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(54) Title: DNA ANTIBODY CONSTRUCTS AND METHOD OF USING SAME

(57) Abstract: Disclosed is a composition including a recombinant nucleic acid sequence that encodes an antibody. Also disclosed is a method of generating a synthetic antibody in a subject by administering the composition to the subject. The disclosure also provides a method of preventing and/or treating disease in a subject using said composition and method of generation.



DNA ANTIBODY CONSTRUCTS AND METHOD OF USING SAME

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Prov. App. No. 61/737,094, filed December 13, 2012, U.S. Prov. App. No. 61/881,376, filed September 23, 2013, and U.S. Prov. App. No. 61/896,646, filed October 28, 2013, all of which are hereby incorporated by reference.

STATEMENT OF GOVERNMENT INTEREST

[0002] This invention was made with government support under contract numbers HHSN272200800063C and 5-P30-AI-045008-13 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

[0003] The present invention relates to a composition comprising a recombinant nucleic acid sequence for generating a synthetic antibody, or fragments thereof, *in vivo*, and a method of preventing and/or treating disease in a subject by administering said composition.

BACKGROUND

[0004] The immunoglobulin molecule comprises two of each type of light (L) and heavy (H) chain, which are covalently linked by disulphide bonds (shown as S-S) between cysteine residues. The variable domains of the heavy chain (VH) and the light chain (VL) contribute to the binding site of the antibody molecule. The heavy-chain constant region is made up of three constant domains (CH1, CH2 and CH3) and the (flexible) hinge region. The light chain also has a constant domain (CL). The variable regions of the heavy and light chains comprise four framework regions (FRs; FR1, FR2, FR3 and FR4) and three complementarity-determining regions (CDRs; CDR1, CDR2 and CDR3). Accordingly, these are very complex genetic systems that have been difficult to assemble *in vivo*.

[0005] Targeted monoclonal antibodies (mAbs) represent one of the most important medical therapeutic advances of the last 25 years. This type of immune based therapy is now used routinely against a host of autoimmune diseases, treatment of cancer as well as infectious diseases. For malignancies, many of the immunoglobulin (Ig) based therapies

currently used are in combination with cytotoxic chemotherapy regimens directed against tumors. This combination approach has significantly improved overall survival. Multiple mAb preparations are licensed for use against specific cancers, including Rituxan (Rituximab), a chimeric mAb targeting CD20 for the treatment of Non-Hodgkins lymphoma and Ipilimumab (Yervoy), a human mAb that blocks CTLA-4 and which has been used for the treatment of melanoma and other malignancies. Additionally, Bevacizumab (Avastin) is another prominent humanized mAb that targets VEGF and tumor neovascularization and has been used for the treatment of colorectal cancer. Perhaps the most high profile mAb for treatment of a malignancy is Trastuzumab (Herceptin), a humanized preparation targeting Her2/neu that has been demonstrated to have considerable efficacy against breast cancer in a subset of patients. Furthermore, a host of mAbs are in use for the treatment of autoimmune and specific blood disorders.

[0006] In addition to cancer treatments, passive transfer of polyclonal Igs mediate protective efficacy against a number of infectious diseases including diphtheria, hepatitis A and B, rabies, tetanus, chicken-pox and respiratory syncytial virus (RSV). In fact, several polyclonal Ig preparations provide temporary protection against specific infectious agents in individuals traveling to disease endemic areas in circumstances when there is insufficient time for protective Igs to be generated through active vaccination. Furthermore, in children with immune deficiency the Palivizumab (Synagis), a mAb, which targets RSV infection, has been demonstrated to clinically protect against RSV.

[0007] The clinical impact of mAb therapy is impressive. However, issues remain that limit the use and dissemination of this therapeutic approach. Some of these include the high cost of production of these complex biologics that can limit their use in the broader population, particularly in the developing world where they could have a great impact. Furthermore, the frequent requirement for repeat administrations of the mAbs to attain and maintain efficacy can be an impediment in terms of logistics and patient compliance. Additionally, the long-term stability of these antibody formulations is frequently short and less than optimal. Thus, there remains a need in the art for a synthetic antibody molecule that can be delivered to a subject in a safe and cost effective manner. Furthermore, synthetic antibody identification and expression methods have been discussed; however, production of the protein still is problematic and expensive.

[0008] Immunotherapy and immunomodulation provide modes of treatment that allow treatment of a disease by working with or modulating or stimulating a subject's immune system to fight off a pathogen or kill a diseased cell. Vaccines provide one class of drugs that

can stimulate both cellular and humoral immune response for prophylaxis, and in some cases therapy, of disease. For example, a vaccine for influenza can help a subject create a memory response to the flu virus and help prevent future infections. However, an existing concern is for pathogens that trigger rapid pathogenesis, where a fast neutralizing antibody response would be beneficial such as, for example, a tropical virus like chikungunya or dengue, or ebola. In such situations, if the subject does not have an established and effective memory response, then a delay in the host humoral response could prove deadly. Moreover, there would be a benefit for immediate production of a neutralizing antibody to help stave off infection from a problematic virus such as HIV before the virus fully infects and settles into the host. There requires a vaccine that could provide immediate memory response, or more preferably a neutralizing antibody response; which then could be paired with a vaccine that stimulates the host immune response for a combination therapy, when necessary.

SUMMARY

[0009] The present invention is directed to a method of generating a synthetic antibody in a subject. The method can comprise administering to the subject a composition comprising a recombinant nucleic acid sequence encoding an antibody or fragment thereof. The recombinant nucleic acid sequence can be expressed in the subject to generate the synthetic antibody.

[0010] The antibody can comprise a heavy chain polypeptide, or fragment thereof, and a light chain polypeptide, or fragment thereof. The heavy chain polypeptide, or fragment thereof, can be encoded by a first nucleic acid sequence and the light chain polypeptide, or fragment thereof, can be encoded by a second nucleic acid sequence. The recombinant nucleic acid sequence can comprise the first nucleic acid sequence and the second nucleic acid sequence. The recombinant nucleic acid sequence can further comprise a promoter for expressing the first nucleic acid sequence and the second nucleic acid sequence as a single transcript in the subject. The promoter can be a cytomegalovirus (CMV) promoter.

[0011] The recombinant nucleic acid sequence can further comprise a third nucleic acid sequence encoding a protease cleavage site. The third nucleic acid sequence can be located between the first nucleic acid sequence and second nucleic acid sequence. The protease of the subject can recognize and cleave the protease cleavage site.

[0012] The recombinant nucleic acid sequence can be expressed in the subject to generate an antibody polypeptide sequence. The antibody polypeptide sequence can comprise the

heavy chain polypeptide, or fragment thereof, the protease cleavage site, and the light chain polypeptide, or fragment thereof. The protease produced by the subject can recognize and cleave the protease cleavage site of the antibody polypeptide sequence thereby generating a cleaved heavy chain polypeptide and a cleaved light chain polypeptide. The synthetic antibody can be generated by the cleaved heavy chain polypeptide and the cleaved light chain polypeptide.

[0013] The recombinant nucleic acid sequence can comprise a first promoter for expressing the first nucleic acid sequence as a first transcript and a second promoter for expressing the second nucleic acid sequence as a second transcript. The first transcript can be translated to a first polypeptide and the second transcript can be translated into a second polypeptide. The synthetic antibody can be generated by the first and second polypeptide. The first promoter and the second promoter can be the same. The promoter can be a cytomegalovirus (CMV) promoter.

[0014] The heavy chain polypeptide can comprise a variable heavy region and a constant heavy region 1. The heavy chain polypeptide can comprise a variable heavy region, a constant heavy region 1, a hinge region, a constant heavy region 2 and a constant heavy region 3. The light chain polypeptide can comprise a variable light region and a constant light region.

