles of the final virus preparation (c) is less than 1 µg/mL.

6. The process of claim 5, wherein the residual host cell DNA of the virus preparation (c) is less than 10 ng/mL and the residual host cell protein of the final virus preparation (c) is less than 100 ng/mL.
7. The process of any of claims 3 to 6, wherein the crude harvest (a) comprising virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b).
8. The process of claim 7, wherein the one or more pre-purification step(s) comprises
 - a) filtration using a filter having a pore size equal to or less than 0.2 µm; and/or
 - b) digestion of host cell genomic DNA by enzymatic treatment; and/or
 - c) ultra/diafiltration using a hollow fiber membrane having a pore size equal to or greater than 300 kDa, preferably equal to or greater than 100 kDa.
9. The process of any one of claims 3 to 8, wherein the concentration of protamine sulphate is 0.5 to 3 mg/ml, more preferably 1 to 2 mg/ml, more preferably 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml, most preferably 1.6 mg/ml or 2.0 mg/ml.
10. The process of any one of claims 3 to 9, wherein the enrichment of infectious virus particles in the virus preparation (c) or any final virus preparation relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.
11. The process of any one of claims 7 to 10, wherein the one or more pre-purification step(s) prior to step (b) of any of claims 7 to 10 is performed using a filter having a pore size equal to or less than 1 µm, preferably 0.2 µm.
12. The process of any one of claims 3 to 11, wherein the residual impurity of the virus preparation (c) is less than 10%.

13. The process of any one of claims 3 to 12, wherein the virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero- α His cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line.
14. The process of claim 13, wherein said cell line is a Vero cell line.
15. The process of any one of claims 3 to 14, wherein said virus is a live virus, an attenuated live virus, a chimeric virus, a modified live virus, or a recombinant live virus.
16. The process of any one of claims 3 to 15, wherein said virus is selected from the group of viruses consisting of a Zika virus, preferably a Zika virus strain of the Asian lineage or an immunogenic variant thereof; an attenuated Chikungunya virus, preferably a Chikungunya virus with a deletion mutation in the non-structural protein 3 (SEQ ID NO: 77); a yellow fever virus, a Dengue virus and a Japanese Encephalitis virus.
17. The process of any one of claims 3 to 16, wherein said process resulting in final virus preparation (c) is followed by an inactivation step, wherein the virus is inactivated preferably by formaldehyde.
18. A composition comprising the virus particles obtainable or obtained by the process of any one of claims 3 to 17, wherein the composition contains protamine at levels below detection in size exclusion chromatography.
19. The composition according to claim 18, wherein the composition contains trace amounts of protamine or fragments thereof detectable by mass spectroscopy or other sensitive methods.
20. The composition according to claim 18 or 19 for treating and/or protecting from an infection.
21. Use of the process according to any one of claims 3 to 17 for manufacturing a composition for immunization against a virus infection.
22. The use according to claim 21, wherein said virus infection is an infection caused by the group of viruses consisting of yellow fever virus, Chikungunya virus and Zika virus.

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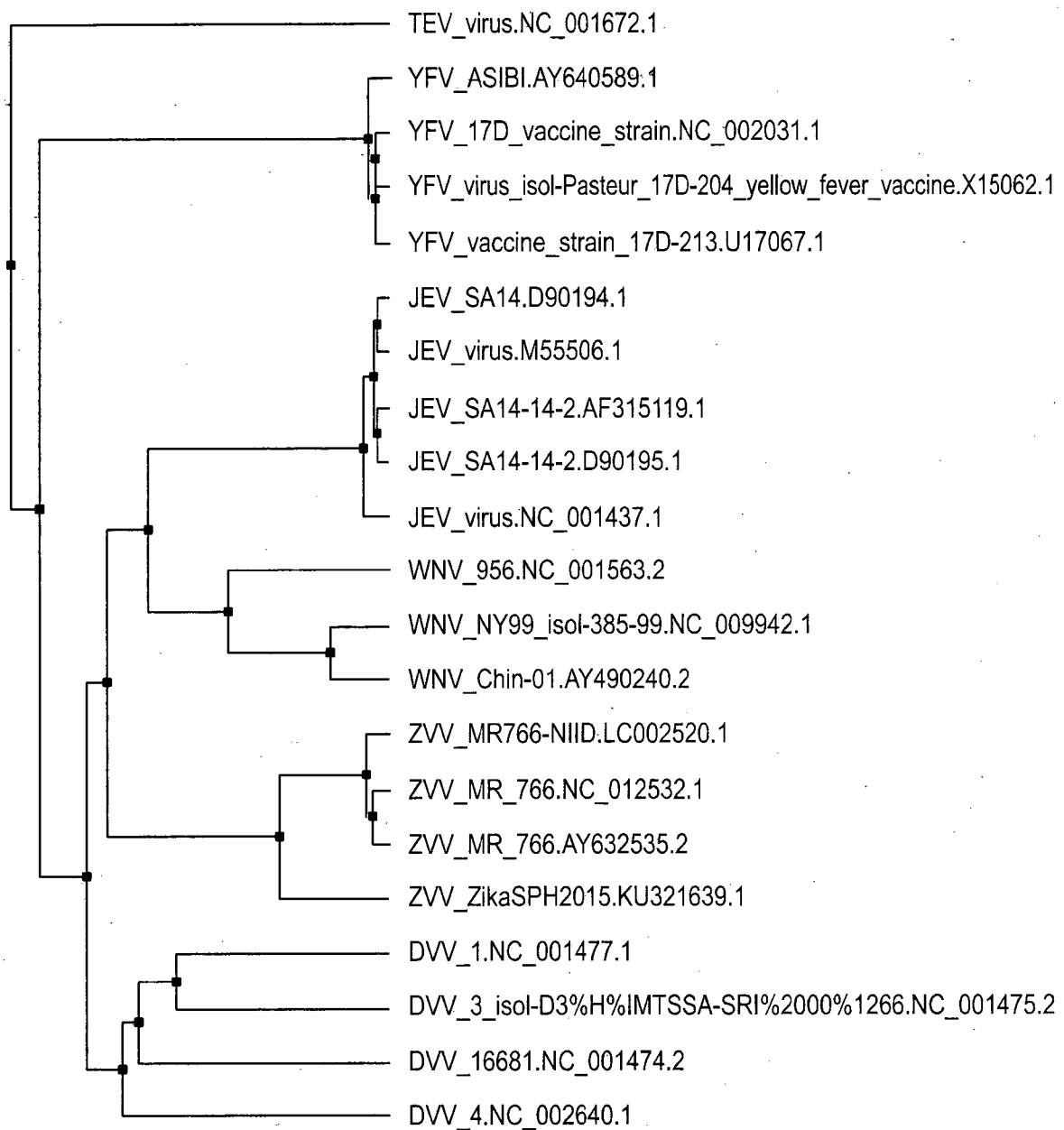


Figure 1

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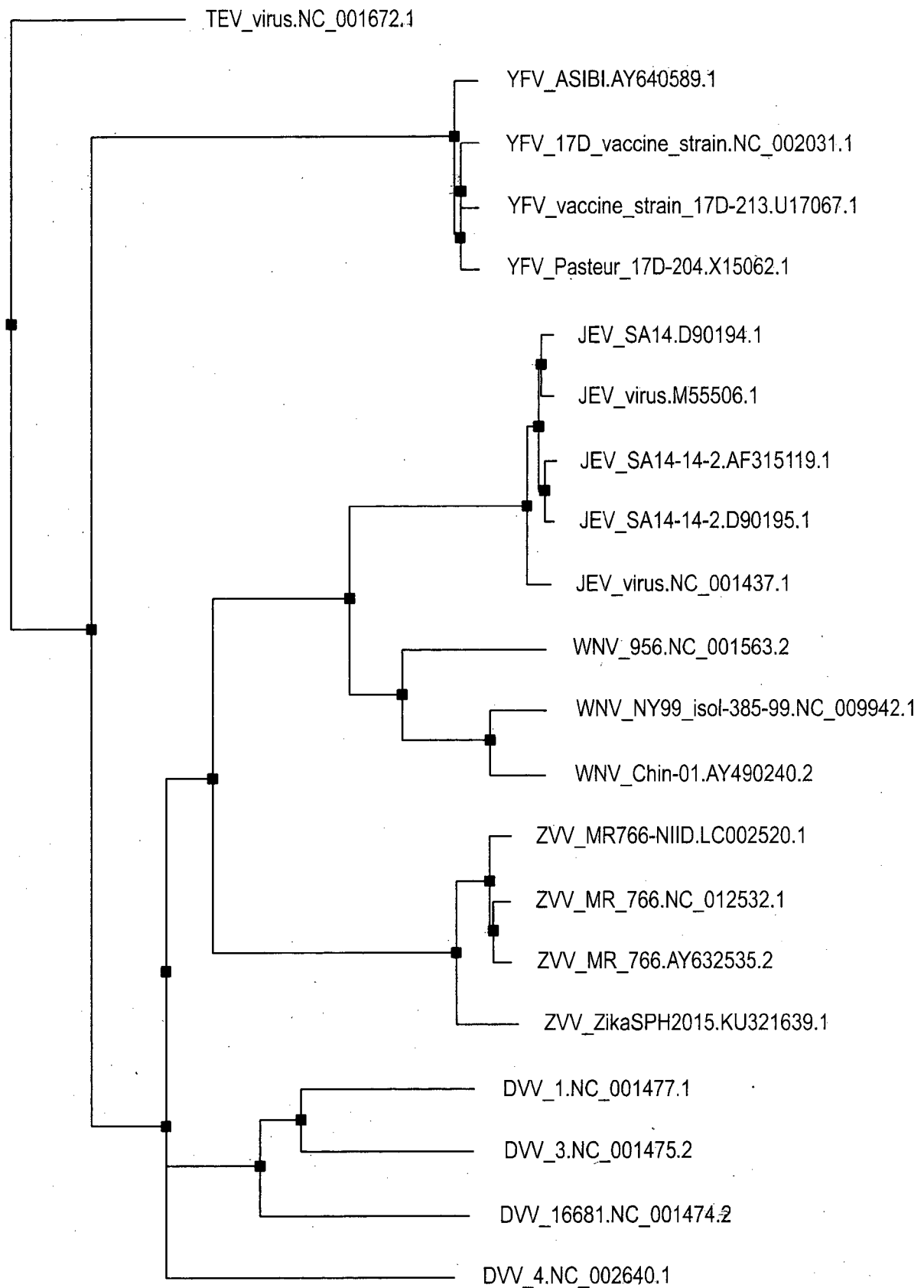


Figure 2

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	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
DW 1NC 001477.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
DW 16681NC 001474.2	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
DW 3NC 001475.2	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
DW 4NC 002640.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
JEV SA14-14-2AF315119.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
JEV SA14-14-2D90195.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
JEV SA14-D90194.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
JEV virus.M55506.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
JEV virus.NC 001437.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
TEV virus.NC 001672.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
WNV 956.NC 001563.2	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
WNV Chin-01.AY490240.2	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
WNV NY99 isol-385-99.NC 009942.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
YFV 17D vaccine strain.NC 002031.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
YFV ASIB.AY640589.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
YFV Pasteur 17D-204.X15062.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
YFV vaccine strain 17D-213.U17067.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
ZW MR766-NIID.LC002520.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
ZW MR 766.AY632535.2	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
ZW MR 766.NC 012532.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
ZWV.ZkaSPH2015.KU321639.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0

 % Identity and higher
 % Identity and higher
 % Identity and higher

Figure 3

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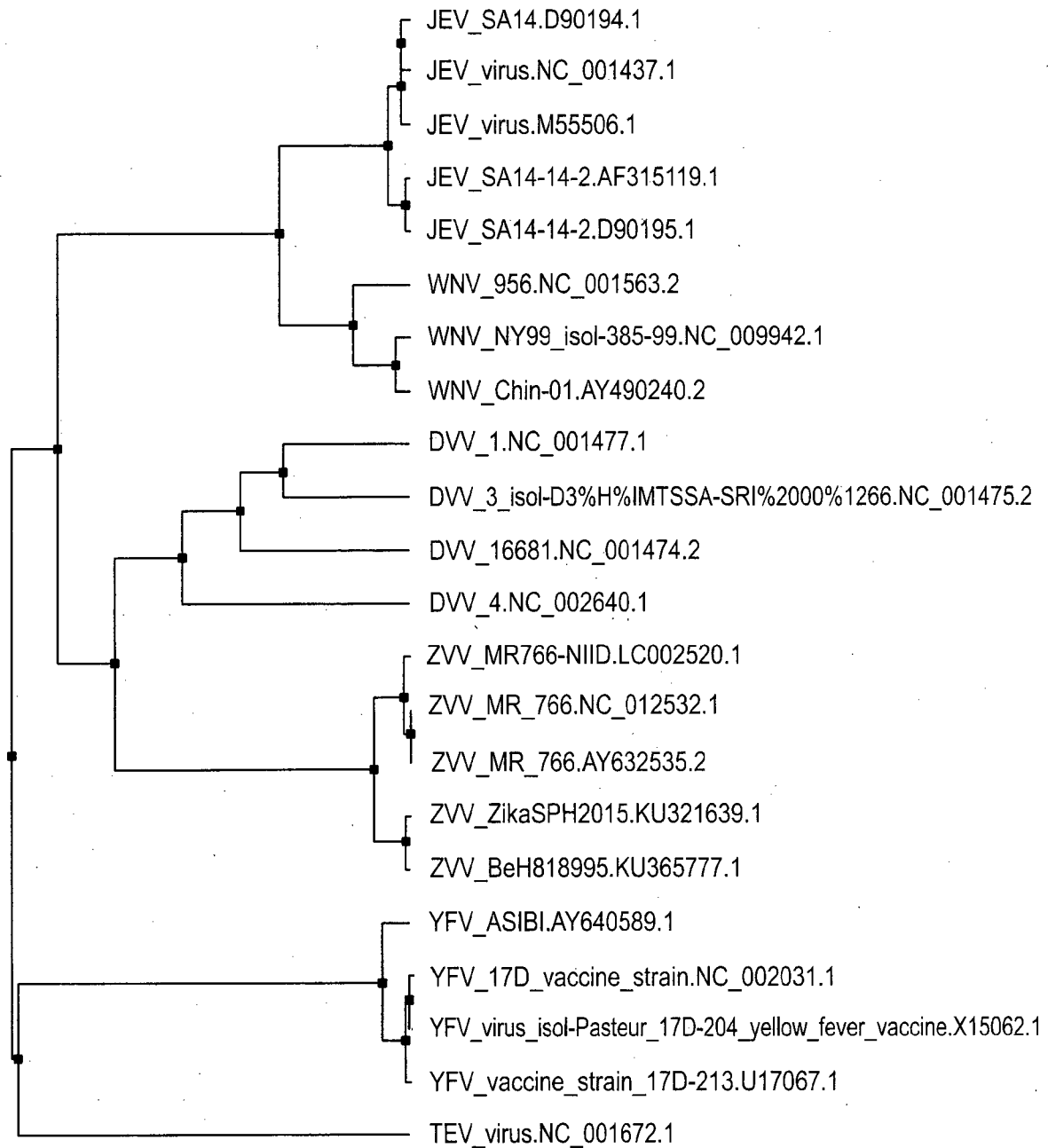


Figure 4

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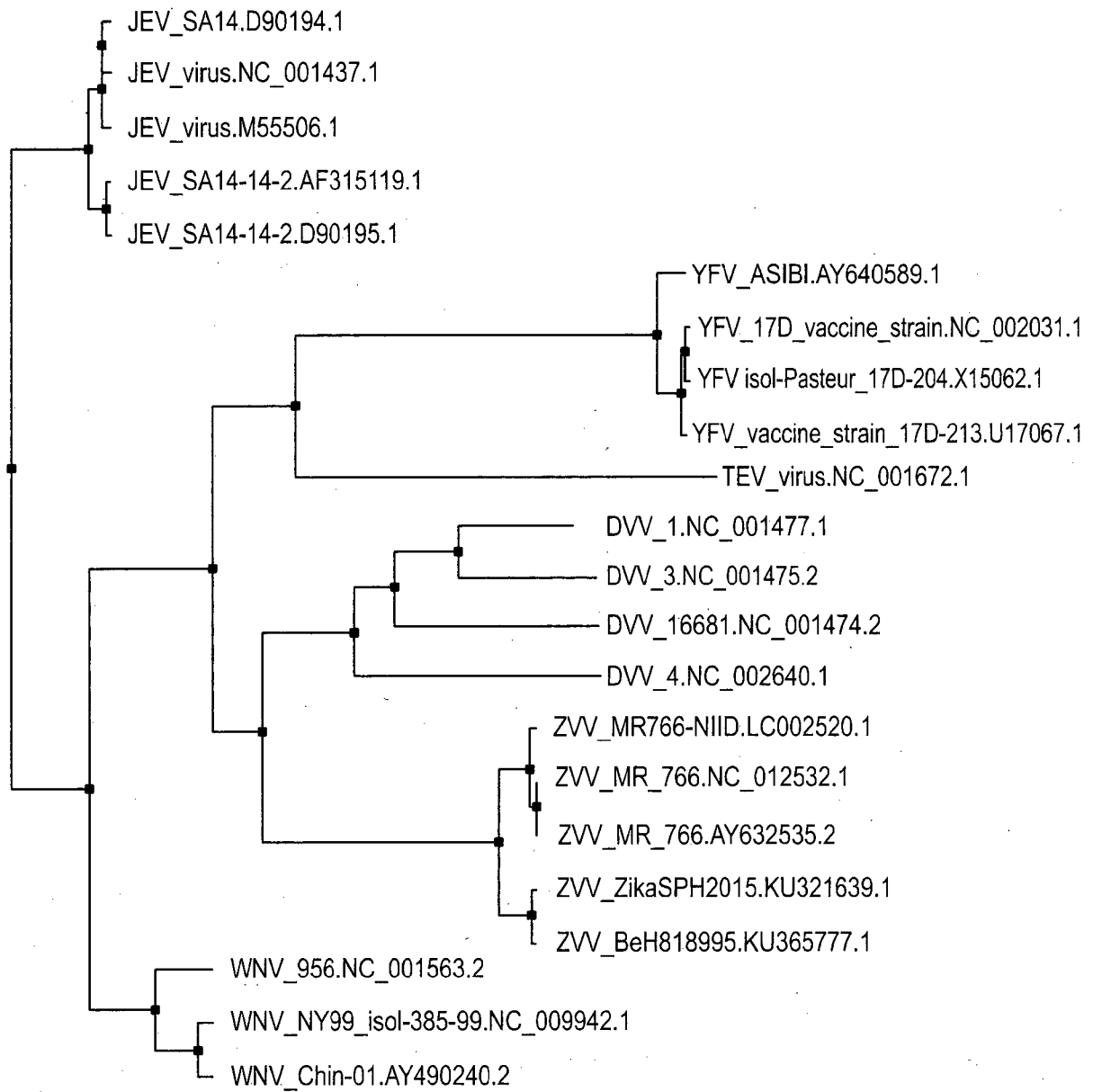


Figure 5

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	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
DV 1.NC 001477.1	0	49.7	46.7	48.0	47.5	49.7	49.7	49.7	50.1	38.3	51.5	50.5	50.9	43.0	43.8	43.0	43.2	57.3	57.9	58.4	58.4	57.1
DV 16681.NC 001474.2	1	68.7	68.7	68.7	68.7	46.7	47.1	46.9	47.5	38.9	48.4	47.6	47.6	43.6	44.4	43.6	43.6	53.6	53.8	54.2	54.2	53.8
DV 3.NC 001475.2	2	77.4	68.7	68.7	68.7	48.0	48.2	48.2	48.6	38.2	46.8	46.7	46.3	41.6	42.2	41.6	41.7	57.3	57.7	58.2	58.2	57.1
DV 4.NC 002640.1	3	63.8	64.0	62.8	60.0	47.5	47.5	47.7	48.1	39.7	49.6	49.6	49.8	40.0	40.6	40.0	40.0	55.8	56.4	56.4	56.4	55.8
JEV SA14-14-2.AF315119.1	4	49.7	46.7	48.0	47.5	49.7	49.7	49.7	50.1	39.6	51.5	50.5	50.9	43.1	42.9	43.1	43.1	54.0	54.8	54.8	54.8	54.0
JEV SA14-14-2.D90195.1	5	49.7	46.7	48.0	47.5	49.7	49.7	49.7	50.1	39.8	51.5	50.5	50.9	43.1	42.9	43.1	43.1	54.0	54.8	54.8	54.8	54.0
JEV SA14.D90194.1	6	50.1	47.1	48.2	47.7	49.7	49.7	49.7	50.1	40.0	51.5	50.5	50.9	43.3	43.3	43.3	43.3	54.4	55.4	55.2	55.2	54.4
JEV virus.M55506.1	7	49.9	46.9	48.2	47.5	49.7	49.7	49.7	50.1	40.0	51.5	50.5	50.9	43.3	43.3	43.3	43.3	54.2	55.2	55.0	55.0	54.2
JEV virus.NC 001437.1	8	50.5	47.5	48.6	48.1	47.8	49.7	49.7	50.1	40.4	51.5	50.5	50.9	43.5	43.5	43.5	43.5	54.8	55.4	55.2	55.2	54.8
TEV virus.NC 001672.1	9	38.3	38.9	38.2	39.7	39.6	39.8	40.0	40.4	40.0	41.3	41.6	41.0	42.3	41.9	42.3	42.3	39.9	39.9	39.6	39.6	39.9
WNV 956.NC 001563.2	10	51.5	48.4	46.8	49.6	46.7	46.7	46.7	47.3	41.3	51.5	50.5	50.9	43.8	43.6	43.8	43.8	55.0	55.0	54.8	54.8	55.0
WNV Chin-01.AY490240.2	11	50.5	47.6	46.7	49.6	46.7	46.7	46.7	47.3	41.6	51.5	50.5	50.9	43.9	43.5	43.9	43.9	54.4	54.4	54.0	54.0	54.4
WNV NY99 isol-385-99.NC 009942.1	12	50.9	47.6	45.3	49.8	46.7	46.7	46.7	47.3	41.0	51.5	50.5	50.9	43.9	43.5	43.9	43.9	54.0	53.8	53.8	53.8	54.0
YFV 17D vaccine strain.NC 002031.1	13	43.0	43.6	41.6	40.0	43.1	43.1	43.3	43.5	42.3	43.8	43.9	43.9	43.9	43.9	43.9	43.9	42.0	43.4	43.5	43.5	42.0
YFV ASIB.AY640589.1	14	43.8	44.4	42.2	40.6	42.9	42.9	43.3	43.5	41.9	43.6	43.5	43.5	43.5	43.5	43.5	43.5	42.4	43.8	43.9	43.9	42.4
YFV isol-Pasteur 17D-204.X15062.1	15	43.0	43.6	41.6	40.0	43.1	43.1	43.3	43.5	42.3	43.8	43.9	43.9	43.9	43.9	43.9	43.9	42.0	43.4	43.5	43.5	42.0
YFV vaccine strain 17D-213.U17067.1	16	43.2	43.6	41.7	40.0	43.1	43.1	43.3	43.5	42.3	43.8	43.9	43.9	43.9	43.9	43.9	43.9	42.0	43.4	43.5	43.5	42.0
ZVVB.H818995.KU365777.1	17	57.3	53.6	57.3	55.8	54.0	54.0	54.4	54.8	39.9	55.0	54.4	54.4	42.0	42.4	42.0	42.0	56.8	56.8	56.4	56.4	56.8
ZVW MR766-NIID.LC002520.1	18	57.9	53.8	57.7	56.4	54.8	54.8	55.4	55.4	39.9	55.0	54.4	54.4	43.4	43.8	43.4	43.4	56.8	56.8	56.4	56.4	56.8
ZVW MR 766.AY632535.2	19	58.4	54.2	58.2	56.4	54.8	54.8	55.2	55.2	39.6	54.8	54.0	53.8	43.5	43.9	43.5	43.5	56.4	56.4	56.0	56.0	56.2
ZVW MR 766.NC 012532.1	20	58.4	54.2	58.2	56.4	54.8	54.8	55.2	55.2	39.6	54.8	54.0	53.8	43.5	43.9	43.5	43.5	56.4	56.4	56.0	56.0	56.2
ZVW ZikaSPH2015.KU321639.1	21	57.1	53.8	57.1	55.8	54.0	54.0	54.4	54.8	39.9	55.0	54.4	54.4	42.0	42.4	42.0	42.0	56.8	56.8	56.4	56.4	56.8

 50 % Identity and higher
 60 % Identity and higher
 70 % Identity and higher

Figure 6

292 AH	G I T D	I E V H G T W S A T	D O K V	V N A P D	S L I	I S U E T V A I D G P	E A	K V Y N	V L T H V K I N O K	S T	C A H L A E E H G D N A	R T Y S	R G V G	G L	S I V A	A K F T 41
292 AH	G I T D	I E V H G T W S A T	O D K V	V N A P D	S L I	I S U E T V A I D R P	E V	K V Y N	V L T H V K I N O K	S T	C A H L A E E H G D N A	R T Y S	R G V G	G L	S I V A	A K F T 41
292 AH	G I T D	I E V H G T W S A T	O D K V	V N A P D	S L I	I S U E T V A I D R P	E V	K V Y N	V L T H V K I N O K	S T	C A H L A E E H G D N A	R T Y S	R G V G	G L	S I V A	A K F T 41
292 AH	G I T D	I E V H G T W S A T	O D K V	V N A P D	S L I	I S U E T V A I D R P	E V	K V Y N	V L T H V K I N O K	S T	C A H L A E E H G D N A	R T Y S	R G V G	G L	S I V A	A K F T 41
307 FN	L G M G N R D I	I E A S A T W D L V	G D S C L	I N A N D	T L	V R M I N I E A S O L	E V	S Y Y H	S V T D I S T V A R	P I T	C A H I E K R A D S S V	Q G F T	D R G V G	G L	S I D T	A K F S 426
307 FN	L G M G N R D I	I E A S A T W D L V	G D S C L	I N A N D	T L	V R M I N I E A S O L	E V	S Y Y H	S V T D I S T V A R	P I T	C A H I E K R A D S S V	Q G F T	D R G V G	G L	S I D T	A K F S 426
307 FN	L G M G N R D I	I E A S A T W D L V	G D S C L	I N A N D	T L	V R M I N I E A S O L	E V	S Y Y H	S V T D I S T V A R	P I T	C A H I E K R A D S S V	Q G F T	D R G V G	G L	S I D T	A K F S 426
307 FN	L G M G N R D I	I E A S A T W D L V	G D S C L	I N A N D	T L	V R M I N I E A S O L	E V	S Y Y H	S V T D I S T V A R	P I T	C A H I E K R A D S S V	Q G F T	D R G V G	G L	S I D T	A K F S 426
307 FN	L G M G N R D I	I E A S A T W D L V	G D S C L	I N A N D	T L	V R M I N I E A S O L	E V	S Y Y H	S V T D I S T V A R	P I T	C A H I E K R A D S S V	Q G F T	D R G V G	G L	S I D T	A K F S 426
294 MR	V G I G N	H V E L S A T W	H G S V	T A K O I	T L	F E L K T E V T N P	V L	K L I E	K I S N T T D S R	P I O	E A T L V E E O D T H F V	E R T F V	R G V G	G L	S L I T	A K F K 413
287 MR	I G M S N	D I V E S G S W D I V	H G S V	T A K I P	T L	F E E K T E A Q P	T L	K Y I E	K L T N T T T E S R	P I O	E P S L N E E O O K R F V	H S M V R	R G V G	G L	G I V T	A M F R 406
313 MR	V G V G N	D I V E S G S A T W	H G G V	T A K O I	T L	F E L K T A K E V	L L	T Y I E	S I N S I T A T R	P I O	E A P L V E E O O Q Q Y I	R D V V R	R G V G	G L	G V V T	A K F S 433
292 SR	T H L E N	D I V T Q T I T R	L G G V	T A E G	S M E D A I	Y Q E N P A K T	E Y	L H	K I S O T K V A A R	P I M	E A T L A E E H Q G G T V	R D O S R	R G V G	G L	S L V T	A K F Q 413
306 FN	L G M S N	D I V E S A T W	G D S V	I N S K D	T	V K M N N E A A N L	D V	S Y Y L	S V S D L S T R A A	P I M	E A H I E K R A D P A F V	Q G V V R	R G V G	G L	S I V A	V K A A 411
306 FN	L G M S N	D I V E S A T W	G D S V	I N S K D	T	V K M N N E A A N L	D V	S Y Y L	S V S D L S T R A A	P I M	E A H I E K R A D P A F V	Q G V V R	R G V G	G L	S I D T	A K F A 425
306 FN	L G M S N	D I V E S A T W	G D S V	I N S K D	T	V K M N N E A A N L	D V	S Y Y L	S V S D L S T R A A	P I M	E A H I E K R A D P A F V	Q G V V R	R G V G	G L	S I D T	A K F A 425
326 IR	I G V S N	D I V E S G T W D V	H G G V	V N A Q D	T V	I E L V T T V S N M	E V	S Y Y E	S I S D M A S D S R	P I O	E A Y L D K Q S D T Q V Y	R T L V R	R G V G	G L	S L V T	A K F T 445
326 IR	I G V S N	D I V E S G T W D V	H G G V	V N A Q D	T V	I E L V T T V S N M	E V	S Y Y E	S I S D M A S D S R	P I O	E A Y L D K Q S D T Q V Y	R T L V R	R G V G	G L	S L V T	A K F A 445
326 IR	I G V S N	D I V E S G T W D V	H G G V	V N A Q D	T V	I E L V T T V S N M	E V	S Y Y E	S I S D M A S D S R	P I O	E A Y L D K Q S D T Q V Y	R T L V R	R G V G	G L	S L V T	A K F T 445
326 IR	I G V S N	D I V E S G T W D V	H G G V	V N A Q D	T V	I E L V T T V S N M	E V	S Y Y E	S I S D M A S D S R	P I O	E A Y L D K Q S D T Q V Y	R T L V R	R G V G	G L	S L V T	A K F T 445
308 IR	I G V S N	D I V E S G T W D V	H G G V	V N A Q D	T V	I E L V T T V S N M	E V	S Y Y E	S I S D M A S D S R	P I O	E A Y L D K Q S D T Q V Y	R T L V R	R G V G	G L	S L V T	A K F A 427
412	--AKSMSLFEVDOTKIO	V I R A O L	V B A K O E	-----	NWNTD I K T L K F D A L S G S O E A E F	T G T	K A T L E	Q V O T A V D F G N S Y I A E M E K	-----	E S W I	D R Q	A Q	L T	Q S G S G G	514	
412	--AKSMSLFEVDOTKIO	V I R A O L	V B A K O E	-----	NWNTD I K T L K F D A L S G S O E E F	I G Y	K A T L E	Q V O T A V D F G N S Y I A E M E T	-----	E S W I	D R Q	A Q	L T	Q S G S G G	514	
412	--AKSMSLFEVDOTKIO	V I R A O L	V B A K O E	-----	NWNTD I K T L K F D A L S G S O E E F	I G Y	K A T L E	Q V O T A V D F G N S Y I A E M E T	-----	E S W I	D R Q	A Q	L T	Q S G S G G	514	
412	--AKSMSLFEVDOTKIO	V I R A O L	V B A K O E	-----	NWNTD I K T L K F D A L S G S O E E F	I G Y	K A T L E	Q V O T A V D F G N S Y I A E M E T	-----	E S W I	D R Q	A Q	L T	Q S G S G G	514	
427	--TSKA I G R T I O P E N I K	E V G I F V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
427	--TSKA I G R T I O P E N I K	E V G I F V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
427	--TSKA I G R T I O P E N I K	E V G I F V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
427	--TSKA I G R T I O P E N I K	E V G I F V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
427	--TSKA I G R T I O P E N I K	E V G I F V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
417	--K K M E G K V Q P E N I K	S V I T V T	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
417	--K K M E G K V Q P E N I K	S V I T V T	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
433	--S G K I T G N L V I E N I L	T V I T V T	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
414	--L E P I E G K V Q P E N I K	T V I T V T	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
412	--E A K K K A T G H V Y D A N K I V	T V K V E P T	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
428	--T T K A T G W I I K E N I K	E V A I F V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
428	--T T K A T G W I I K E N I K	E V A I F V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
428	--T T K A T G W I I K E N I K	E V A I F V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
446	--S K K M T G K S I Q P E N I L	R I M L S V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
446	--S K K M T G K S I Q P E N I L	R I M L S V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
446	--S K K M T G K S I Q P E N I L	R I M L S V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
446	--S K K M T G K S I Q P E N I L	R I M L S V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
446	--S K K M T G K S I Q P E N I L	R I M L S V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537
428	--S K K M T G K S I Q P E N I L	R I M L S V	-----	TTTSENHGNYS A Q V G A S O A K F T	V T P I N A P S I	I T L K G O Y	E V T L O D	EP R S	L N T E A F Y V N T V G S	-----	K S F L	H R E	F H	L A	T S P S S T	537

[illegible]

Figure 7 (Part 2 of 3)

9/35

748	M	G	A	V	L	I	W	V	G	I	N	T	R	N	M	T	M	S	M	I	L	V	G	V	I	M	M	F	L	S	L	G	V	G	A	
748	M	G	A	V	L	I	W	V	G	I	N	T	R	N	M	T	M	S	M	I	L	V	G	V	I	M	M	F	L	S	L	G	V	G	A	
748	M	G	A	V	L	I	W	V	G	I	N	T	R	N	M	T	M	S	M	I	L	V	G	V	I	M	M	F	L	S	L	G	V	G	A	
748	M	G	A	V	L	I	W	V	G	I	N	T	R	N	M	T	M	S	M	I	L	V	G	V	I	M	M	F	L	S	L	G	V	G	A	
770	M	G	A	L	L	L	M	G	V	N	A	R	D	R	S	I	A	L	A	F	L	A	T	G	V	L	V	F	L	A	T	N	H	A	H	
770	M	G	A	L	L	L	M	G	V	N	A	R	D	R	S	I	A	L	A	F	L	A	T	G	V	L	V	F	L	A	T	N	H	A	H	
770	M	G	A	L	L	L	M	G	V	N	A	R	D	R	S	I	A	L	A	F	L	A	T	G	V	L	V	F	L	A	T	N	H	A	H	
770	M	G	A	L	L	L	M	G	V	N	A	R	D	R	S	I	A	L	A	F	L	A	T	G	V	L	V	F	L	A	T	N	H	A	H	
770	M	G	A	L	L	L	M	G	V	N	A	R	D	R	S	I	A	L	A	F	L	A	T	G	V	L	V	F	L	A	T	N	H	A	H	
752	I	G	L	L	T	W	L	G	L	N	S	R	S	T	S	L	S	M	T	C	I	A	V	G	M	T	L	Y	L	G	V	M	Q	A		
745	I	G	V	I	T	W	I	G	M	N	S	R	S	T	S	L	S	V	T	L	V	L	V	G	I	V	T	L	Y	L	G	V	M	Q	A	
771	I	G	F	L	V	W	I	G	T	N	S	R	N	T	S	M	A	M	T	C	I	A	V	G	I	T	L	F	L	G	F	T	M	Q	A	
750	I	G	V	L	T	W	I	G	L	N	S	K	N	T	S	M	S	F	S	C	I	A	I	S	I	I	T	L	Y	L	G	A	V	Q	A	
751	L	G	V	A	L	A	M	L	G	L	N	M	R	N	P	T	M	S	M	S	F	L	A	S	G	L	V	L	A	M	T	L	G	V	A	
766	L	G	A	L	L	M	G	I	N	A	R	D	R	S	I	A	M	T	F	L	A	V	S	G	V	L	L	F	L	S	V	N	H	A		
770	L	G	A	L	L	M	G	I	N	A	R	D	R	S	I	A	L	T	F	L	A	V	S	G	V	L	L	F	L	S	V	N	H	A		
770	L	G	A	L	L	M	G	I	N	A	R	D	R	S	I	A	L	T	F	L	A	I	G	G	V	L	L	F	L	S	V	N	H	A		
793	I	G	T	L	V	W	L	G	L	N	T	K	N	G	S	I	S	L	T	C	L	A	L	G	G	V	M	I	F	L	S	T	A	M	S	A
793	I	G	T	L	L	M	W	L	G	L	N	T	K	N	G	S	I	S	L	M	C	L	A	L	G	V	L	I	F	L	S	T	A	M	S	A
789	I	G	T	L	V	W	L	G	L	N	T	K	N	G	S	I	S	L	T	C	L	A	L	G	G	V	M	I	F	L	S	T	A	M	S	A
789	I	G	T	L	V	W	L	G	L	N	T	K	N	G	S	I	S	L	T	C	L	A	L	G	G	V	M	I	F	L	S	T	A	M	S	A
775	I	G	T	L	M	W	L	G	L	N	T	K	N	G	S	I	S	L	M	C	L	A	L	G	G	V	L	I	F	L	S	T	A	M	S	A