[0015] The recombinant nucleic acid sequence can further comprise a Kozak sequence. The recombinant nucleic acid sequence can further comprise an immunoglobulin (Ig) signal peptide. The Ig signal peptide can comprise an IgE or IgG signal peptide.

[0016] The recombinant nucleic acid sequence can comprise a nucleic acid sequence encoding at least one amino acid sequence of SEQ ID NOs:1, 2, 5, 41, 43, 45, 46, 47, 48, 49, 51, 53, 55, 57, 59, and 61. The recombinant nucleic acid sequence can comprise at least one nucleic acid sequence of SEQ ID NOs:3, 4, 6, 7, 40, 42, 44, 50, 52, 54, 56, 58, 60, 62, and 63.

[0017] The present invention is also directed to a method of generating a synthetic antibody in a subject. The method can comprise administering to the subject a composition comprising a first recombinant nucleic acid sequence encoding a heavy chain polypeptide, or fragment thereof, and a second recombinant nucleic acid sequence encoding a light chain polypeptide, or fragment thereof. The first recombinant nucleic acid sequence can be expressed in the subject to generate a first polypeptide and the second recombinant nucleic acid can be expressed in the subject to generate a second polypeptide. The synthetic antibody can be generated by the first and second polypeptides.

[0018] The first recombinant nucleic acid sequence can further comprise a first promoter for expressing the first polypeptide in the subject. The second recombinant nucleic acid sequence can further comprise a second promoter for expressing the second polypeptide in the subject. The first promoter and second promoter can be the same. The promoter can be a cytomegalovirus (CMV) promoter.

[0019] The heavy chain polypeptide can comprise a variable heavy region and a constant heavy region 1. The heavy chain polypeptide can comprise a variable heavy region, a constant heavy region 1, a hinge region, a constant heavy region 2 and a constant heavy region 3. The light chain polypeptide can comprise a variable light region and a constant light region.

[0020] The first recombinant nucleic acid sequence and the second recombinant nucleic acid sequence can further comprise a Kozak sequence. The first recombinant nucleic acid sequence and the second recombinant nucleic acid sequence can further comprise an immunoglobulin (Ig) signal peptide. The Ig signal peptide can comprise an IgE or IgG signal peptide.

[0021] The present invention is further directed to method of preventing or treating a disease in a subject. The method can comprise generating a synthetic antibody in a subject according to one of the above methods. The synthetic antibody can be specific for a foreign antigen. The foreign antigen can be derived from a virus. The virus can be Human immunodeficiency virus (HIV), Chikungunya virus (CHIKV) or Dengue virus.

[0022] The virus can be HIV. The recombinant nucleic acid sequence can comprise a nucleic acid sequence encoding at least one amino acid sequence of SEQ ID NOs:1, 2, 5, 46, 47, 48, 49, 51, 53, 55, and 57. The recombinant nucleic acid sequence can comprise at least one nucleic acid sequence of SEQ ID NOs:3, 4, 6, 7, 50, 52, 55, 56, 62, and 63.

[0023] The virus can be CHIKV. The recombinant nucleic acid sequence can comprise a nucleic acid sequence encoding at least one amino acid sequence of SEQ ID NOs:59 and 61. The recombinant nucleic acid sequence can comprise at least one nucleic acid sequence of SEQ ID NOs:58 and 60.

[0024] The virus can be Dengue virus. The recombinant nucleic acid sequence can comprise a nucleic acid sequence encoding at least one amino acid sequence of SEQ ID NO:45. The recombinant nucleic acid sequence comprises at least one nucleic acid sequence of SEQ ID NO:44.

[0025] The synthetic antibody can be specific for a self-antigen. The self-antigen can be Her2. The recombinant nucleic acid sequence can comprise a nucleic acid sequence

encoding at least one amino acid sequence of SEQ ID NOs:41 and 43. The recombinant nucleic acid sequence can comprise at least one nucleic acid sequence of SEQ ID NOs:40 and 42.

[0026] An aspect of the invention herein described includes the nucleotide products described herein, which in some instances are comprised of one nucleotide construct, and in some instances are comprised of two distinct nucleotide constructs.

[0027] An aspect of the invention relates to methods of treating a from infection by a pathogen, comprising administering a nucleotide sequence encoding a synthetic antibody specific for the pathogen, and in some instances also administering an antigen of the pathogen to generate an immune response in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] FIG. 1 shows the nucleic acid sequence encoding an IgG heavy chain as described in Example 1.

[0029] FIG. 2 shows the nucleic acid sequence encoding an IgG light chain as described in Example 1.

[0030] FIG. 3 shows a graph plotting time (hours) vs. OD 450 nm (1:100 dilution of tissue culture supernatant).

[0031] FIG. 4 shows an image of a Western blot.

[0032] FIG. 5 shows generation and confirmation of expression of pHIV-1 Env-Fab. (A & B) Circular plasmid map of pHIV-1 Env Fab anti-gp120 Fab expressing construct were designed using VRC01 heavy (H) and light (L) variable chain Ig genes. Several modifications were included when constructing the Fab plasmids in order to increase the level of expression. The Fab VL and VH fragment genes, as shown, were cloned separately between the BamH1 and Xho1 restriction sites of the pVax1 vector. (C) In vitro expression of pHIV-1 Env Fab. The graph indicated the temporal kinetics of expression of the pHIV-1 Env Fab after transfection of 293T cells. The values indicated, indicative of expression, are mean OD450nm \pm SD of triplicate wells. As a control 293T cells were also transfected with the pVax1 backbone.

[0033] FIG. 6 shows measurement of temporal generation of anti HIV Env specific Fab by pHIV-1 Env Fab. (A) Time course of generation of anti-HIV1 Fab. After administration of pHIV-1 Env Fab, production of the specific Fab was measured over 10 days in the sera at a final dilution of 1:100 by ELISA and presented as OD450nm. Sera from pVax1 administered

mice were used as a negative control. (B) Comparative measurement of anti-gp120 antibody responses after immunization with recombinant gp120 (rgp120). As described in Example 2, mice were immunized with a single injection of rgp120 followed by measurement of production of anti-gp120 antibodies up to 10 days and presented as OD450nm values. PBS was used as a negative control injection for this study. (C) Confirmation of HIV1Env-Fab binding by immunoblot analysis. As indicated in Example, either 5 or 10 μ g of gp120 were subjected to SDS-PAGE and nitrocellulose blotting followed by incubation of the blots with sera from pHIV-1 Env Fab administered mice. The immunoblot indicated that the experimental sera recognized bound rgp120, confirming the specificity of the generated Fab. (D) Temporal quantitation of human IgG1Fab, measured as IgG1 in mouse sera following pHIV-1Env-Fab administration. IgG1 was measured by a standard ELISA kit, at the time points indicated, and expressed as Fab (μ g/mL) \pm SD. Sera from pVax1-administered mice were used as a negative control. Sera samples were analyzed at the time points indicated on the x-axis. The arrow shown in the graphs displayed in (A), (B) and (D) indicate the point of DNA plasmid administration.