YFV_ASBI.AY640589.1/292-784
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YFV_vaccine_strain_17D-213.U17067.1/292-784
YFV_isol-Pasteur_17D-204.X15062.1/292-784
IEV_SA14.D90194.1/307-806
IEV_virus_NC_001437.1/307-806
IEV_virus.M55506.1/307-806
IEV_SA14-14-2.AF315119.1/307-806
IEV_SA14-14-2.D90195.1/307-806
DWV_1.NC_001477.1/294-788
DWV_16681.NC_001474.2/287-781
DWV_4.NC_002640.1/313-807
DWV_3.NC_001475.2/294-786
TEV_virus_NC_001672.1/292-787
WNV_956.NC_001563.2/306-802
WNV_NY99_isol-385-99.NC_009942.1/306-806
WNV_Chin-01.AY490240.2/306-806
ZVV_MR766-NIID.LC002520.1/326-829
ZVV_ZikaSPH2015.KU321639.1/326-829
ZVV_MR_766.NC_012532.1/326-825
ZVV_MR_766.AY632535.2/326-825
ZVV_BeH818995.KU365777.1/308-811

Figure 7 (Part 3 of 3)

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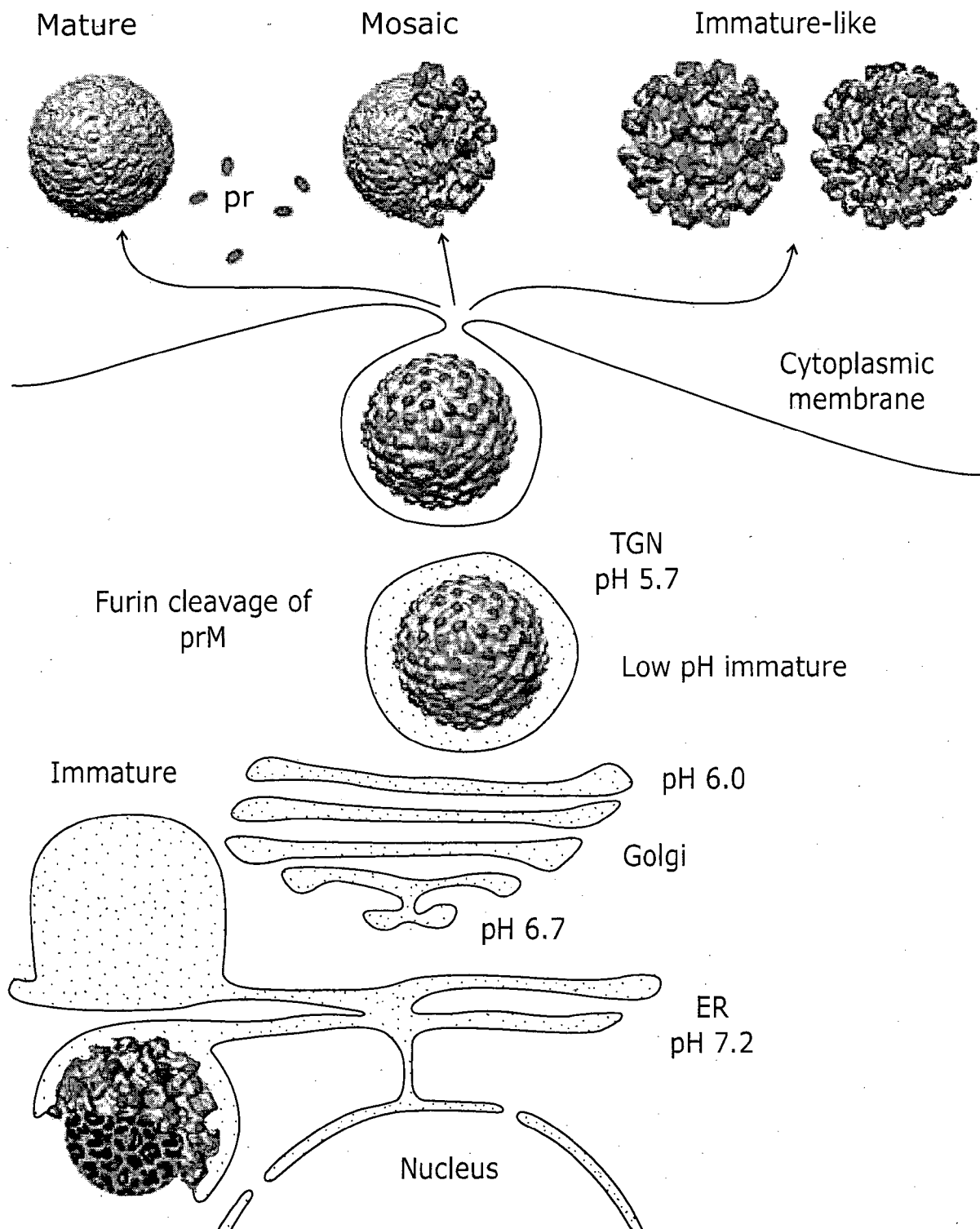


Figure 8

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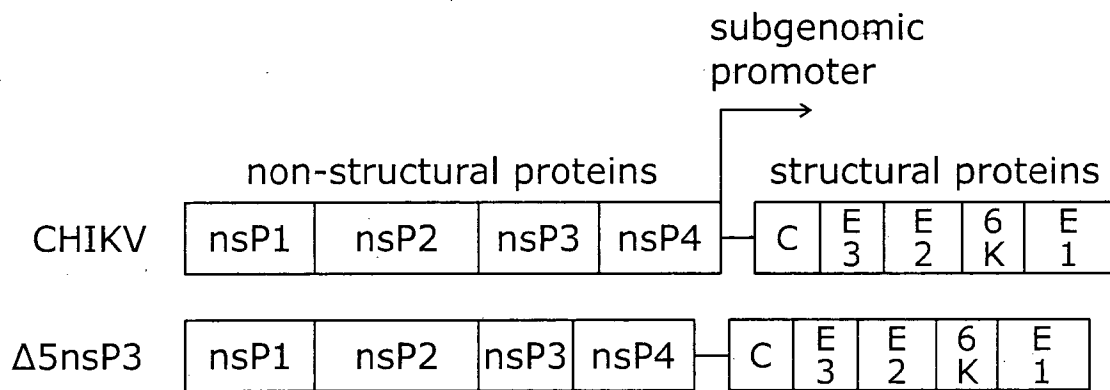


Figure 9

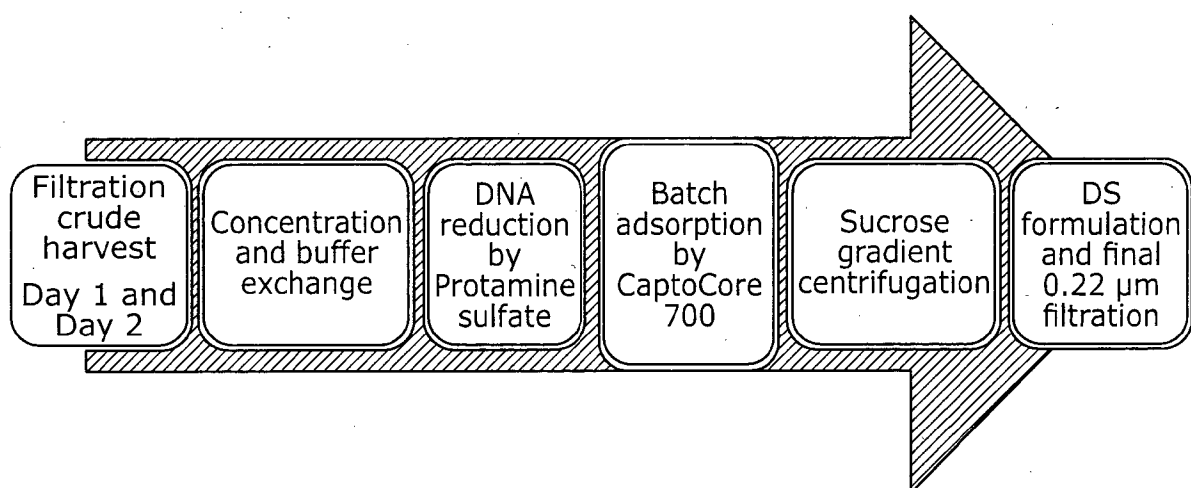


Figure 10

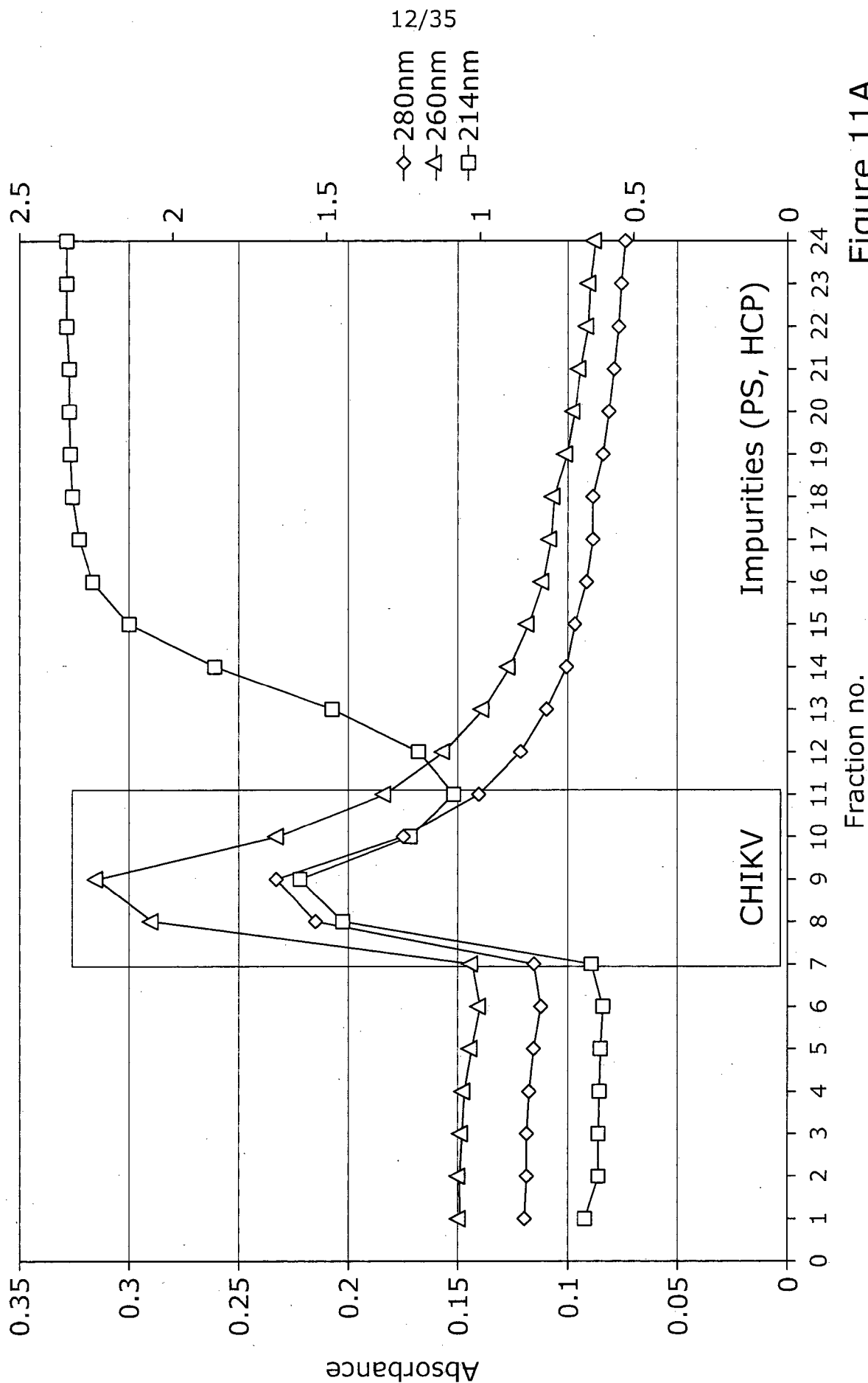


Figure 11A

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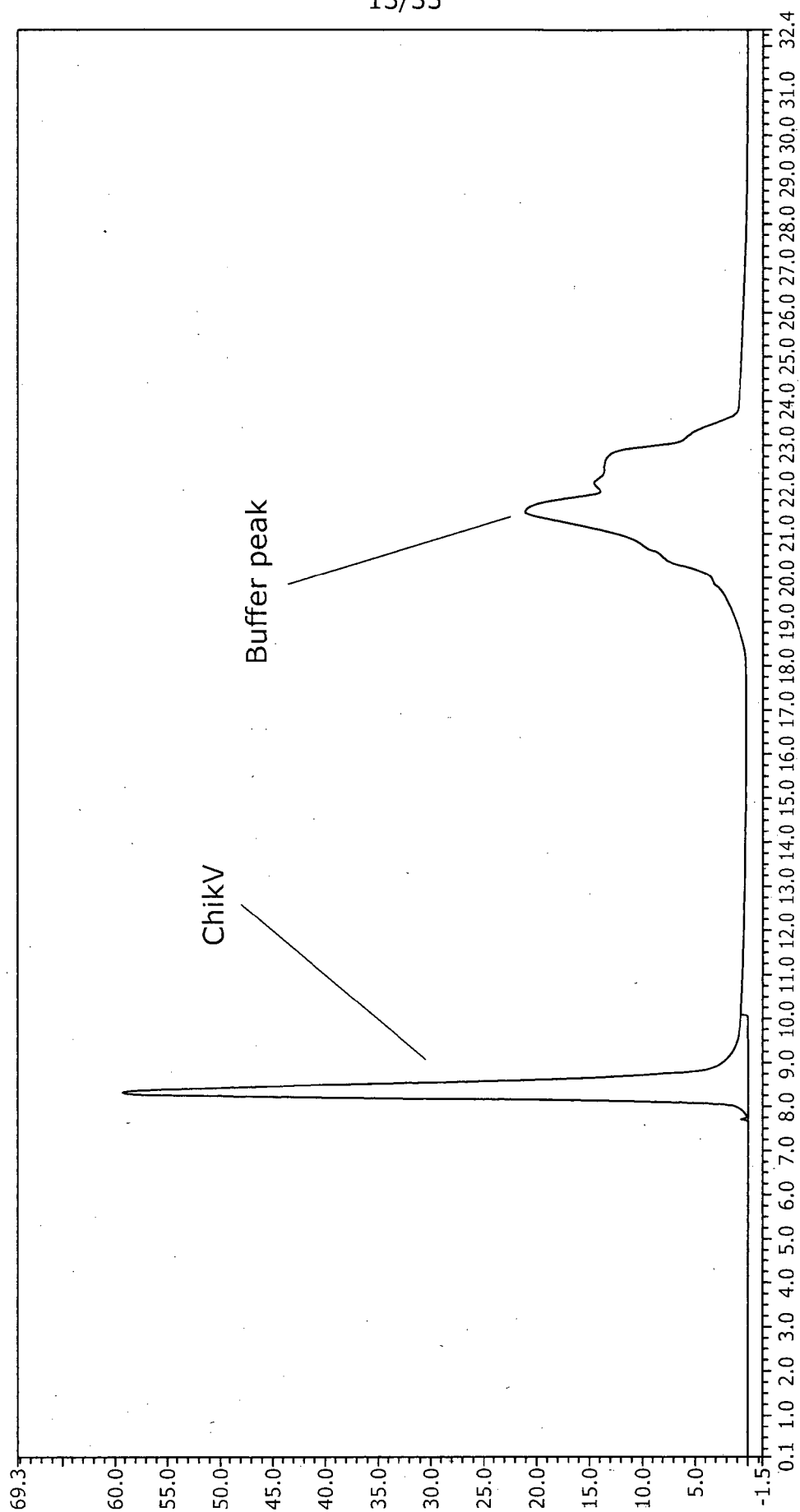
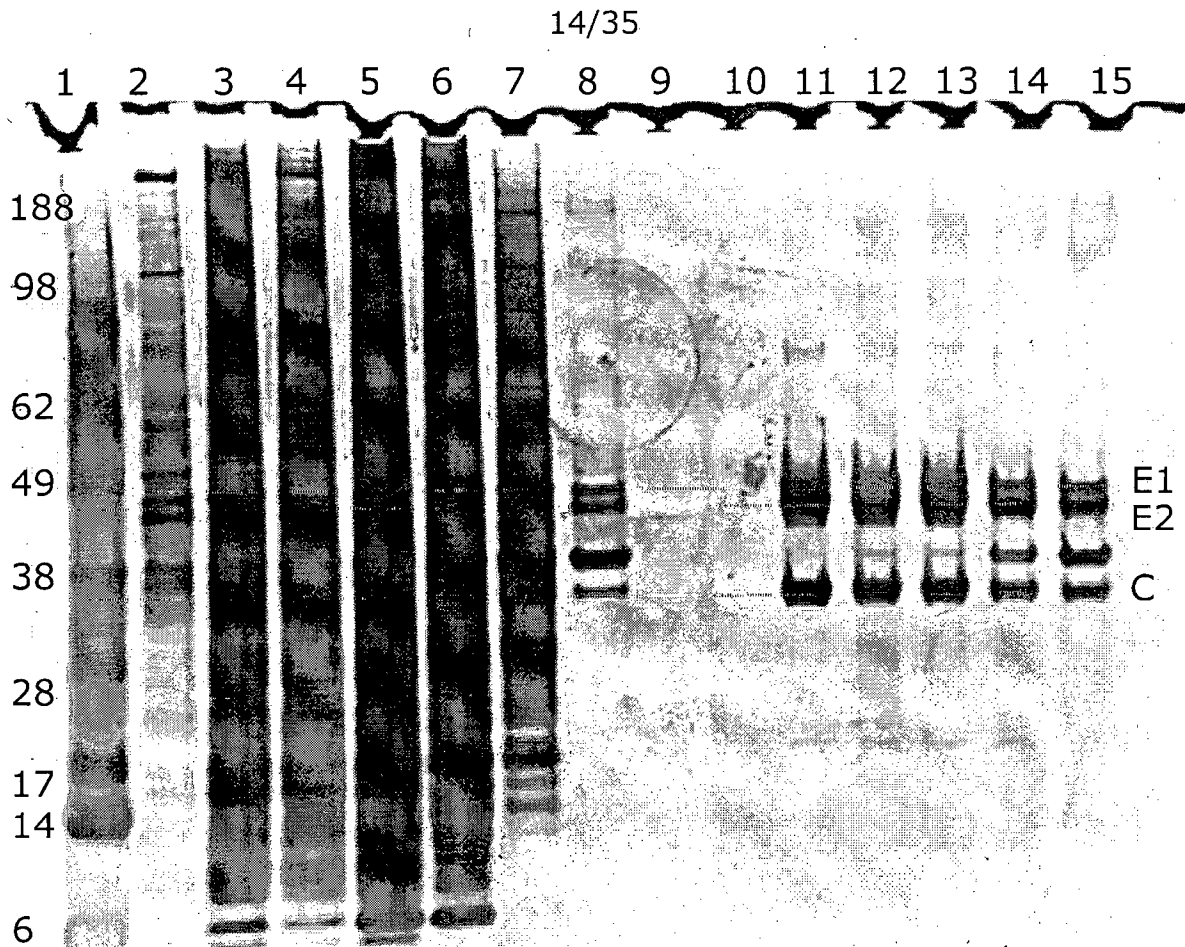