[0034] FIG. 7 shows FACS binding analysis HIV1 Env Fab to clade A HIV Env glycoprotein. (A) FACS scans indicating binding of anti-HIV1Env-Fab to HIV-1 clade A Env glycoprotein. DNA expressing either a consensus (pCon-Env-A) or “optimized” (pOpt-Env-A) HIV-1 clade A envelope was transfected into 293T cells. Two days post transfection, cells were stained with either purified native VRC01 Ig, sera generated from pHIV-1 Env Fab (collected 48 hours after a single plasmid administration) or control Ig generated from pIgG-E1M2 administration. Sera and VRC01 antibody were diluted 1:4 or 1:100, respectively in 50 μ l of PBS and incubated at room temperature for 30 minutes. Cells were then stained with the appropriate secondary phycoerythrin (PE) conjugated Igs and subsequently gated for FACS analysis as singlet and live cells. The percent binding of positive cells was indicated in each of the scans. (B) Graphical representation of the FACS binding data. The number of stained cells (i.e. indicative of expression levels) in each of the Ig/sera tested groups was divided by the background staining values and presented as percent of specific binding on the y-axis as a function of the different HIV clade A Env preparations tested.

[0035] FIG. 8 shows time course of neutralization of HIV-1 by sera from pHIV-1Env-Fab administered mice. Sera used for analysis of neutralization activity sera were collected at the time points indicated in the graphs. The neutralization analysis was conducted in TZM-BL cells using a panel of HIV-1 pseudotyped viruses: Bal26 (Panel A; clade B, Tier 1), Q23Env17 (Panel B; clade A, Tier 1), SF162S (Panel C; clade B, Tier 1), and ZM53M (Panel

D; clade C, Tier 2). Cells were infected at an MOI of 0.01 as delineated in Example 2 and incubated in the presence of sera (final dilution of 1:50) containing Fab generated from pHIV-1 Env Fab administration. Percent neutralization values are shown, the calculation of which was described in Example 2. As well, horizontal lines are provided in each of the graphs, indicating the approximate time points at which the experimental sera mediated 50% viral neutralization.

[0036] FIG. 9 shows the nucleic acid sequence encoding the heavy chain (VH-CH1) of the HIV-1 Env Fab described in Examples 2-7.

[0037] FIG. 10 shows the nucleic acid sequence encoding the light chain (VL-CL) of the HIV-1 Env Fab described in Examples 2-7.

[0038] FIG. 11 shows immunofluorescence of cells transfected with a plasmid encoding HIV Env. The cells were stained with preparations from pVAX1 (left panel) or pHIV-Env-Fab (right panel).

[0039] FIG. 12 shows a graph plotting type of antigen vs. sera concentration (ng/mL).

[0040] FIG. 13 shows a schematic of a construct encoding a synthetic human IgG1 antibody.

[0041] FIG. 14 shows a schematic of the assembled antibody (upon expression) that is encoded by the construct of FIG. 13.

[0042] FIG. 15 shows the amino acid sequence of the VRC01 IgG.

[0043] FIG. 16 shows (A) a schematic of the construct encoding HIV-1 Env-PG9 Ig; (B) a schematic of the vector containing the construct of (A); and (C) an image of a stained gel.

[0044] FIG. 17 shows (A) a schematic of the construct encoding HIV-1 Env-4E10 Ig; (B) a schematic of the vector containing the construct of (A); and (C) an image of a stained gel.

[0045] FIG. 18 shows the amino acid sequence of HIV-1 Env-PG9 Ig before cleavage by furin.

[0046] FIG. 19 shows the amino acid sequence of HIV-1 Env-4E10 Ig before cleavage by furin.

[0047] FIG. 20 shows (A) a schematic of a construct encoding the heavy (VH-CH1) chain of CHIKV-Env-Fab; and (B) a schematic of a construct encoding the heavy (VL-CL) chain of CHIKV-Env-Fab.

[0048] FIG. 21 shows a schematic of an expression vector containing the construct encoding the heavy (VH-CH1) or light (VL-CL) chain of CHIKV-Env-Fab.

[0049] FIG. 22 shows a graph plotting time in hours (hr) vs. OD450 nm.

[0050] FIG. 23 shows an image of an immunoblot.

[0051] FIG. 24 shows a schematic of the timing of DNA administration and obtaining the pre-bleed and bleeds.

[0052] FIG. 25 shows a graph plotting time in days vs. OD450 nm.

[0053] FIG. 26 shows a graph plotting days after challenge vs. percent survival.

[0054] FIG. 27 shows a graph plotting mouse group vs. pg/mL of TNF- α .

[0055] FIG. 28 shows a graph plotting mouse group vs. pg/mL of IL-6.

[0056] FIG. 29 shows a schematic illustrating a construct encoding a VH-CH1 and under the control of a promoter.

[0057] FIG. 30 shows a schematic illustrating a construct encoding a VL-CL and under the control of a promoter.

[0058] FIG. 31 shows a schematic illustrating the construct encoding a VH-CH1 or VL-CL of the anti-Her-2 Fab cloned into an expression vector.

[0059] FIG. 32 shows the nucleic acid sequence encoding the VH-CH1 of the anti-Her-2 Fab.

[0060] FIG. 33 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 32 (i.e., the amino acid sequence of the VH-CH1 of the anti-Her-2 Fab).

[0061] FIG. 34 shows the nucleic acid sequence encoding the VL-CL of the anti-Her-2 Fab.

[0062] FIG. 35 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 34 (i.e., the amino acid sequence of the VL-CL of the anti-Her-2 Fab).

[0063] FIG. 36 shows a graph plotting type of transfected cell vs. IgG concentration ($\mu\text{g/mL}$).

[0064] FIG. 37 shows a schematic illustrating a construct encoding the variable heavy region (VH), variable heavy constant region 1 (CH1), hinge region, variable heavy constant region 2 (CH2), variable heavy constant 3 (CH3) of an immunoglobulin G (IgG) heavy chain and encoding the variable light region (VL) and variable light constant region (CL) of an IgG light chain. The heavy and light chains of the IgG are separated by a protease cleavage site and each is preceded by a signal peptide (encoded by leader sequence).

[0065] FIG. 38 shows a nucleic acid sequence encoding the anti-Dengue virus (DENV) human IgG.

[0066] FIG. 39 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 39 (i.e., the amino acid sequence of the anti-DENV human IgG). In this amino acid sequence, protease cleavage has not yet occurred to separate the heavy and light chains into two separate polypeptides.

[0067] FIG. 40 shows a graph plotting mouse group vs. OD 450 nm.

[0068] FIG. 41 shows a graph plotting days post-injection vs. human IgG concentration (ng/mL).

[0069] FIG. 42 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 1 (i.e., SEQ ID NO:6). This amino acid sequence is the amino acid sequence of the IgG heavy chain described in Example 1 below.

[0070] FIG. 43 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 2 (i.e., SEQ ID NO:7). This amino acid sequence is the amino acid sequence of the IgG light chain described in Example 1 below.

[0071] FIG. 44 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 9 (i.e., SEQ ID NO:3). This amino acid sequence is the amino acid sequence of the heavy chain (VH-CH1) of HIV-1 Env-Fab described in Examples 2-7.

[0072] FIG. 45 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 10 (i.e., SEQ ID NO:4). This amino acid sequence is the amino acid sequence of the light chain (VL-CL) of HIV-1 Env-Fab described in Examples 2-7.

[0073] FIG. 46 shows the nucleic acid sequence encoding the HIV-1 PG9 single chain Fab (scFab) described in Example 11 below.

[0074] FIG. 47 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 46 (i.e., SEQ ID NO:50). This amino acid sequence is the amino acid sequence of the HIV-1 PG9 scFab described in Example 11 below.

[0075] FIG. 48 shows the nucleic acid sequence encoding the HIV-1 4E10 single chain Fab (scFab) described in Example 13 below.

[0076] FIG. 49 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 48 (i.e., SEQ ID NO:52). This amino acid sequence is the amino acid sequence of the HIV-1 4E10 scFab described in Example 13 below.

[0077] FIG. 50 shows a schematic illustrating a construct encoding the variable heavy region (VH), variable heavy constant region 1 (CH1), hinge region, variable heavy constant region 2 (CH2), variable heavy constant 3 (CH3) of an immunoglobulin G (IgG) heavy chain. The nucleic acid sequence encoding the IgG heavy chain is preceded by a leader sequence.