Figure 11B



Lane	Sample
1	Marker Seeblueplus2
2	CHIKV lot20150812_WVB2015-01_Filtered harvest0.2 μ _24hpi
3	CHIKV lot20150812_WVB2015-01_Filtered harvest0.2 μ _48hpi
4	20150812_CHIKV_DSP_UF/DF_Load
5	20150812_CHIKV_DSP_UF/DF_conc.10x
6	20150813_CHIKV_DSP_UF/DF_conc.&dia. 11x
7	20150813_CHIKV_DSP_PStreatment
8	20150813_CHIKV_DSP_PS&CC700treatment
9	20150813_CHIKV_DSP_SGCFrac F5
10	20150813_CHIKV_DSP_SGCFrac F6
11	20150813_CHIKV_DSP_SGCPoolF7-F10
12	20150813_CHIKV_DSP_SGCPoolF7-F11 (final pool)
13	20150813_CHIKV_DSP_SGCPoolF7-F12
14	20150813_CHIKV_DSP_SGCFrac F13
15	20150813_CHIKV_DSP_SGCFrac F14

Figure 11C

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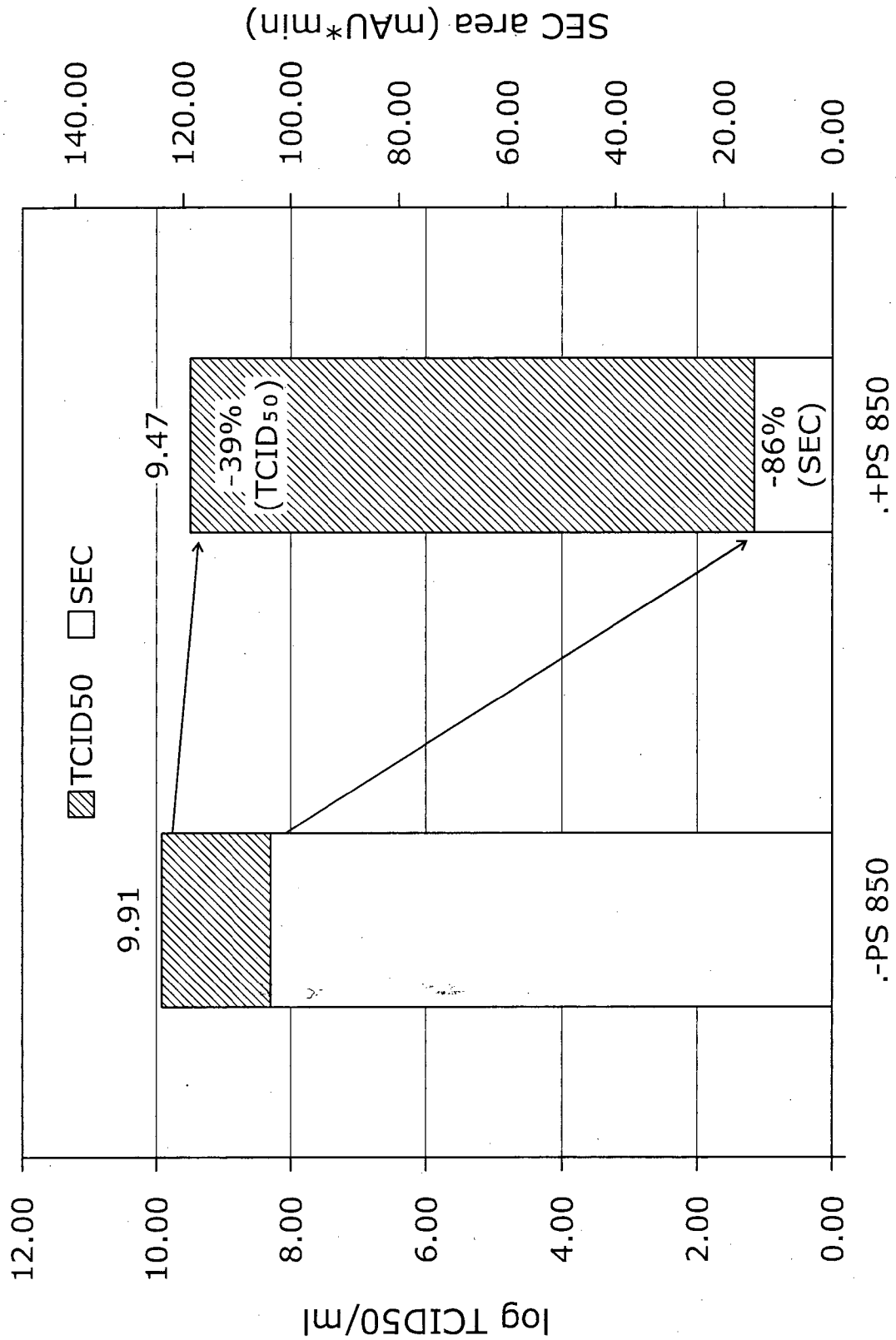


Figure 12A

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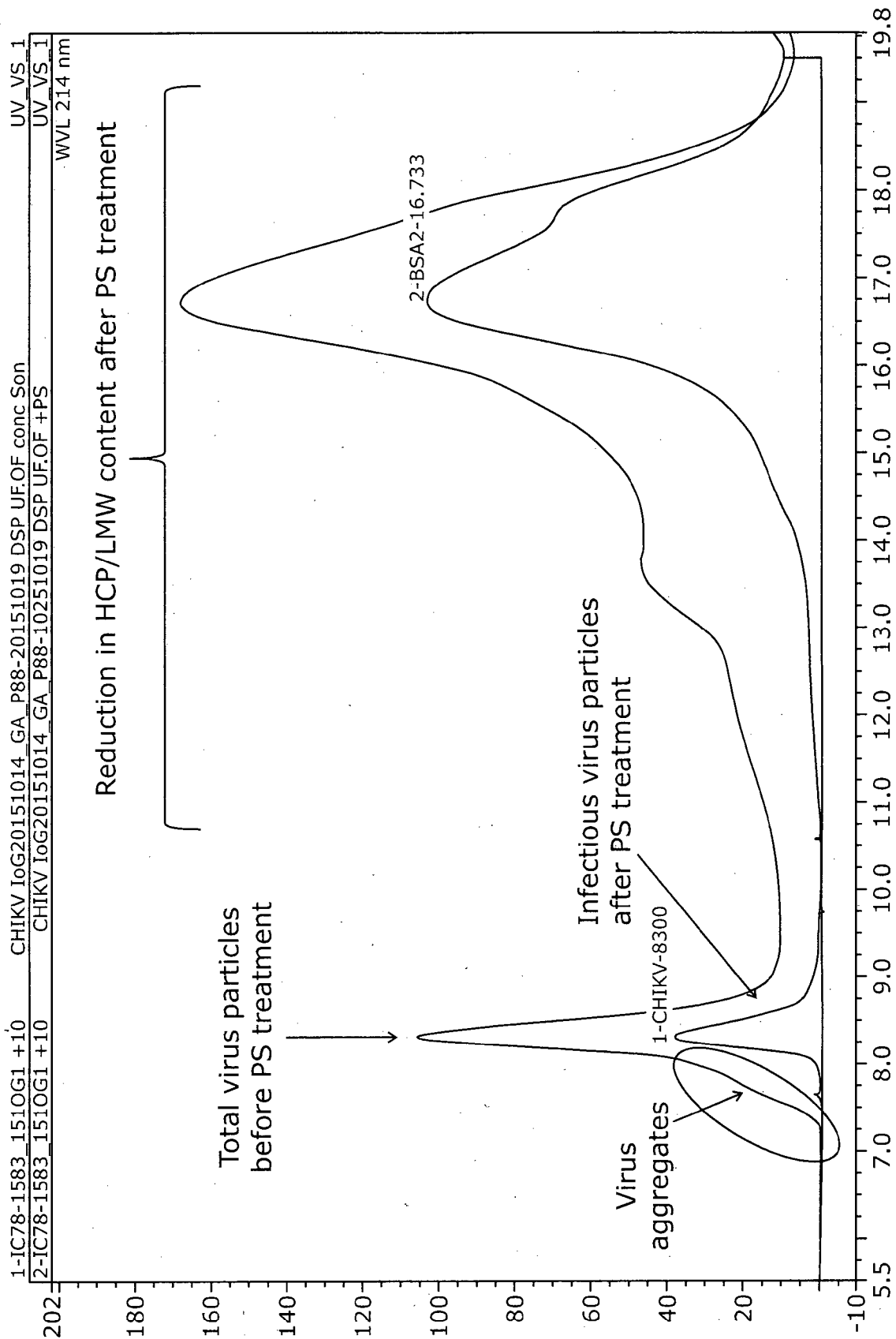


Figure 12B

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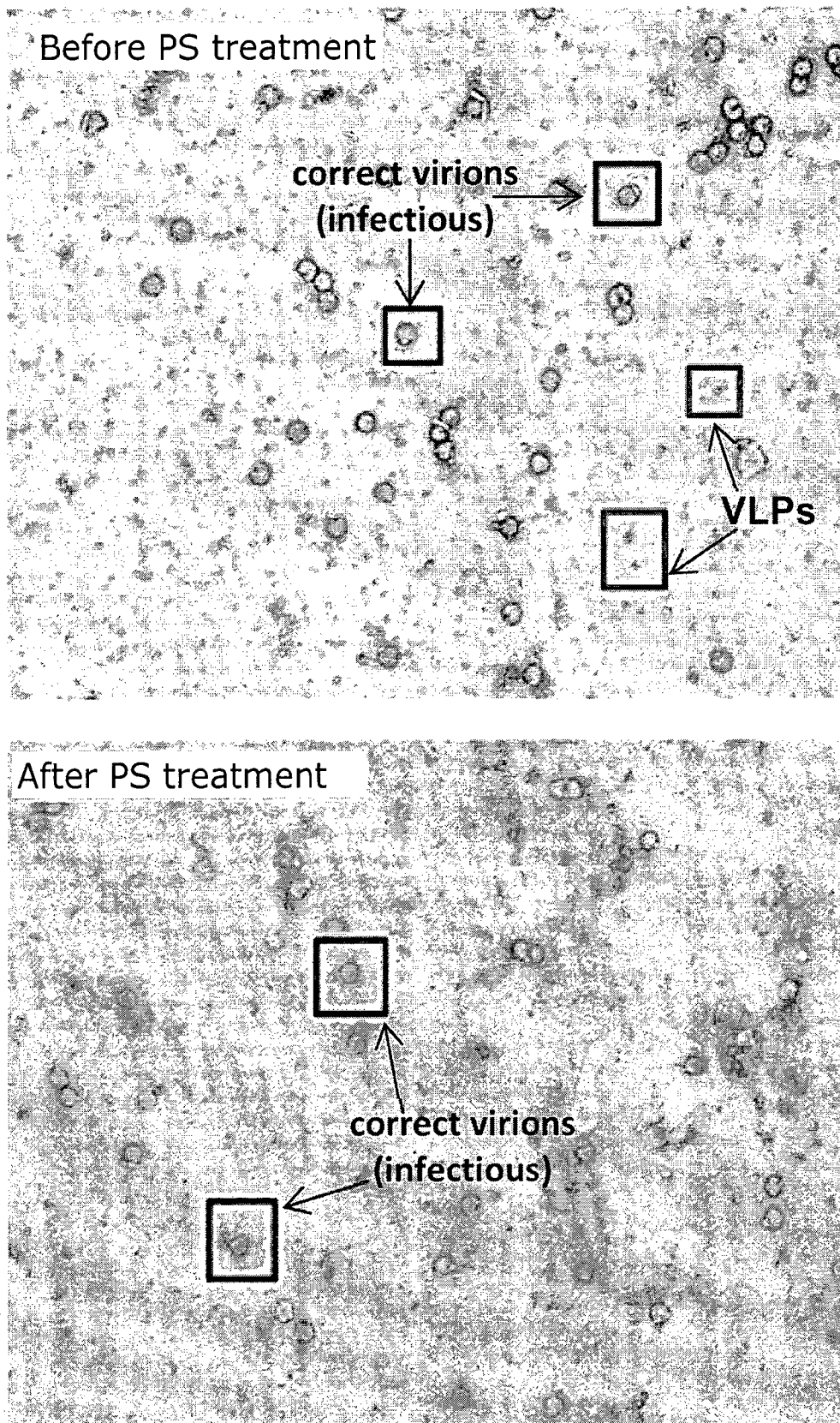


Figure 13

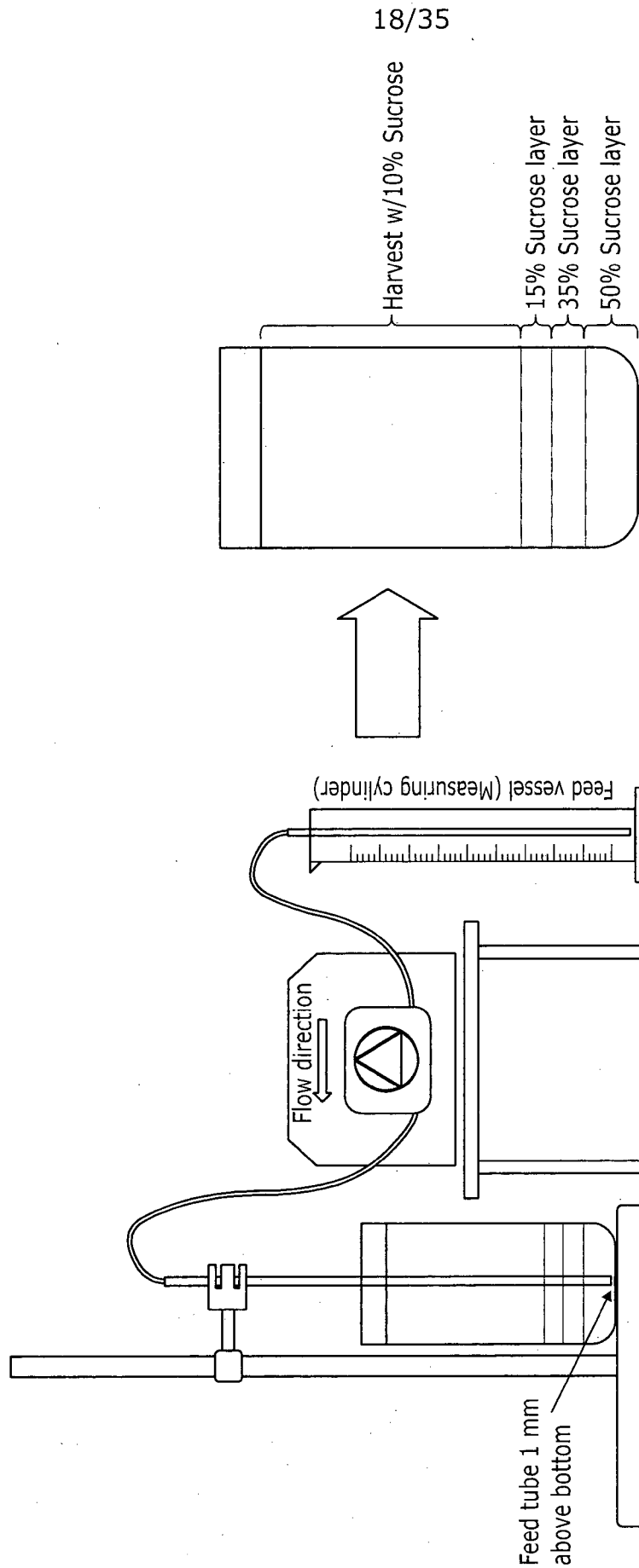


Figure 14

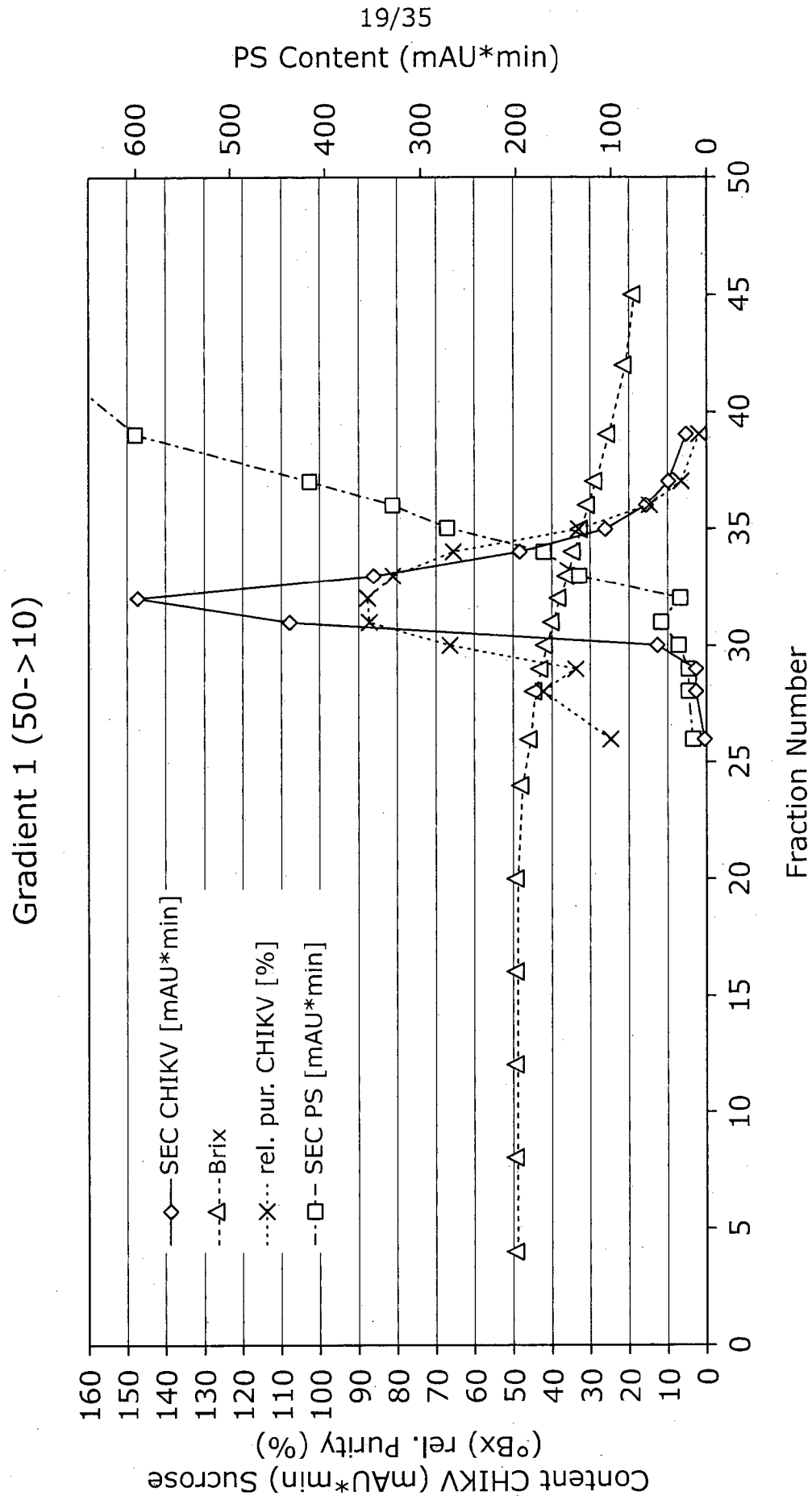


Figure 15A

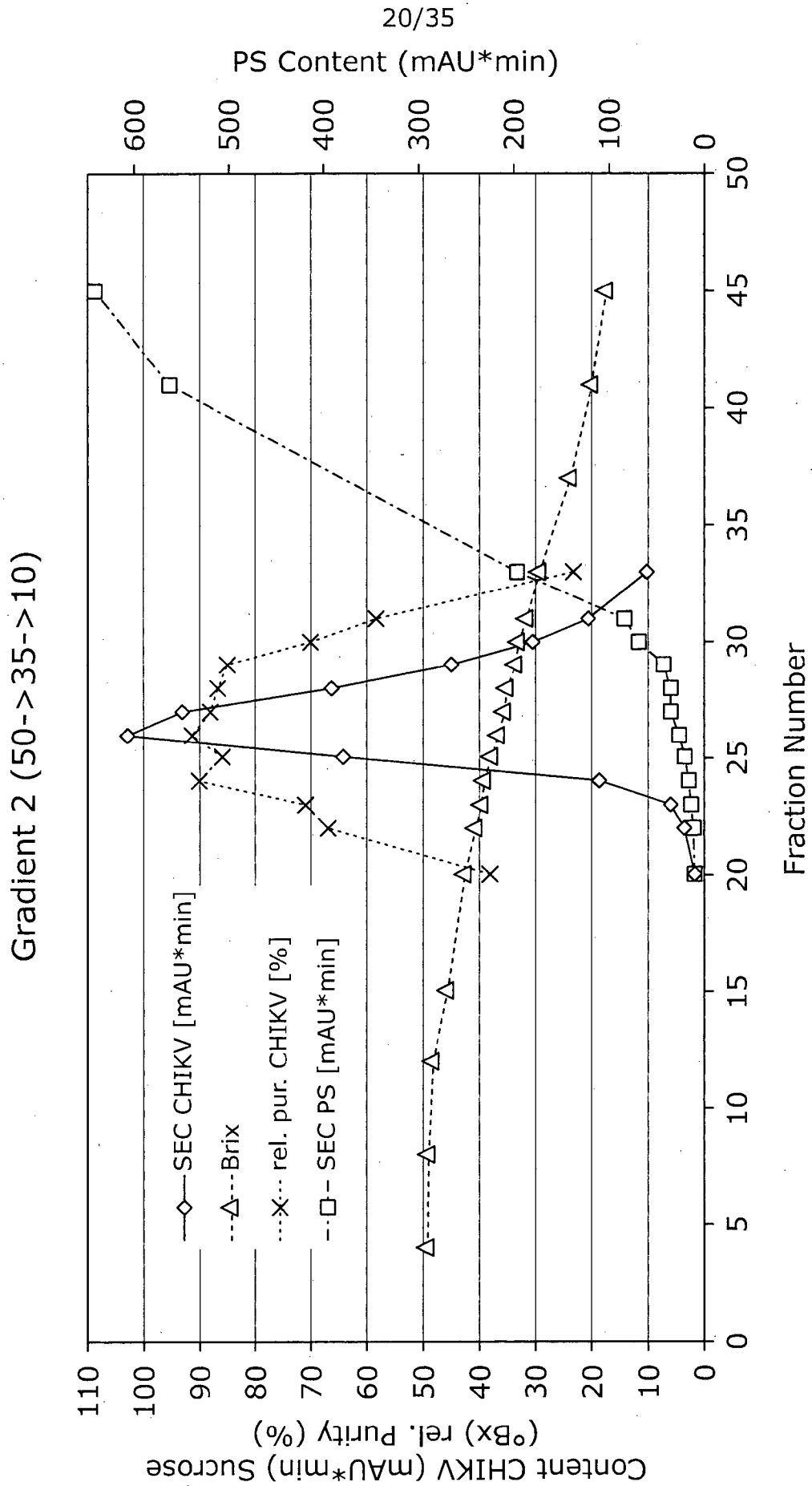


Figure 15B

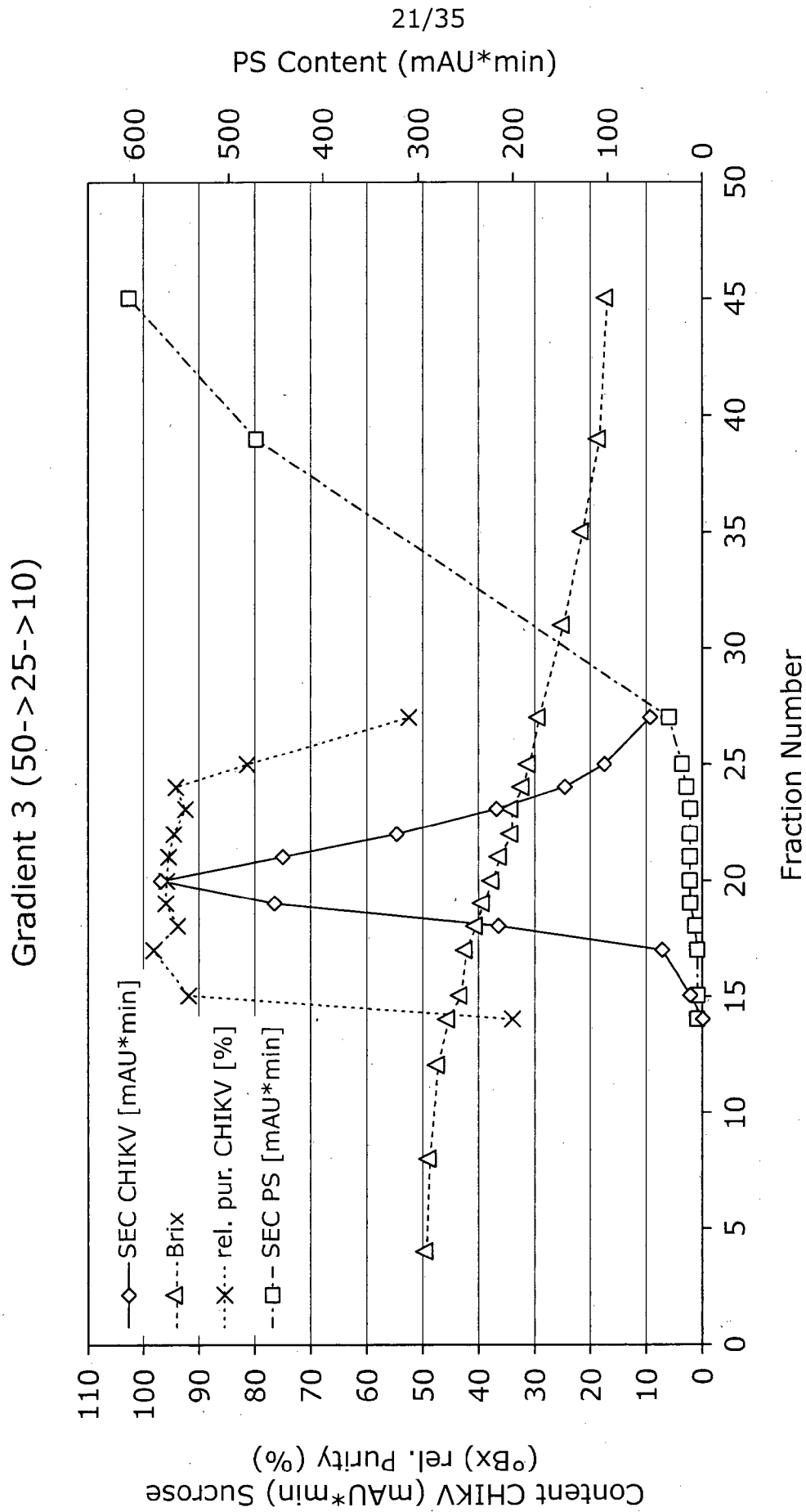


Figure 15C

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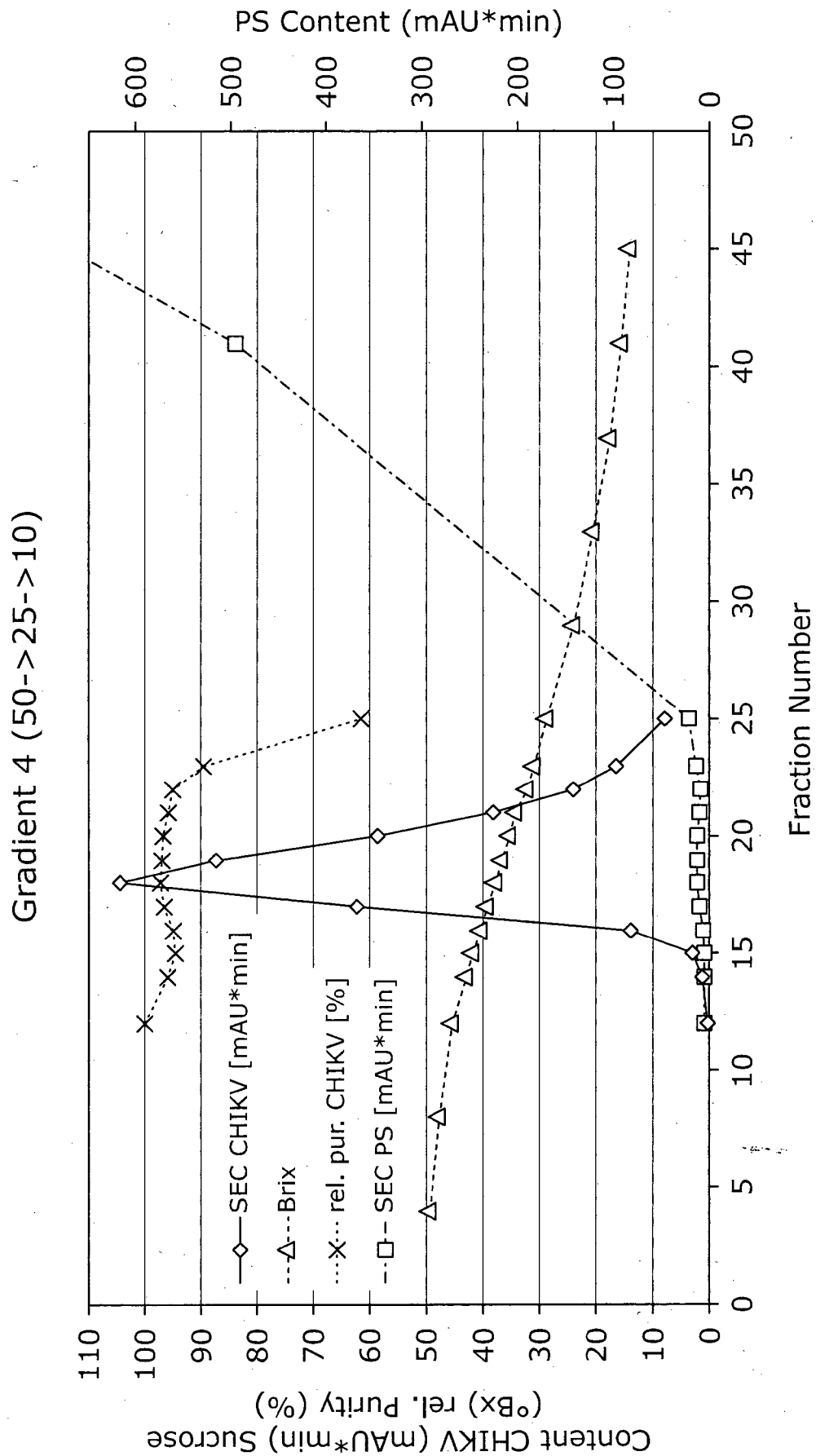