[0078] FIG. 51 shows a schematic illustrating a construct encoding the variable light region (VL) and variable light constant region (CL) of an IgG light chain. The nucleic acid sequence encoding the IgG light chain is preceded by a leader sequence.

[0079] FIG. 52 shows the nucleic acid sequence encoding the HIV-1 VRC01 IgG1 heavy chain described in Example 9 below.

[0080] FIG. 53 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 52 (i.e., SEQ ID NO:54). This amino acid sequence is the amino acid sequence of the HIV-1 VRC01 IgG1 heavy chain described in Example 9 below.

[0081] FIG. 54 shows the nucleic acid sequence encoding the HIV-1 VRC01 IgG light chain described in Example 9 below.

[0082] FIG. 55 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 54 (i.e., SEQ ID NO:56). This amino acid sequence is the amino acid sequence of the HIV-1 VRC01 IgG light chain described below in Example 9.

[0083] FIG. 56 shows the nucleic acid sequence encoding the heavy chain (VH-CH1) of the CHIKV-Env-Fab described below in Example 14.

[0084] FIG. 57 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 56 (i.e., SEQ ID NO:58). This amino acid sequence is the amino acid sequence of the heavy chain (VH-CH1) of the CHIKV-Env-Fab described in Example 14 below.

[0085] FIG. 58 shows the nucleic acid sequence encoding the light chain (VL-CL) of the CHIKV-Env-Fab described below in Example 14.

[0086] FIG. 59 shows the amino acid sequence encoded by the nucleic acid sequence of FIG. 58 (i.e., SEQ ID NO:60). This amino acid sequence is the amino acid sequence of the light chain (VL-CL) of the CHIKV-Env-Fab described in Example 14 below.

[0087] FIG. 60 shows the nucleic acid sequence encoding HIV-1 Env-4E10 Ig described in Example 12 below

[0088] FIG. 61 shows the nucleic acid sequence encoding HIV-1 Env-PG9 Ig described in Example 10 below.

[0089] FIG. 62 shows the nucleic acid sequence encoding VRC01 IgG (SEQ ID NO:64).

DETAILED DESCRIPTION

[0090] The present invention relates to a composition comprising a recombinant nucleic acid sequence encoding an antibody, a fragment thereof, a variant thereof, or a combination thereof. The composition can be administered to a subject in need thereof to facilitate in vivo expression and formation of a synthetic antibody.

[0091] In particular, the heavy chain and light chain polypeptides expressed from the recombinant nucleic acid sequences can assemble into the synthetic antibody. The heavy chain polypeptide and the light chain polypeptide can interact with one another such that assembly results in the synthetic antibody being capable of binding the antigen, being more

immunogenic as compared to an antibody not assembled as described herein, and being capable of eliciting or inducing an immune response against the antigen.

[0092] Additionally, these synthetic antibodies are generated more rapidly in the subject than antibodies that are produced in response to antigen induced immune response. The synthetic antibodies are able to effectively bind and neutralize a range of antigens. The synthetic antibodies are also able to effectively protect against and/or promote survival of disease.

1. Definitions

[0093] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[0094] The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms “a,” “and” and “the” include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments “comprising,” “consisting of” and “consisting essentially of,” the embodiments or elements presented herein, whether explicitly set forth or not.

[0095] “Antibody” may mean an antibody of classes IgG, IgM, IgA, IgD or IgE, or fragments, fragments or derivatives thereof, including Fab, F(ab')₂, Fd, and single chain antibodies, and derivatives thereof. The antibody may be an antibody isolated from the serum sample of mammal, a polyclonal antibody, affinity purified antibody, or mixtures thereof which exhibits sufficient binding specificity to a desired epitope or a sequence derived therefrom.

[0096] “Antibody fragment” or “fragment of an antibody” as used interchangeably herein refers to a portion of an intact antibody comprising the antigen-binding site or variable region. The portion does not include the constant heavy chain domains (i.e. CH₂, CH₃, or CH₄, depending on the antibody isotype) of the Fc region of the intact antibody. Examples

of antibody fragments include, but are not limited to, Fab fragments, Fab' fragments, Fab'-SH fragments, F(ab')₂ fragments, Fd fragments, Fv fragments, diabodies, single-chain Fv (scFv) molecules, single-chain polypeptides containing only one light chain variable domain, single-chain polypeptides containing the three CDRs of the light-chain variable domain, single-chain polypeptides containing only one heavy chain variable region, and single-chain polypeptides containing the three CDRs of the heavy chain variable region.

[0097] “Antigen” refers to proteins that have the ability to generate an immune response in a host. An antigen may be recognized and bound by an antibody. An antigen may originate from within the body or from the external environment.

[0098] “Coding sequence” or “encoding nucleic acid” as used herein may mean refers to the nucleic acid (RNA or DNA molecule) that comprise a nucleotide sequence which encodes an antibody as set forth herein. The coding sequence may further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to whom the nucleic acid is administered. The coding sequence may further include sequences that encode signal peptides.

[0099] “Complement” or “complementary” as used herein may mean a nucleic acid may mean Watson-Crick (e.g., A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

[00100] “Constant current” as used herein to define a current that is received or experienced by a tissue, or cells defining said tissue, over the duration of an electrical pulse delivered to same tissue. The electrical pulse is delivered from the electroporation devices described herein. This current remains at a constant amperage in said tissue over the life of an electrical pulse because the electroporation device provided herein has a feedback element, preferably having instantaneous feedback. The feedback element can measure the resistance of the tissue (or cells) throughout the duration of the pulse and cause the electroporation device to alter its electrical energy output (e.g., increase voltage) so current in same tissue remains constant throughout the electrical pulse (on the order of microseconds), and from pulse to pulse. In some embodiments, the feedback element comprises a controller.

[00101] “Current feedback” or “feedback” as used herein may be used interchangeably and may mean the active response of the provided electroporation devices, which comprises measuring the current in tissue between electrodes and altering the energy output delivered by the EP device accordingly in order to maintain the current at a constant level. This constant level is preset by a user prior to initiation of a pulse sequence or electrical treatment.

The feedback may be accomplished by the electroporation component, e.g., controller, of the electroporation device, as the electrical circuit therein is able to continuously monitor the current in tissue between electrodes and compare that monitored current (or current within tissue) to a preset current and continuously make energy-output adjustments to maintain the monitored current at preset levels. The feedback loop may be instantaneous as it is an analog closed-loop feedback.

[00102] “Decentralized current” as used herein may mean the pattern of electrical currents delivered from the various needle electrode arrays of the electroporation devices described herein, wherein the patterns minimize, or preferably eliminate, the occurrence of electroporation related heat stress on any area of tissue being electroporated.

[00103] “Electroporation,” “electro-permeabilization,” or “electro-kinetic enhancement” (“EP”) as used interchangeably herein may refer to the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane; their presence allows biomolecules such as plasmids, oligonucleotides, siRNA, drugs, ions, and water to pass from one side of the cellular membrane to the other.

[00104] “Endogenous antibody” as used herein may refer to an antibody that is generated in a subject that is administered an effective dose of an antigen for induction of a humoral immune response.

[00105] “Feedback mechanism” as used herein may refer to a process performed by either software or hardware (or firmware), which process receives and compares the impedance of the desired tissue (before, during, and/or after the delivery of pulse of energy) with a present value, preferably current, and adjusts the pulse of energy delivered to achieve the preset value. A feedback mechanism may be performed by an analog closed loop circuit.