Figure 15D

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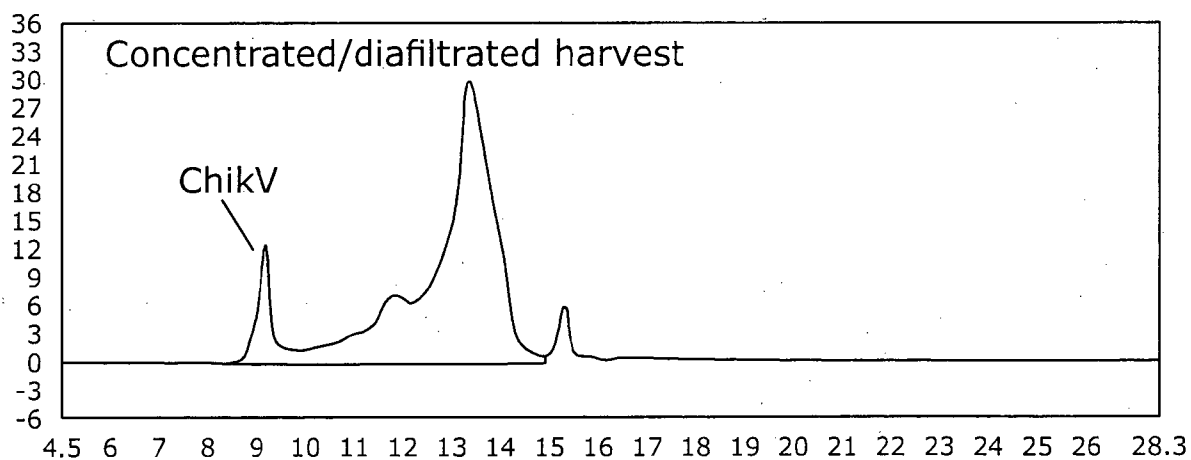
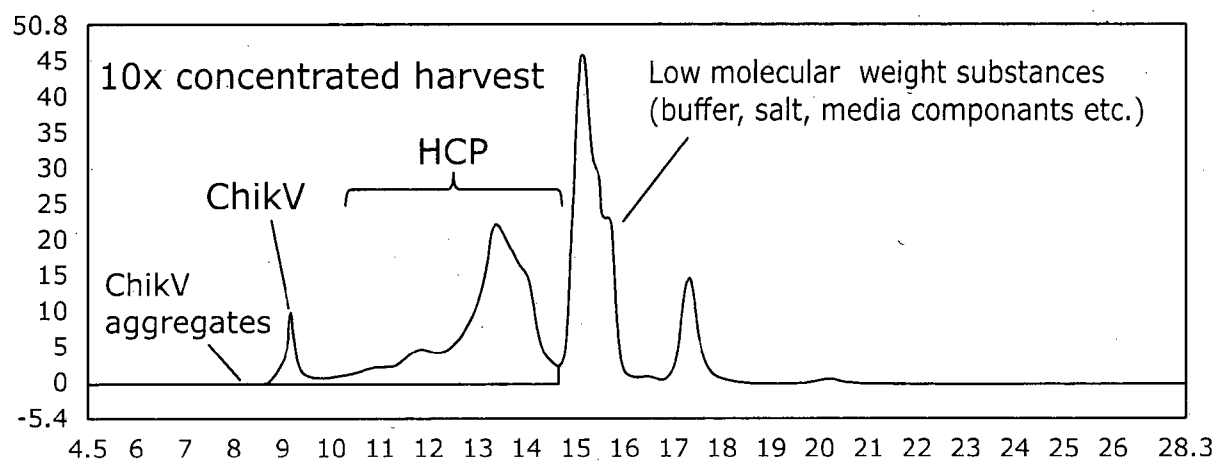
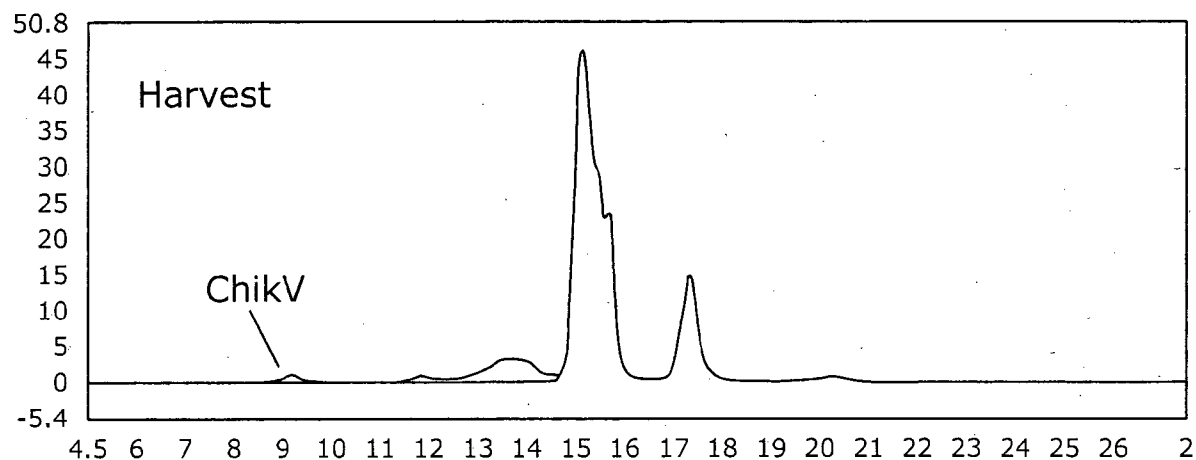


Figure 16 (Part 1 of 2)

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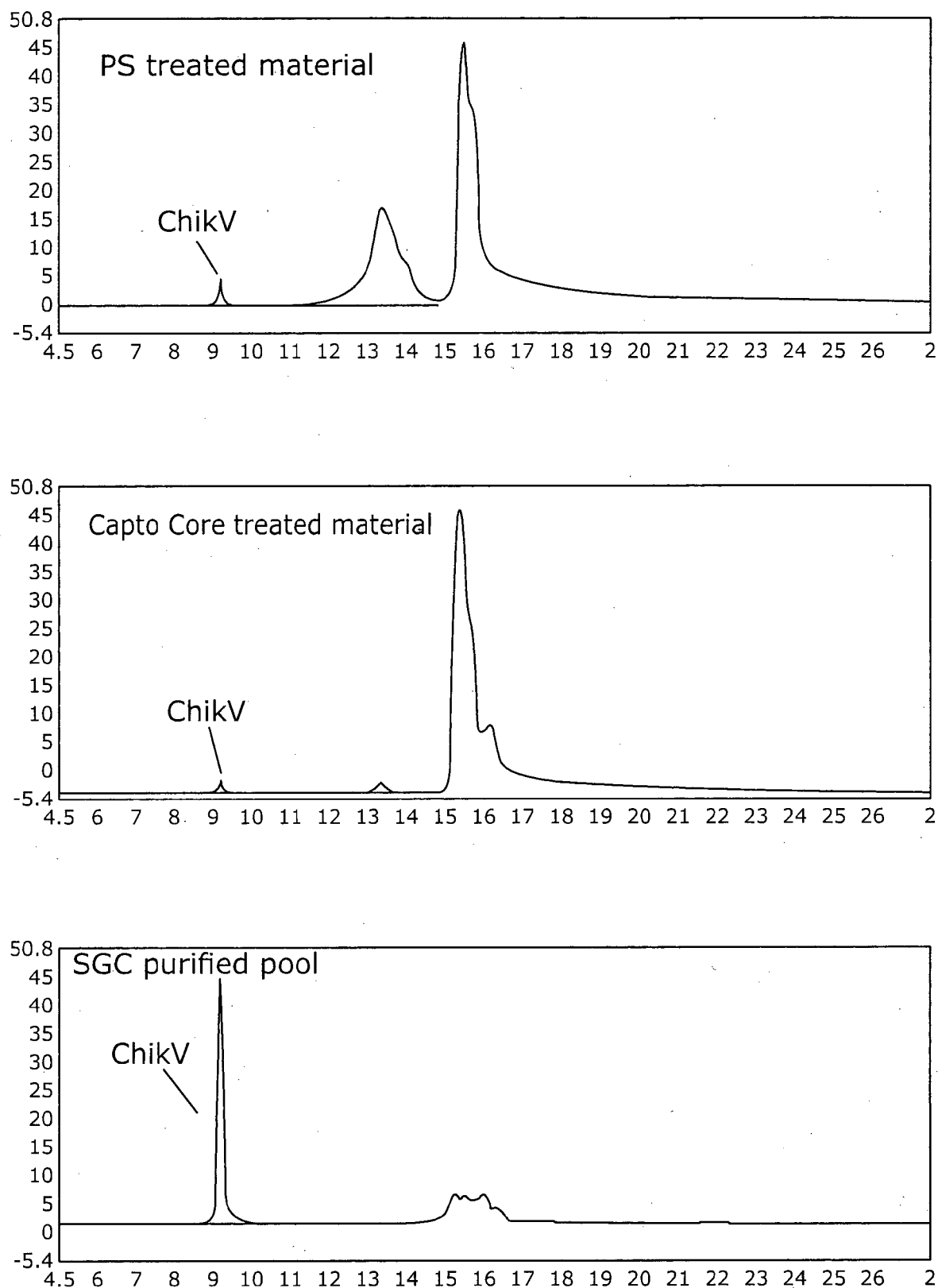


Figure 16 (Part 2 of 2)

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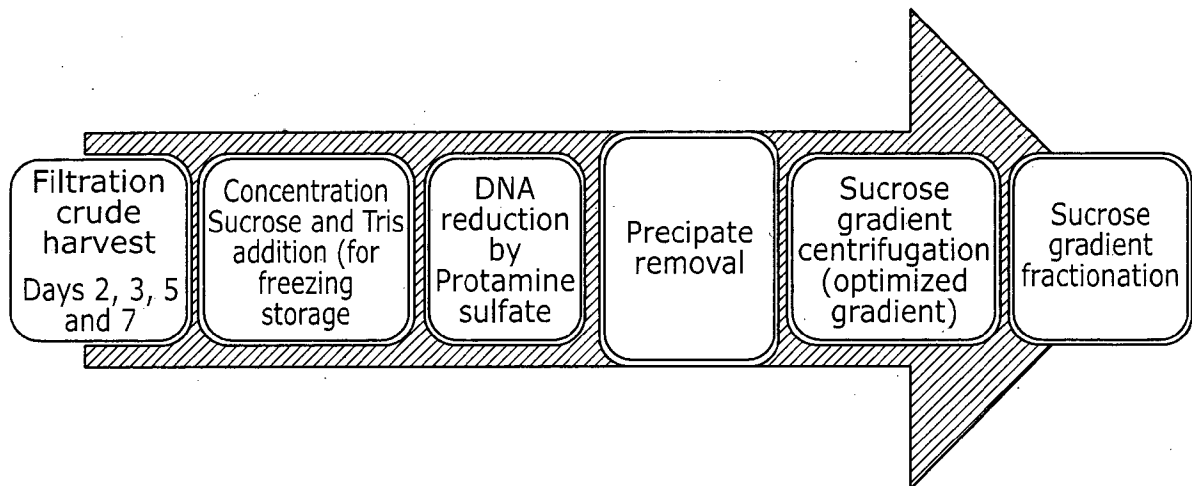


Figure 17A

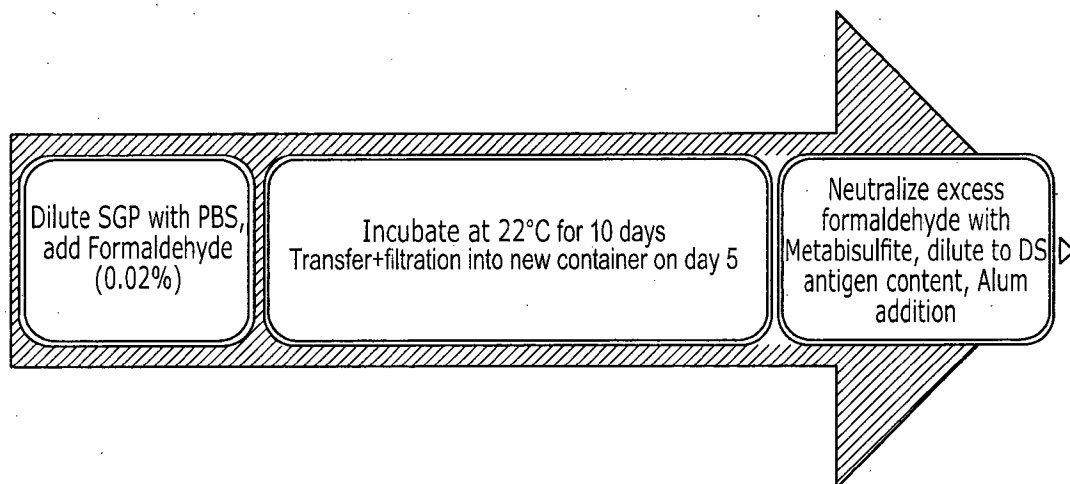


Figure 17B

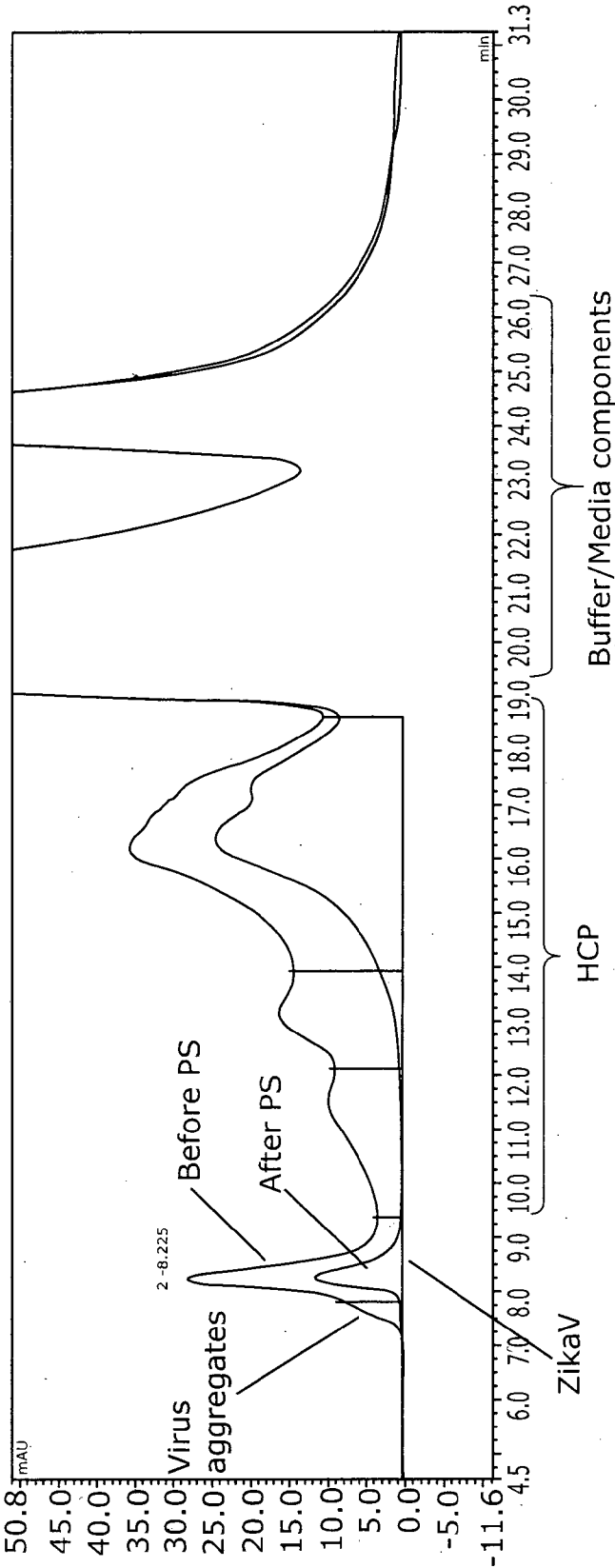
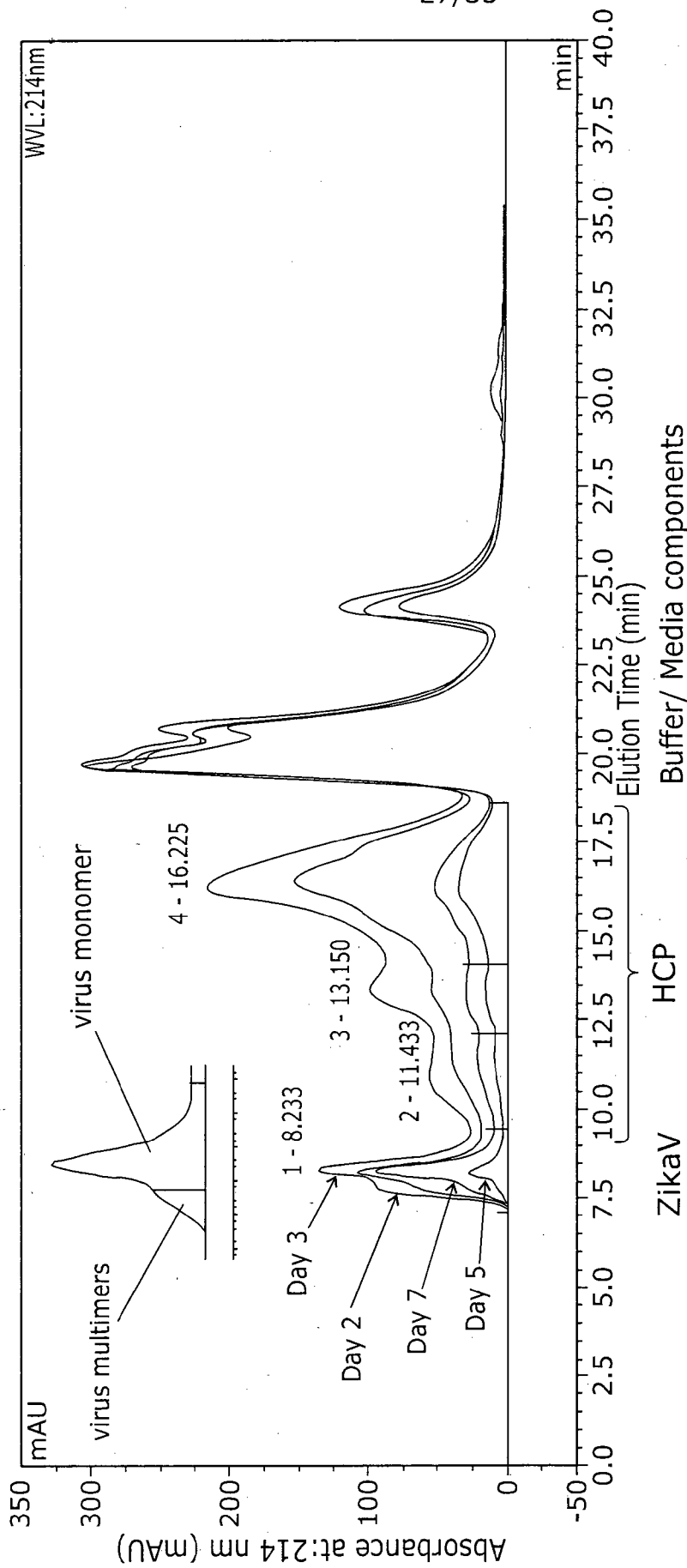


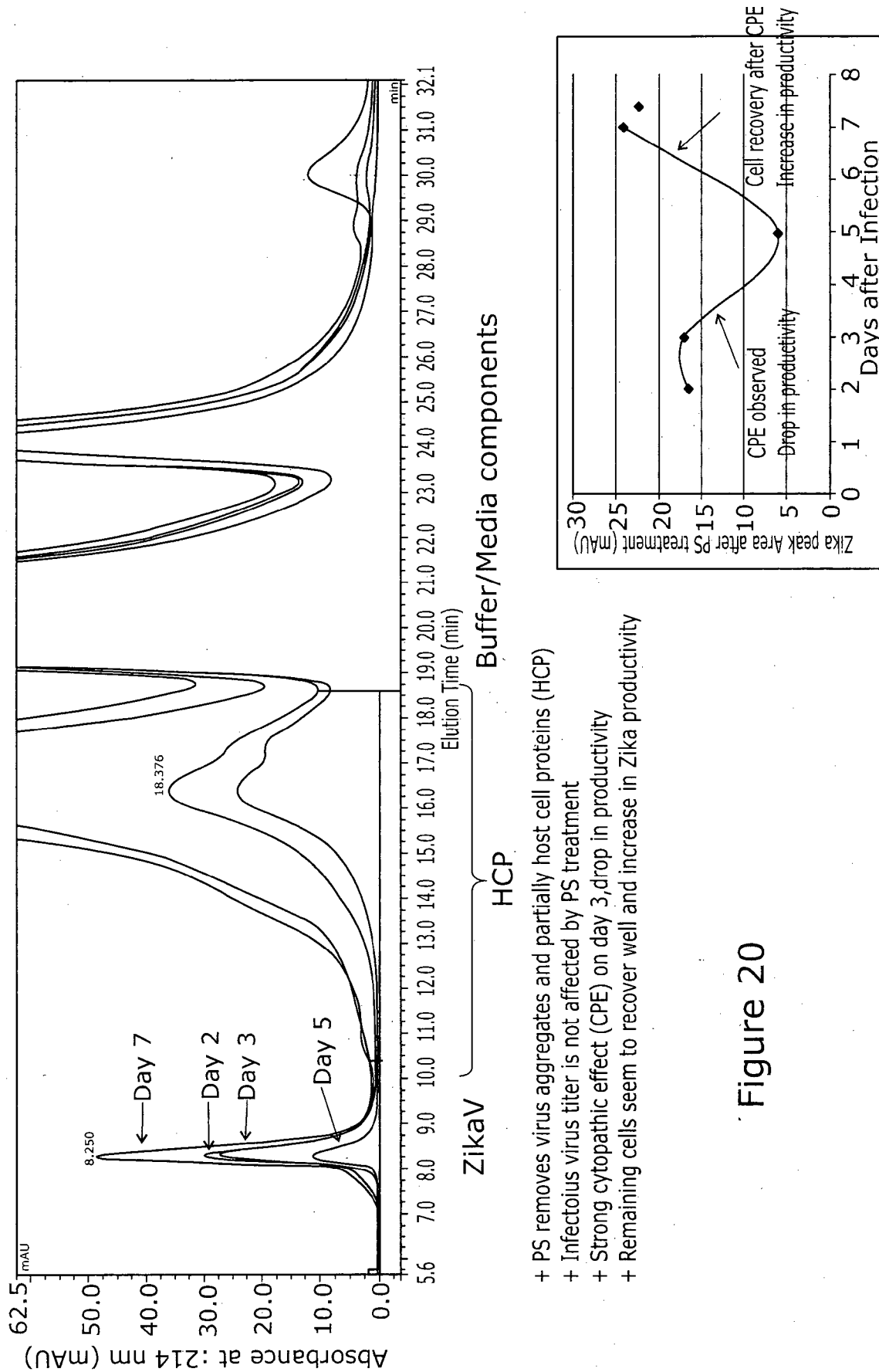
Figure 18



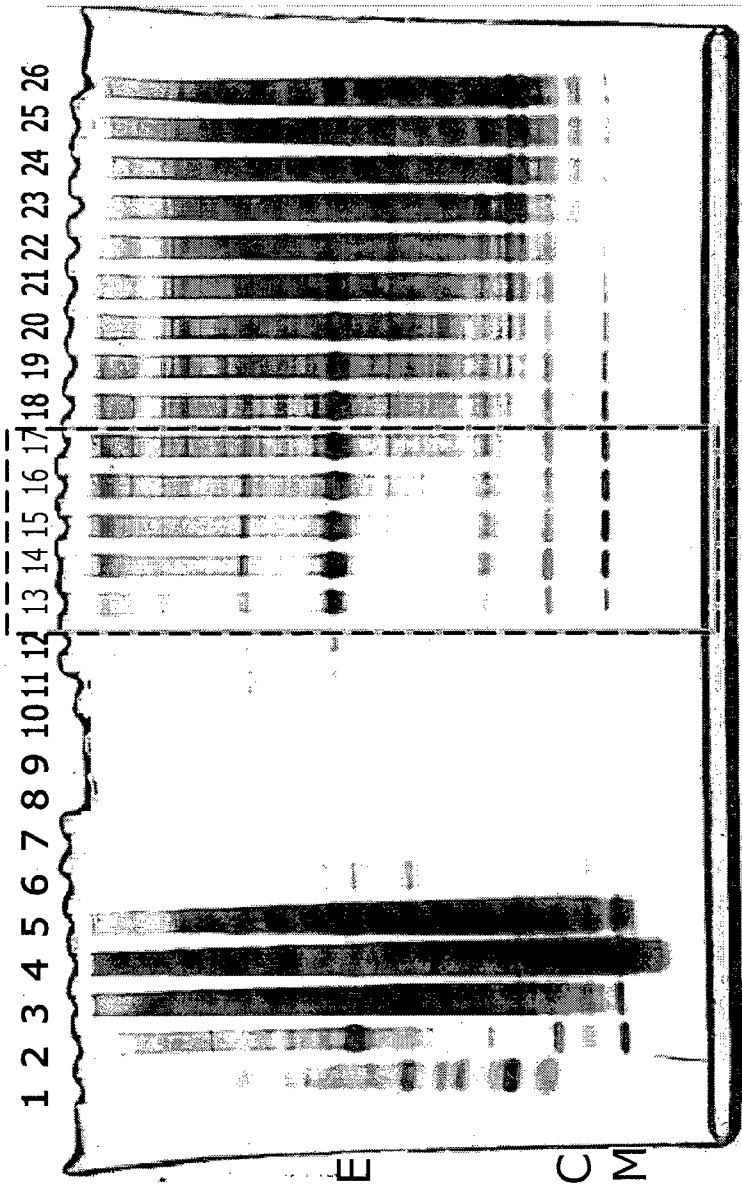
- + Similar virus concentration in harvests Day 2, Day 3, Day 7; lower virus concentration in harvest Day 5
- + Day 2 and Day 3 harvests contained more HCP than Days 5/7 (correlates with observed CPE)
- + Minor amount of virus multimers observed

Figure 19

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Lane	Sample
1	Molecular weight Marker
2	JEV Sucrose Gradient Pool (Reference)
3	Zika Harvest pool (day 2,3,5,7)
4	Zika Harvest pool (day 2,3,5,7) concentrated 300x
5	Zika Harvest pool (day 2,3,5,7) concentrated 300x + Protamine sulfate
6	Ultrafiltration Permeate
7	SGC Bottle 1, Fraction 4
8	SGC Bottle 1, Fraction 5
9	SGC Bottle 1, Fraction 6
10	SGC Bottle 1, Fraction 7
11	SGC Bottle 1, Fraction 8
12	SGC Bottle 1, Fraction 9
13	SGC Bottle 1, Fraction 10
14	SGC Bottle 1, Fraction 11
15	SGC Bottle 1, Fraction 12
16	SGC Bottle 1, Fraction 13
17	SGC Bottle 1, Fraction 14
18	SGC Bottle 1, Fraction 15
19	SGC Bottle 1, Fraction 16
20	SGC Bottle 1, Fraction 17
21	SGC Bottle 1, Fraction 18
22	SGC Bottle 1, Fraction 19
23	SGC Bottle 1, Fraction 20
24	SGC Bottle 1, Fraction 21
25	SGC Bottle 1, Fraction 22
26	SGC Bottle 1, Fraction 23



Pooled fractions F10-F14
(10mL total volume pere bottle)

Figure 21

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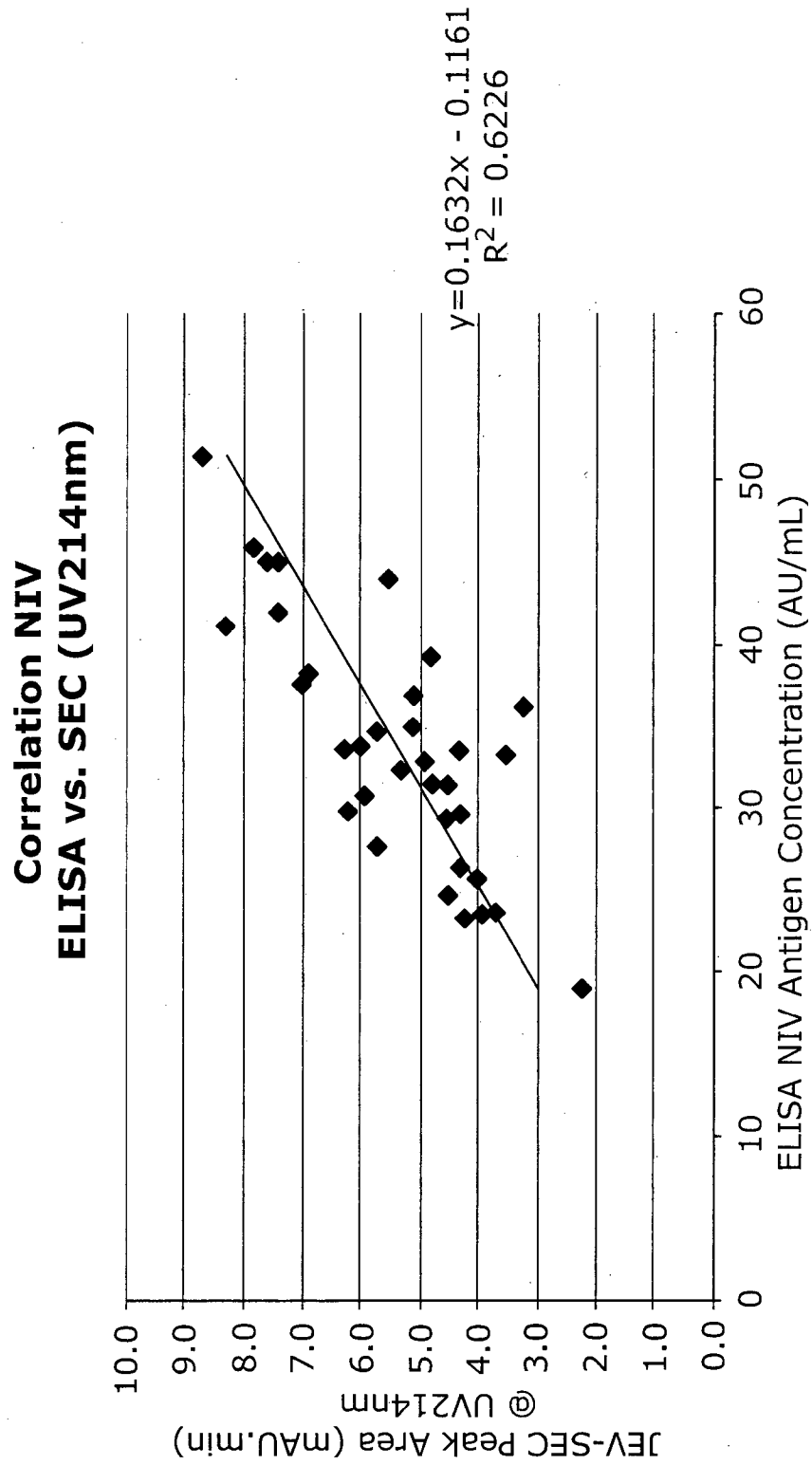