[00106] “Fragment” may mean a polypeptide fragment of an antibody that is function, i.e., can bind to desired target and have the same intended effect as a full length antibody. A fragment of an antibody may be 100% identical to the full length except missing at least one amino acid from the N and/or C terminal, in each case with or without signal peptides and/or a methionine at position 1. Fragments may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length antibody, excluding any heterologous signal peptide added. The fragment may comprise a fragment of a polypeptide that is 95% or more, 96% or more, 97% or more, 98% or more or 99% or more

identical to the antibody and additionally comprise an N terminal methionine or heterologous signal peptide which is not included when calculating percent identity. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a fragment of an antibody.

[00107] A fragment of a nucleic acid sequence that encodes an antibody may be 100% identical to the full length except missing at least one nucleotide from the 5' and/or 3' end, in each case with or without sequences encoding signal peptides and/or a methionine at position 1. Fragments may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length coding sequence, excluding any heterologous signal peptide added. The fragment may comprise a fragment that encode a polypeptide that is 95% or more, 96% or more, 97% or more, 98% or more or 99% or more identical to the antibody and additionally optionally comprise sequence encoding an N terminal methionine or heterologous signal peptide which is not included when calculating percent identity. Fragments may further comprise coding sequences for an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The coding sequence encoding the N terminal methionine and/or signal peptide may be linked to a fragment of coding sequence.

[00108] "Genetic construct" as used herein refers to the DNA or RNA molecules that comprise a nucleotide sequence which encodes a protein, such as an antibody. The coding sequence includes initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of the individual to whom the nucleic acid molecule is administered. As used herein, the term "expressible form" refers to gene constructs that contain the necessary regulatory elements operable linked to a coding sequence that encodes a protein such that when present in the cell of the individual, the coding sequence will be expressed.

[00109] "Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences, may mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage may be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences

to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) may be considered equivalent. Identity may be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

[00110] “Impedance” as used herein may be used when discussing the feedback mechanism and can be converted to a current value according to Ohm's law, thus enabling comparisons with the preset current.

[00111] “Immune response” as used herein may mean the activation of a host's immune system, e.g., that of a mammal, in response to the introduction of one or more nucleic acids and/or peptides. The immune response can be in the form of a cellular or humoral response, or both.

[00112] “Nucleic acid” or “oligonucleotide” or “polynucleotide” as used herein may mean at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid may be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that may hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

[00113] Nucleic acids may be single stranded or double stranded, or may contain portions of both double stranded and single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods.

[00114] “Operably linked” as used herein may mean that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter may be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene may be approximately the same as the distance between that promoter

and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance may be accommodated without loss of promoter function.

[00115] A “peptide,” “protein,” or “polypeptide” as used herein can mean a linked sequence of amino acids and can be natural, synthetic, or a modification or combination of natural and synthetic.

[00116] “Promoter” as used herein may mean a synthetic or naturally-derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter may comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter may also comprise distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A promoter may be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter may regulate the expression of a gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents. Representative examples of promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, SV40 early promoter or SV 40 late promoter and the CMV IE promoter.

[00117] “Signal peptide” and “leader sequence” are used interchangeably herein and refer to an amino acid sequence that can be linked at the amino terminus of a protein set forth herein. Signal peptides/leader sequences typically direct localization of a protein. Signal peptides/leader sequences used herein preferably facilitate secretion of the protein from the cell in which it is produced. Signal peptides/leader sequences are often cleaved from the remainder of the protein, often referred to as the mature protein, upon secretion from the cell. Signal peptides/leader sequences are linked at the N terminus of the protein.

[00118] “Stringent hybridization conditions” as used herein may mean conditions under which a first nucleic acid sequence (e.g., probe) will hybridize to a second nucleic acid sequence (e.g., target), such as in a complex mixture of nucleic acids. Stringent conditions are sequence dependent and will be different in different circumstances. Stringent conditions may be selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m may be the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the

target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m , 50% of the probes are occupied at equilibrium). Stringent conditions may be those in which the salt concentration is less than about 1.0 M sodium ion, such as about 0.01-1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., about 10-50 nucleotides) and at least about 60°C for long probes (e.g., greater than about 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal may be at least 2 to 10 times background hybridization. Exemplary stringent hybridization conditions include the following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C.

[00119] “Subject” and “patient” as used herein interchangeably refers to any vertebrate, including, but not limited to, a mammal (e.g., cow, pig, camel, llama, horse, goat, rabbit, sheep, hamsters, guinea pig, cat, dog, rat, and mouse, a non-human primate (for example, a monkey, such as a cynomolgous or rhesus monkey, chimpanzee, etc) and a human). In some embodiments, the subject may be a human or a non-human. The subject or patient may be undergoing other forms of treatment.

[00120] “Substantially complementary” as used herein may mean that a first sequence is at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the complement of a second sequence over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or that the two sequences hybridize under stringent hybridization conditions.

[00121] “Substantially identical” as used herein may mean that a first and second sequence are at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% over a region of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100 or more nucleotides or amino acids, or with respect to nucleic acids, if the first sequence is substantially complementary to the complement of the second sequence.

[00122] “Synthetic antibody” as used herein refers to an antibody that is encoded by the recombinant nucleic acid sequence described herein and is generated in a subject.

[00123] “Treatment” or “treating,” as used herein can mean protecting of a subject from a disease through means of preventing, suppressing, repressing, or completely eliminating the

disease. Preventing the disease involves administering a vaccine of the present invention to a subject prior to onset of the disease. Suppressing the disease involves administering a vaccine of the present invention to a subject after induction of the disease but before its clinical appearance. Repressing the disease involves administering a vaccine of the present invention to a subject after clinical appearance of the disease.

[00124] “Variant” used herein with respect to a nucleic acid may mean (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto.

[00125] “Variant” with respect to a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Variant may also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte et al., J. Mol. Biol. 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ± 2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions may be performed with amino acids having hydrophilicity values within ± 2 of each other. Both the hydropathic index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are

understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

[00126] A variant may be a nucleic acid sequence that is substantially identical over the full length of the full gene sequence or a fragment thereof. The nucleic acid sequence may be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the gene sequence or a fragment thereof. A variant may be an amino acid sequence that is substantially identical over the full length of the amino acid sequence or fragment thereof. The amino acid sequence may be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the amino acid sequence or a fragment thereof.

[00127] “Vector” as used herein may mean a nucleic acid sequence containing an origin of replication. A vector may be a plasmid, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector may be a DNA or RNA vector. A vector may be either a self-replicating extrachromosomal vector or a vector which integrates into a host genome.

[00128] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

2. Composition

[00129] The present invention relates to a composition comprising a recombinant nucleic acid sequence encoding an antibody, a fragment thereof, a variant thereof, or a combination thereof. The composition, when administered to a subject in need thereof, can result in the generation of a synthetic antibody in the subject. The synthetic antibody can bind a target molecule (i.e., an antigen) present in the subject. Such binding can neutralize the antigen, block recognition of the antigen by another molecule, for example, a protein or nucleic acid, and elicit or induce an immune response to the antigen.

[00130] The synthetic antibody can treat, prevent, and/or protect against disease in the subject administered the composition. The synthetic antibody by binding the antigen can treat, prevent, and/or protect against disease in the subject administered the composition. The synthetic antibody can promote survival of the disease in the subject administered the composition. The synthetic antibody can provide at least about 50%, 55%, 60%, 65%, 70%,

75%, 80%, 85%, 90%, 95%, or 100% survival of the disease in the subject administered the composition. In other embodiments, the synthetic antibody can provide at least about 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80% survival of the disease in the subject administered the composition.

[00131] The composition can result in the generation of the synthetic antibody in the subject within at least about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 20 hours, 25 hours, 30 hours, 35 hours, 40 hours, 45 hours, 50 hours, or 60 hours of administration of the composition to the subject. The composition can result in generation of the synthetic antibody in the subject within at least about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, or 10 days of administration of the composition to the subject. The composition can result in generation of the synthetic antibody in the subject within about 1 hour to about 6 days, about 1 hour to about 5 days, about 1 hour to about 4 days, about 1 hour to about 3 days, about 1 hour to about 2 days, about 1 hour to about 1 day, about 1 hour to about 72 hours, about 1 hour to about 60 hours, about 1 hour to about 48 hours, about 1 hour to about 36 hours, about 1 hour to about 24 hours, about 1 hour to about 12 hours, or about 1 hour to about 6 hours of administration of the composition to the subject.

[00132] The composition, when administered to the subject in need thereof, can result in the generation of the synthetic antibody in the subject more quickly than the generation of an endogenous antibody in a subject who is administered an antigen to induce a humoral immune response. The composition can result in the generation of the synthetic antibody at least about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, or 10 days before the generation of the endogenous antibody in the subject who was administered an antigen to induce a humoral immune response.

[00133] The composition of the present invention can have features required of effective compositions such as being safe so that the composition does not cause illness or death; being protective against illness; and providing ease of administration, few side effects, biological stability and low cost per dose.

3. Recombinant Nucleic Acid Sequence

[00134] As described above, the composition can comprise a recombinant nucleic acid sequence. The recombinant nucleic acid sequence can encode the antibody, a fragment thereof, a variant thereof, or a combination thereof. The antibody is described in more detail below.

[00135] The recombinant nucleic acid sequence can be a heterologous nucleic acid sequence. The recombinant nucleic acid sequence can include at least one heterologous nucleic acid sequence or one or more heterologous nucleic acid sequences.

[00136] The recombinant nucleic acid sequence can be an optimized nucleic acid sequence. Such optimization can increase or alter the immunogenicity of the antibody. Optimization can also improve transcription and/or translation. Optimization can include one or more of the following: low GC content leader sequence to increase transcription; mRNA stability and codon optimization; addition of a kozak sequence (e.g., GCC ACC) for increased translation; addition of an immunoglobulin (Ig) leader sequence encoding a signal peptide; and eliminating to the extent possible cis-acting sequence motifs (i.e., internal TATA boxes).

a. Recombinant Nucleic Acid Sequence Construct

[00137] The recombinant nucleic acid sequence can include one or more recombinant nucleic acid sequence constructs. The recombinant nucleic acid sequence construct can include one or more components, which are described in more detail below.

[00138] The recombinant nucleic acid sequence construct can include a heterologous nucleic acid sequence that encodes a heavy chain polypeptide, a fragment thereof, a variant thereof, or a combination thereof. The recombinant nucleic acid sequence construct can include a heterologous nucleic acid sequence that encodes a light chain polypeptide, a fragment thereof, a variant thereof, or a combination thereof. The recombinant nucleic acid sequence construct can also include a heterologous nucleic acid sequence that encodes a protease or peptidase cleavage site. The recombinant nucleic acid sequence construct can include one or more leader sequences, in which each leader sequence encodes a signal peptide. The recombinant nucleic acid sequence construct can include one or more promoters, one or more introns, one or more transcription termination regions, one or more initiation codons, one or more termination or stop codons, and/or one or more polyadenylation signals. The recombinant nucleic acid sequence construct can also include one or more linker or tag sequences. The tag sequence can encode a hemagglutinin (HA) tag.

(1) Heavy Chain Polypeptide

[00139] The recombinant nucleic acid sequence construct can include the heterologous nucleic acid encoding the heavy chain polypeptide, a fragment thereof, a variant thereof, or a combination thereof. The heavy chain polypeptide can include a variable heavy chain (VH) region and/or at least one constant heavy chain (CH) region. The at least one constant heavy

chain region can include a constant heavy chain region 1 (CH1), a constant heavy chain region 2 (CH2), and a constant heavy chain region 3 (CH3), and/or a hinge region.

[00140] In some embodiments, the heavy chain polypeptide can include a VH region and a CH1 region. In other embodiments, the heavy chain polypeptide can include a VH region, a CH1 region, a hinge region, a CH2 region, and a CH3 region.

[00141] The heavy chain polypeptide can include a complementarity determining region ("CDR") set. The CDR set can contain three hypervariable regions of the VH region. Proceeding from N-terminus of the heavy chain polypeptide, these CDRs are denoted "CDR1," "CDR2," and "CDR3," respectively. CDR1, CDR2, and CDR3 of the heavy chain polypeptide can contribute to binding or recognition of the antigen.

(2) Light Chain Polypeptide

[00142] The recombinant nucleic acid sequence construct can include the heterologous nucleic acid sequence encoding the light chain polypeptide, a fragment thereof, a variant thereof, or a combination thereof. The light chain polypeptide can include a variable light chain (VL) region and/or a constant light chain (CL) region.

[00143] The light chain polypeptide can include a complementarity determining region ("CDR") set. The CDR set can contain three hypervariable regions of the VL region. Proceeding from N-terminus of the light chain polypeptide, these CDRs are denoted "CDR1," "CDR2," and "CDR3," respectively. CDR1, CDR2, and CDR3 of the light chain polypeptide can contribute to binding or recognition of the antigen.

(3) Protease Cleavage Site

[00144] The recombinant nucleic acid sequence construct can include the heterologous nucleic acid sequence encoding the protease cleavage site. The protease cleavage site can be recognized by a protease or peptidase. The protease can be an endopeptidase or endoprotease, for example, but not limited to, furin, elastase, HtrA, calpain, trypsin, chymotrypsin, trypsin, and pepsin. The protease can be furin. In other embodiments, the protease can be a serine protease, a threonine protease, cysteine protease, aspartate protease, metalloprotease, glutamic acid protease, or any protease that cleaves an internal peptide bond (i.e., does not cleave the N-terminal or C-terminal peptide bond).

[00145] The protease cleavage site can include one or more amino acid sequences that promote or increase the efficiency of cleavage. The one or more amino acid sequences can

promote or increase the efficiency of forming or generating discrete polypeptides. The one or more amino acids sequences can include a 2A peptide sequence.

(4) Linker Sequence

[00146] The recombinant nucleic acid sequence construct can include one or more linker sequences. The linker sequence can spatially separate or link the one or more components described herein. In other embodiments, the linker sequence can encode an amino acid sequence that spatially separates or links two or more polypeptides.

(5) Promoter

[00147] The recombinant nucleic acid sequence construct can include one or more promoters. The one or more promoters may be any promoter that is capable of driving gene expression and regulating gene expression. Such a promoter is a cis-acting sequence element required for transcription via a DNA dependent RNA polymerase. Selection of the promoter used to direct gene expression depends on the particular application. The promoter may be positioned about the same distance from the transcription start in the recombinant nucleic acid sequence construct as it is from the transcription start site in its natural setting.

However, variation in this distance may be accommodated without loss of promoter function.

[00148] The promoter may be operably linked to the heterologous nucleic acid sequence encoding the heavy chain polypeptide and/or light chain polypeptide. The promoter may be a promoter shown effective for expression in eukaryotic cells. The promoter operably linked to the coding sequence may be a CMV promoter, a promoter from simian virus 40 (SV40), such as SV40 early promoter and SV40 later promoter, a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter, Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. The promoter may also be a promoter from a human gene such as human actin, human myosin, human hemoglobin, human muscle creatine, human polyhedrin, or human metallothionein.