Figure 22

Comparison of harvest schedules

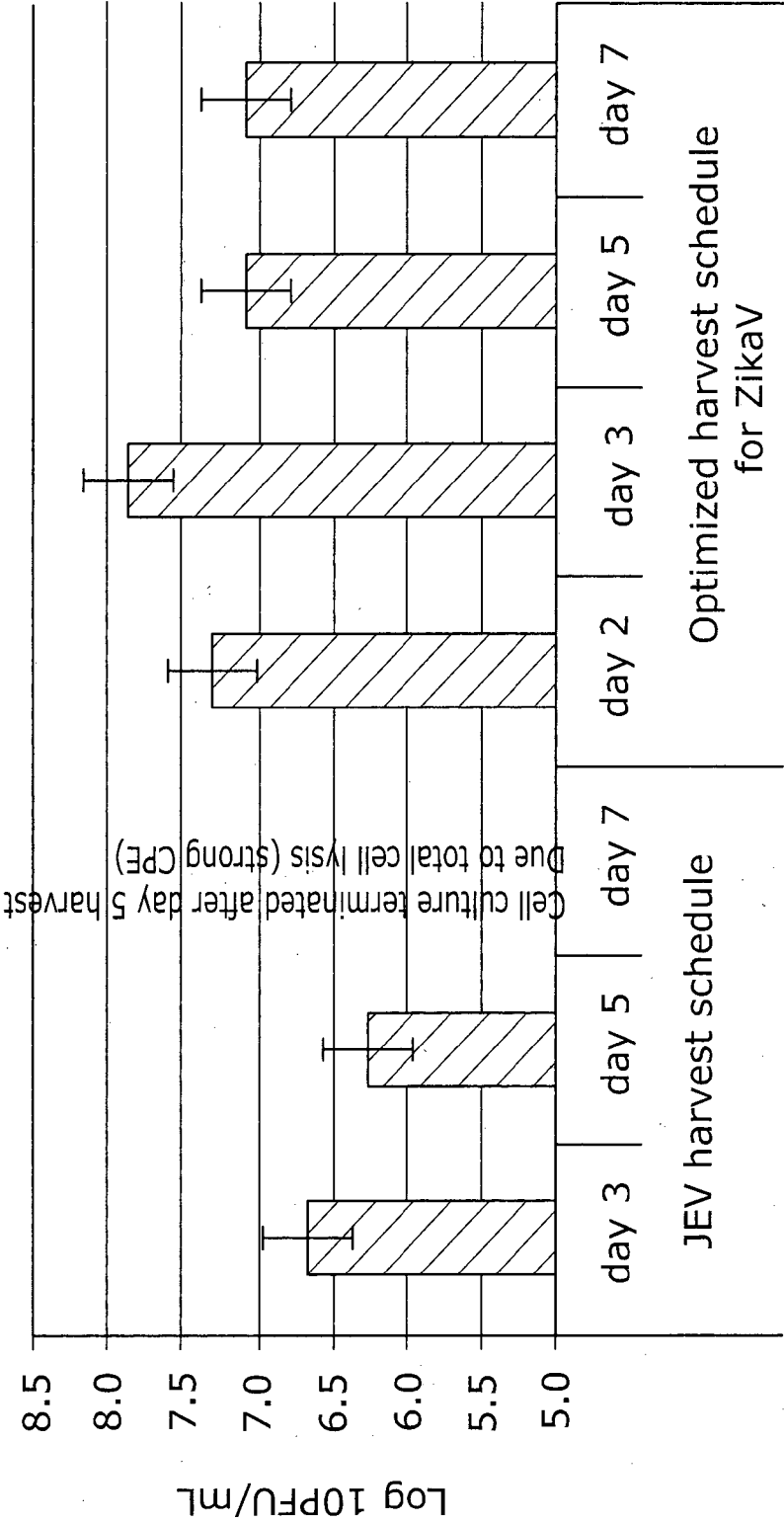


Figure 23

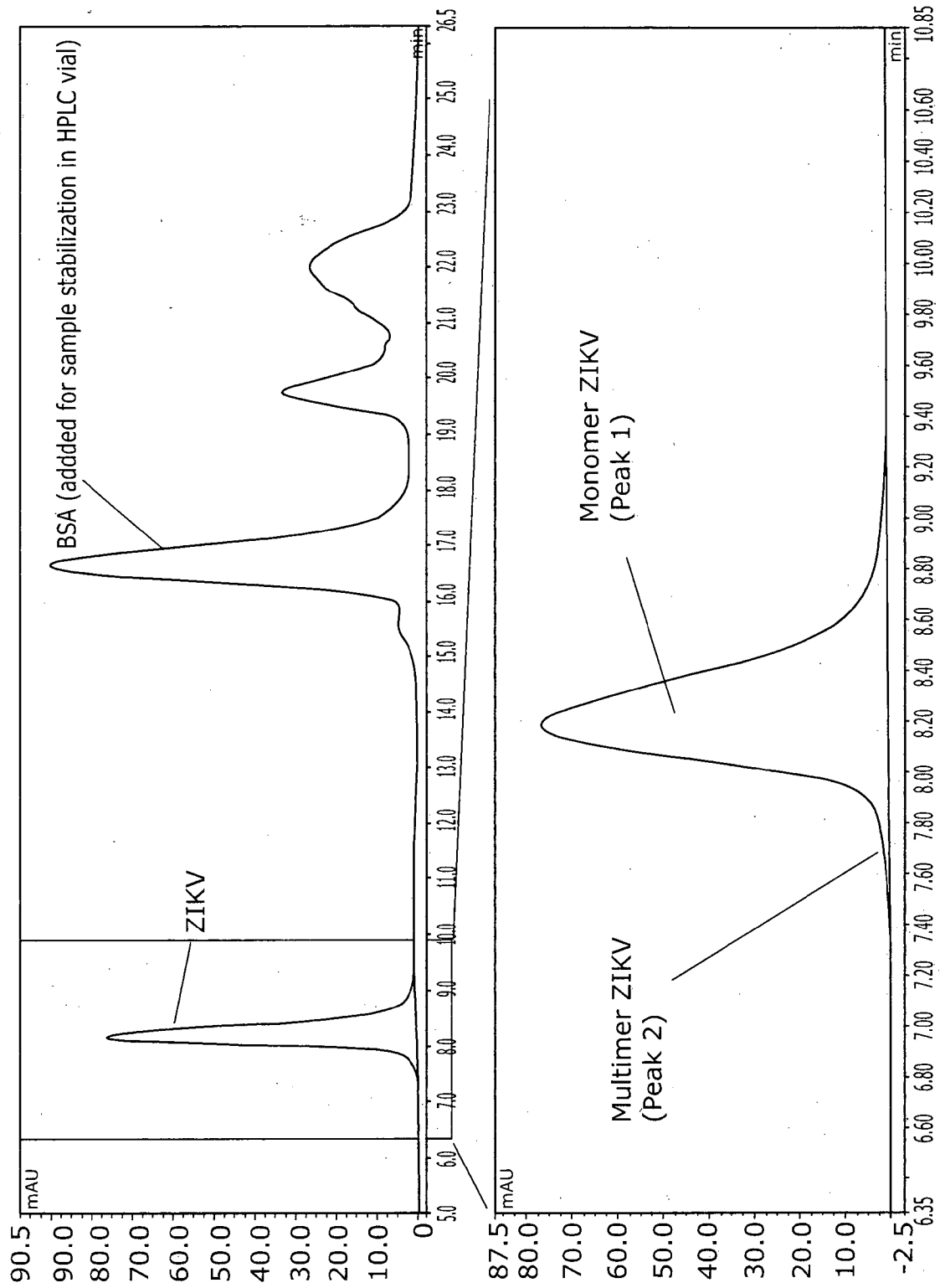


Figure 24

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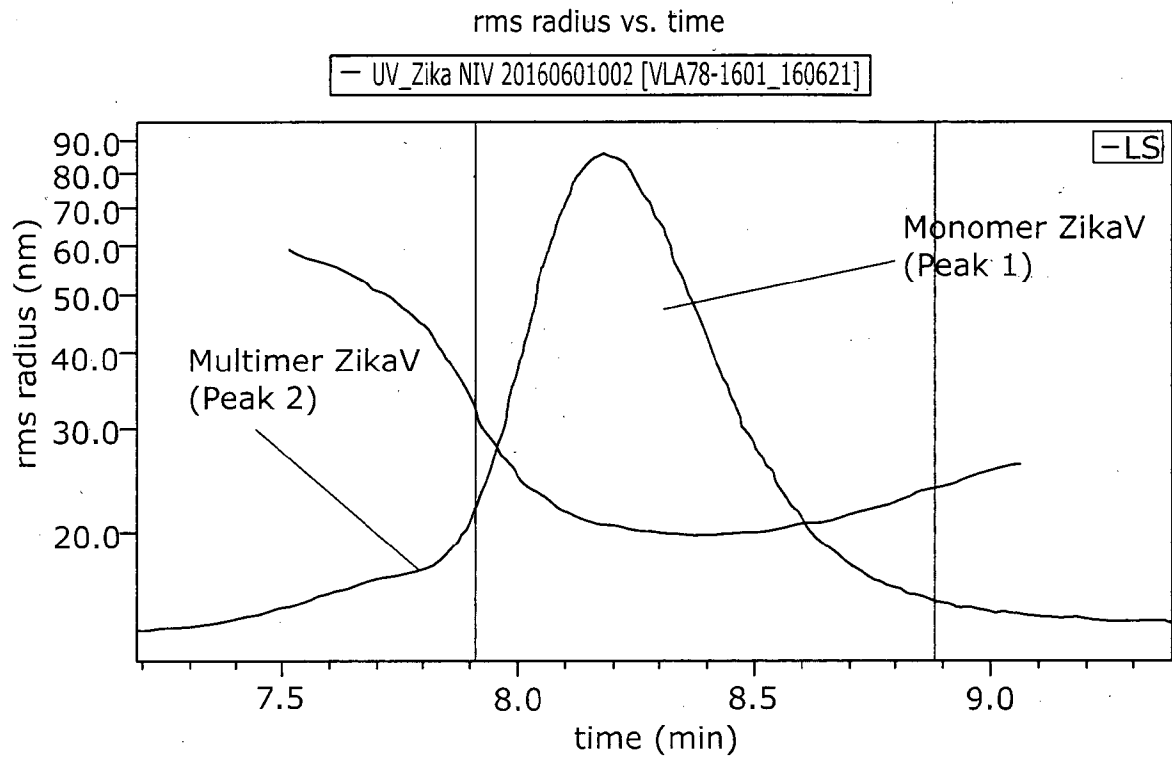


Figure 25

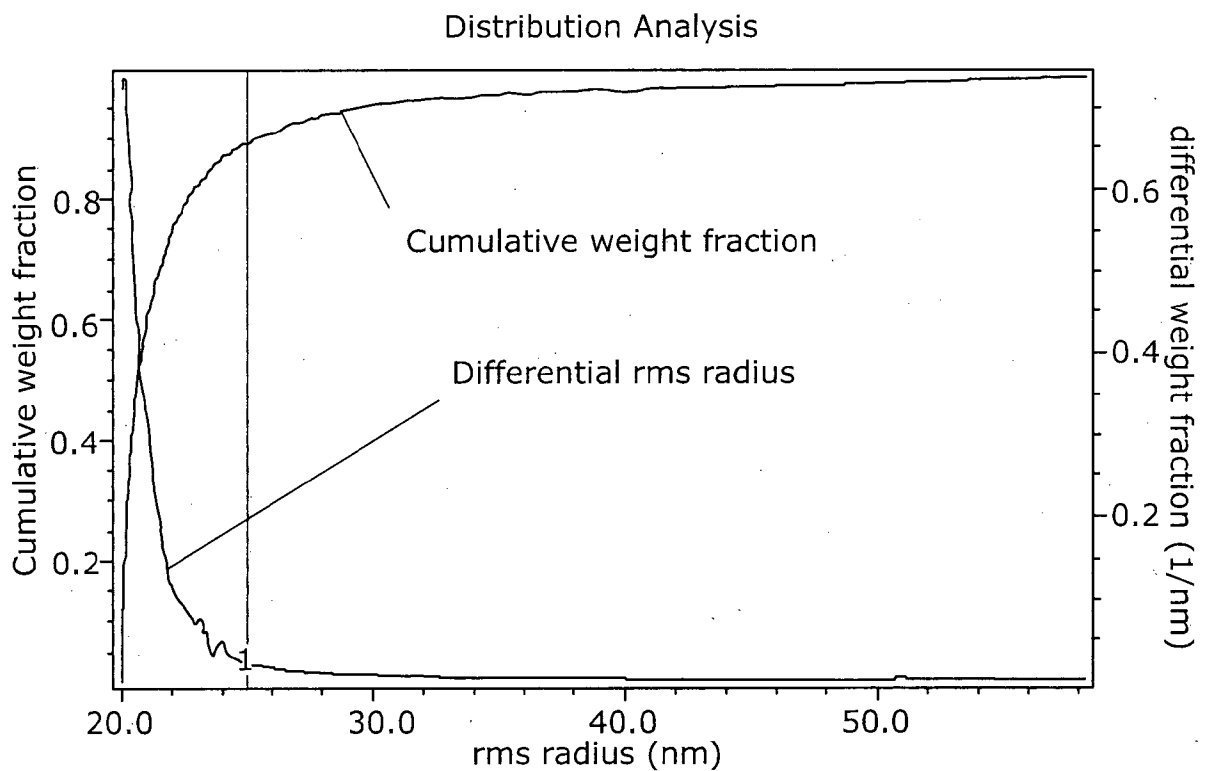


Figure 26

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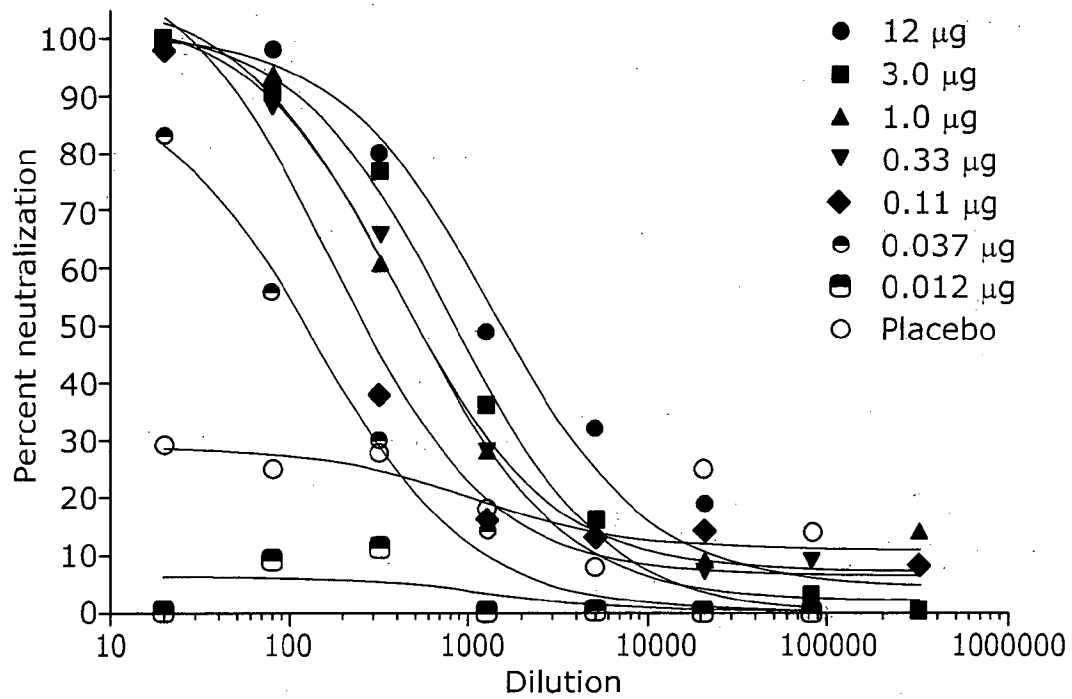


Figure 27

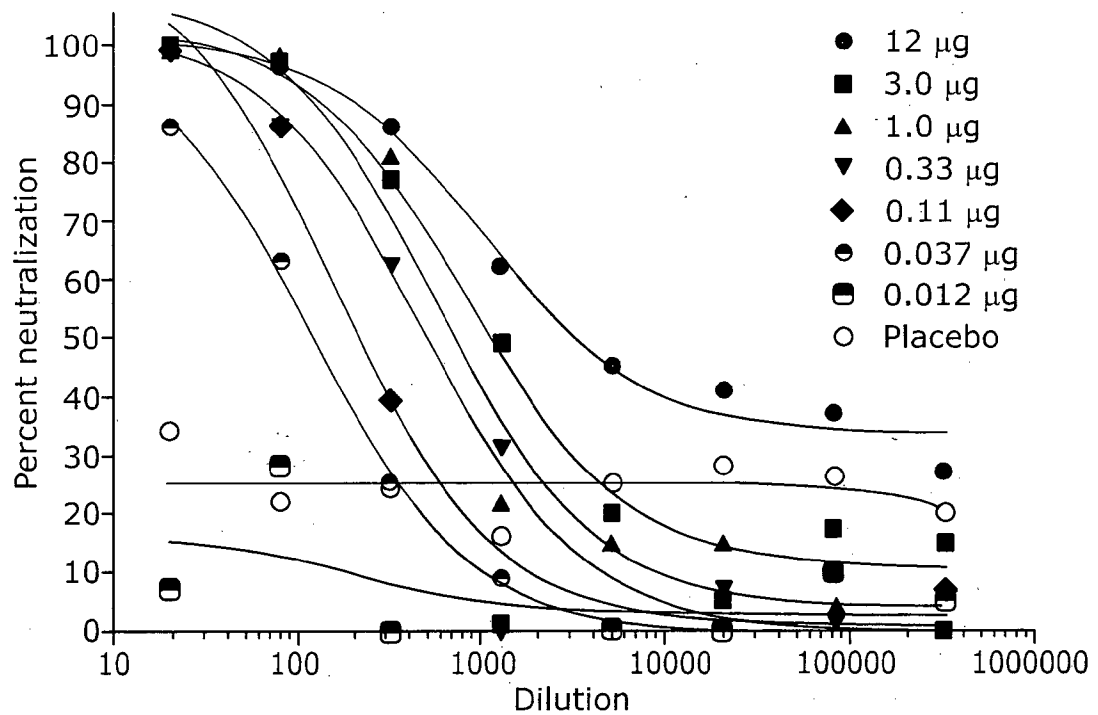


Figure 28

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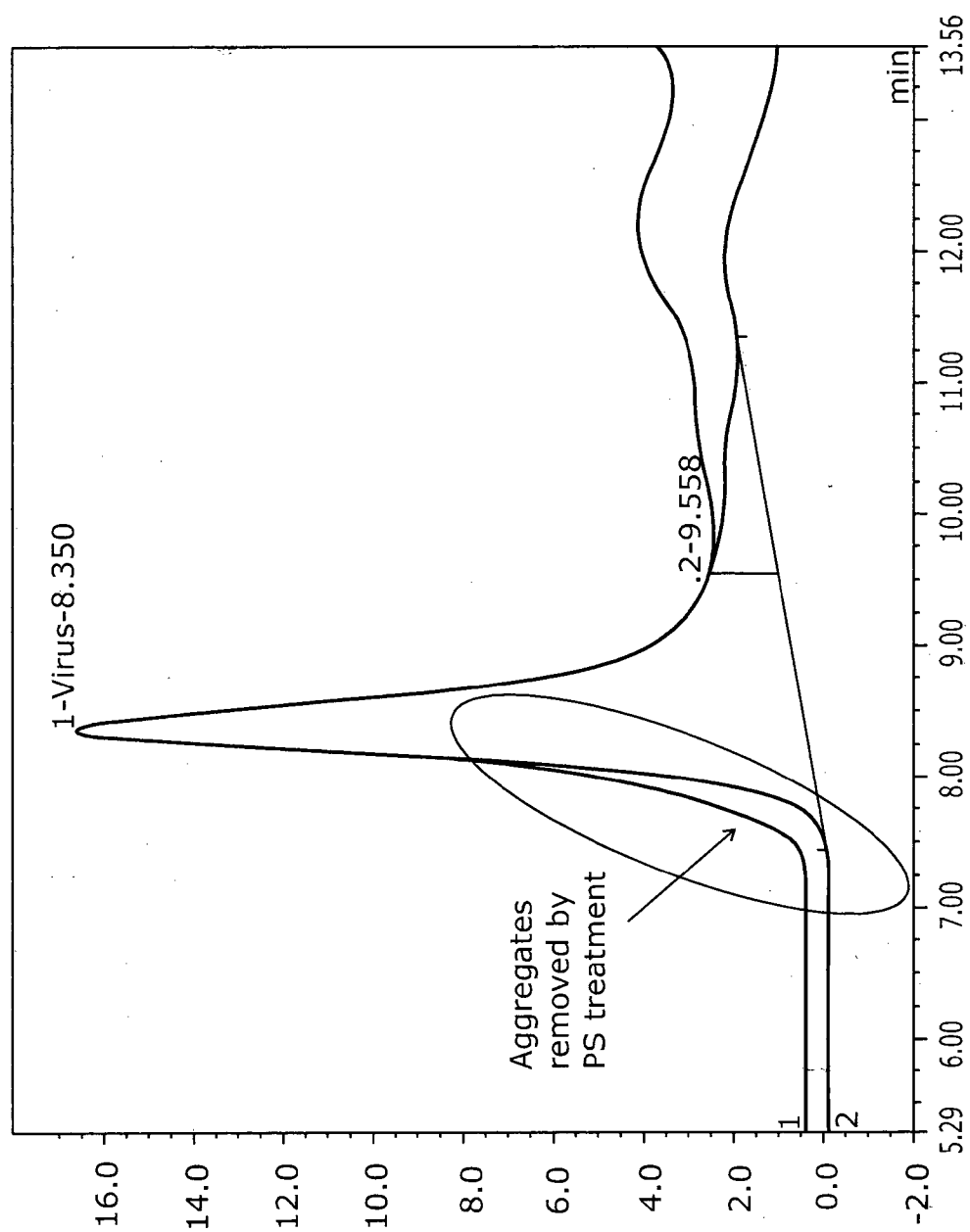


Figure 29

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/082663

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/12 C07K14/005
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SRIVASTAVA A K ET AL: "A purified inactivated Japanese encephalitis virus vaccine made in vero cells", VACCINE, vol. 19, no. 31, 14 August 2001 (2001-08-14), pages 4557-4565, XP027321987, ISSN: 0264-410X [retrieved on 2001-08-14] cited in the application	3-21
Y	abstract page 4559, left-hand column, paragraph 3 - page 4560, left-hand column, paragraph 2 page 4564, left-hand column, paragraph 2 ----- -/-	22



Further documents are listed in the continuation of Box C.



See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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Date of the actual completion of the international search

21 March 2017

Date of mailing of the international search report

19/04/2017

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Authorized officer

Lewis, Birgit

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/082663

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	B. D. COX ET AL: "Predicting Zika virus structural biology: Challenges and opportunities for intervention", ANTIVIRAL CHEMISTRY & CHEMOTHERAPY., vol. 24, no. 3-4, 1 August 2015 (2015-08-01), pages 118-126, XP055328128, GB ISSN: 0956-3202, DOI: 10.1177/2040206616653873 the whole document -----	22
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X,P	WO 2016/145149 A1 (THE US SECRETARY OF THE ARMY ON BEHALF OF THE WALTER REED ARMY INST OF) 15 September 2016 (2016-09-15) page 5, lines 8-17 paragraphs [0037] - [0040] paragraph [0048] claims 1-19 -----	3-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2016/082663

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