[00149] The promoter can be a constitutive promoter or an inducible promoter, which initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism, the promoter can also be specific to a particular tissue or organ or stage of development. The promoter may also be a tissue specific promoter, such

as a muscle or skin specific promoter, natural or synthetic. Examples of such promoters are described in US patent application publication no. US20040175727, the contents of which are incorporated herein in its entirety.

[00150] The promoter can be associated with an enhancer. The enhancer can be located upstream of the coding sequence. The enhancer may be human actin, human myosin, human hemoglobin, human muscle creatine or a viral enhancer such as one from CMV, FMDV, RSV or EBV. Polynucleotide function enhances are described in U.S. Patent Nos. 5,593,972, 5,962,428, and W094/016737, the contents of each are fully incorporated by reference.

(6) Intron

[00151] The recombinant nucleic acid sequence construct can include one or more introns. Each intron can include functional splice donor and acceptor sites. The intron can include an enhancer of splicing. The intron can include one or more signals required for efficient splicing.

(7) Transcription Termination Region

[00152] The recombinant nucleic acid sequence construct can include one or more transcription termination regions. The transcription termination region can be downstream of the coding sequence to provide for efficient termination. The transcription termination region can be obtained from the same gene as the promoter described above or can be obtained from one or more different genes.

(8) Initiation Codon

[00153] The recombinant nucleic acid sequence construct can include one or more initiation codons. The initiation codon can be located upstream of the coding sequence. The initiation codon can be in frame with the coding sequence. The initiation codon can be associated with one or more signals required for efficient translation initiation, for example, but not limited to, a ribosome binding site.

(9) Termination Codon

[00154] The recombinant nucleic acid sequence construct can include one or more termination or stop codons. The termination codon can be downstream of the coding sequence. The termination codon can be in frame with the coding sequence. The termination

codon can be associated with one or more signals required for efficient translation termination.

(10) Polyadenylation Signal

[00155] The recombinant nucleic acid sequence construct can include one or more polyadenylation signals. The polyadenylation signal can include one or more signals required for efficient polyadenylation of the transcript. The polyadenylation signal can be positioned downstream of the coding sequence. The polyadenylation signal may be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β -globin polyadenylation signal. The SV40 polyadenylation signal may be a polyadenylation signal from a pCEP4 plasmid (Invitrogen, San Diego, CA).

(11) Leader Sequence

The recombinant nucleic acid sequence construct can include one or more leader sequences. The leader sequence can encode a signal peptide. The signal peptide can be an immunoglobulin (Ig) signal peptide, for example, but not limited to, an IgG signal peptide and a IgE signal peptide. In some example, the leader sequence is an IgE leader IgE leader sequence SEQ ID NO:65: atggactgga ctggattct gttcctggtc gccgccgcaa ctcgcgtgca tagc, which encodes protein SEQ ID NO:66: Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val His Ser.

b. Arrangement of the Recombinant Nucleic Acid Sequence Construct

[00156] As described above, the recombinant nucleic acid sequence can include one or more recombinant nucleic acid sequence constructs, in which each recombinant nucleic acid sequence construct can include one or more components. The one or more components are described in detail above. The one or more components, when included in the recombinant nucleic acid sequence construct, can be arranged in any order relative to one another. In some embodiments, the one or more components can be arranged in the recombinant nucleic acid sequence construct as described below.

(1) Arrangement 1

[00157] In one arrangement, a first recombinant nucleic acid sequence construct can include the heterologous nucleic acid sequence encoding the heavy chain polypeptide and a

second recombinant nucleic acid sequence construct can include the heterologous nucleic acid sequence encoding the light chain polypeptide.

[00158] The first recombinant nucleic acid sequence construct can be placed in a vector. The second recombinant nucleic acid sequence construct can be placed in a second or separate vector. Placement of the recombinant nucleic acid sequence construct into the vector is described in more detail below.

[00159] The first recombinant nucleic acid sequence construct can also include the promoter, intron, transcription termination region, initiation codon, termination codon, and/or polyadenylation signal. The first recombinant nucleic acid sequence construct can further include the leader sequence, in which the leader sequence is located upstream (or 5') of the heterologous nucleic acid sequence encoding the heavy chain polypeptide. Accordingly, the signal peptide encoded by the leader sequence can be linked by a peptide bond to the heavy chain polypeptide.

[00160] The second recombinant nucleic acid sequence construct can also include the promoter, initiation codon, termination codon, and polyadenylation signal. The second recombinant nucleic acid sequence construct can further include the leader sequence, in which the leader sequence is located upstream (or 5') of the heterologous nucleic acid sequence encoding the light chain polypeptide. Accordingly, the signal peptide encoded by the leader sequence can be linked by a peptide bond to the light chain polypeptide.

[00161] Accordingly, one example of arrangement 1 can include the first vector (and thus first recombinant nucleic acid sequence construct) encoding the heavy chain polypeptide that includes VH and CH1, and the second vector (and thus second recombinant nucleic acid sequence construct) encoding the light chain polypeptide that includes VL and CL. A second example of arrangement 1 can include the first vector (and thus first recombinant nucleic acid sequence construct) encoding the heavy chain polypeptide that includes VH, CH1, hinge region, CH2, and CH3, and the second vector (and thus second recombinant nucleic acid sequence construct) encoding the light chain polypeptide that includes VL and CL.

(2) Arrangement 2

[00162] In a second arrangement, the recombinant nucleic acid sequence construct can include the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide. The heterologous nucleic acid sequence encoding the heavy chain polypeptide can be positioned upstream (or 5') of the heterologous nucleic acid sequence encoding the light chain polypeptide.

Alternatively, the heterologous nucleic acid sequence encoding the light chain polypeptide can be positioned upstream (or 5') of the heterologous nucleic acid sequence encoding the heavy chain polypeptide.

[00163] The recombinant nucleic acid sequence construct can be placed in the vector as described in more detail below.

[00164] The recombinant nucleic acid sequence construct can include the heterologous nucleic acid sequence encoding the protease cleavage site and/or the linker sequence. If included in the recombinant nucleic acid sequence construct, the heterologous nucleic acid sequence encoding the protease cleavage site can be positioned between the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide. Accordingly, the protease cleavage site allows for separation of the heavy chain polypeptide and the light chain polypeptide into distinct polypeptides upon expression. In other embodiments, if the linker sequence is included in the recombinant nucleic acid sequence construct, then the linker sequence can be positioned between the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide.

[00165] The recombinant nucleic acid sequence construct can also include the promoter, intron, transcription termination region, initiation codon, termination codon, and/or polyadenylation signal. The recombinant nucleic acid sequence construct can include one or more promoters. The recombinant nucleic acid sequence construct can include two promoters such that one promoter can be associated with the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the second promoter can be associated with the heterologous nucleic acid sequence encoding the light chain polypeptide. In still other embodiments, the recombinant nucleic acid sequence construct can include one promoter that is associated with the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide.

[00166] The recombinant nucleic acid sequence construct can further include two leader sequences, in which a first leader sequence is located upstream (or 5') of the heterologous nucleic acid sequence encoding the heavy chain polypeptide and a second leader sequence is located upstream (or 5') of the heterologous nucleic acid sequence encoding the light chain polypeptide. Accordingly, a first signal peptide encoded by the first leader sequence can be linked by a peptide bond to the heavy chain polypeptide and a second signal peptide encoded by the second leader sequence can be linked by a peptide bond to the light chain polypeptide.

[00167] Accordingly, one example of arrangement 2 can include the vector (and thus recombinant nucleic acid sequence construct) encoding the heavy chain polypeptide that includes VH and CH1, and the light chain polypeptide that includes VL and CL, in which the linker sequence is positioned between the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide.

[00168] A second example of arrangement of 2 can include the vector (and thus recombinant nucleic acid sequence construct) encoding the heavy chain polypeptide that includes VH and CH1, and the light chain polypeptide that includes VL and CL, in which the heterologous nucleic acid sequence encoding the protease cleavage site is positioned between the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide.

[00169] A third example of arrangement 2 can include the vector (and thus recombinant nucleic acid sequence construct) encoding the heavy chain polypeptide that includes VH, CH1, hinge region, CH2, and CH3, and the light chain polypeptide that includes VL and CL, in which the linker sequence is positioned between the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide.

[00170] A forth example of arrangement of 2 can include the vector (and thus recombinant nucleic acid sequence construct) encoding the heavy chain polypeptide that includes VH, CH1, hinge region, CH2, and CH3, and the light chain polypeptide that includes VL and CL, in which the heterologous nucleic acid sequence encoding the protease cleavage site is positioned between the heterologous nucleic acid sequence encoding the heavy chain polypeptide and the heterologous nucleic acid sequence encoding the light chain polypeptide.

c. Expression from the Recombinant Nucleic Acid Sequence Construct

[00171] As described above, the recombinant nucleic acid sequence construct can include, amongst the one or more components, the heterologous nucleic acid sequence encoding the heavy chain polypeptide and/or the heterologous nucleic acid sequence encoding the light chain polypeptide. Accordingly, the recombinant nucleic acid sequence construct can facilitate expression of the heavy chain polypeptide and/or the light chain polypeptide.

[00172] When arrangement 1 as described above is utilized, the first recombinant nucleic acid sequence construct can facilitate the expression of the heavy chain polypeptide and the second recombinant nucleic acid sequence construct can facilitate expression of the light

chain polypeptide. When arrangement 2 as described above is utilized, the recombinant nucleic acid sequence construct can facilitate the expression of the heavy chain polypeptide and the light chain polypeptide.

[00173] Upon expression, for example, but not limited to, in a cell, organism, or mammal, the heavy chain polypeptide and the light chain polypeptide can assemble into the synthetic antibody. In particular, the heavy chain polypeptide and the light chain polypeptide can interact with one another such that assembly results in the synthetic antibody being capable of binding the antigen. In other embodiments, the heavy chain polypeptide and the light chain polypeptide can interact with one another such that assembly results in the synthetic antibody being more immunogenic as compared to an antibody not assembled as described herein. In still other embodiments, the heavy chain polypeptide and the light chain polypeptide can interact with one another such that assembly results in the synthetic antibody being capable of eliciting or inducing an immune response against the antigen.

d. Vector

[00174] The recombinant nucleic acid sequence construct described above can be placed in one or more vectors. The one or more vectors can contain an origin of replication. The one or more vectors can be a plasmid, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. The one or more vectors can be either a self-replication extra chromosomal vector, or a vector which integrates into a host genome.

[00175] The one or more vectors can be a heterologous expression construct, which is generally a plasmid that is used to introduce a specific gene into a target cell. Once the expression vector is inside the cell, the heavy chain polypeptide and/or light chain polypeptide that are encoded by the recombinant nucleic acid sequence construct is produced by the cellular-transcription and translation machinery ribosomal complexes. The one or more vectors can express large amounts of stable messenger RNA, and therefore proteins.

(1) Expression Vector

[00176] The one or more vectors can be a circular plasmid or a linear nucleic acid. The circular plasmid and linear nucleic acid are capable of directing expression of a particular nucleotide sequence in an appropriate subject cell. The one or more vectors comprising the recombinant nucleic acid sequence construct may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components.

(2) Plasmid

[00177] The one or more vectors can be a plasmid. The plasmid may be useful for transfecting cells with the recombinant nucleic acid sequence construct. The plasmid may be useful for introducing the recombinant nucleic acid sequence construct into the subject. The plasmid may also comprise a regulatory sequence, which may be well suited for gene expression in a cell into which the plasmid is administered.

[00178] The plasmid may also comprise a mammalian origin of replication in order to maintain the plasmid extrachromosomally and produce multiple copies of the plasmid in a cell. The plasmid may be pVAXI, pCEP4 or pREP4 from Invitrogen (San Diego, CA), which may comprise the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region, which may produce high copy episomal replication without integration. The backbone of the plasmid may be pAV0242. The plasmid may be a replication defective adenovirus type 5 (Ad5) plasmid.

[00179] The plasmid may be pSE420 (Invitrogen, San Diego, Calif.), which may be used for protein production in *Escherichia coli* (E.coli). The plasmid may also be p YES2 (Invitrogen, San Diego, Calif.), which may be used for protein production in *Saccharomyces cerevisiae* strains of yeast. The plasmid may also be of the MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, Calif.), which may be used for protein production in insect cells. The plasmid may also be pcDNAI or pcDNA3 (Invitrogen, San Diego, Calif.), which may be used for protein production in mammalian cells such as Chinese hamster ovary (CHO) cells.

(3) Circular and Linear Vector

[00180] The one or more vectors may be circular plasmid, which may transform a target cell by integration into the cellular genome or exist extrachromosomally (e.g., autonomous replicating plasmid with an origin of replication). The vector can be pVAX, pcDNA3.0, or provax, or any other expression vector capable of expressing the heavy chain polypeptide and/or light chain polypeptide encoded by the recombinant nucleic acid sequence construct.

[00181] Also provided herein is a linear nucleic acid, or linear expression cassette ("LEC"), that is capable of being efficiently delivered to a subject via electroporation and expressing the heavy chain polypeptide and/or light chain polypeptide encoded by the recombinant nucleic acid sequence construct. The LEC may be any linear DNA devoid of any phosphate backbone. The LEC may not contain any antibiotic resistance genes and/or a phosphate

backbone. The LEC may not contain other nucleic acid sequences unrelated to the desired gene expression.

[00182] The LEC may be derived from any plasmid capable of being linearized. The plasmid may be capable of expressing the heavy chain polypeptide and/or light chain polypeptide encoded by the recombinant nucleic acid sequence construct. The plasmid can be pNP (Puerto Rico/34) or pM2 (New Caledonia/99). The plasmid may be WLV009, pVAX, pcDNA3.0, or provax, or any other expression vector capable of expressing the heavy chain polypeptide and/or light chain polypeptide encoded by the recombinant nucleic acid sequence construct.

[00183] The LEC can be pcrM2. The LEC can be pcrNP. pcrNP and pcrMR can be derived from pNP (Puerto Rico/34) and pM2 (New Caledonia/99), respectively.

(4) Method of Preparing the Vector

[00184] Provided herein is a method for preparing the one or more vectors in which the recombinant nucleic acid sequence construct has been placed. After the final subcloning step, the vector can be used to inoculate a cell culture in a large scale fermentation tank, using known methods in the art.

[00185] In other embodiments, after the final subcloning step, the vector can be used with one or more electroporation (EP) devices. The EP devices are described below in more detail.

[00186] The one or more vectors can be formulated or manufactured using a combination of known devices and techniques, but preferably they are manufactured using a plasmid manufacturing technique that is described in a licensed, co-pending U.S. provisional application U.S. Serial No. 60/939,792, which was filed on May 23, 2007. In some examples, the DNA plasmids described herein can be formulated at concentrations greater than or equal to 10 mg/mL. The manufacturing techniques also include or incorporate various devices and protocols that are commonly known to those of ordinary skill in the art, in addition to those described in U.S. Serial No. 60/939,792, including those described in a licensed patent, US Patent No. 7,238,522, which issued on July 3, 2007. The above-referenced application and patent, US Serial No. 60/939,792 and US Patent No. 7,238,522, respectively, are hereby incorporated in their entirety.

4. Antibody

[00187] As described above, the recombinant nucleic acid sequence can encode the antibody, a fragment thereof, a variant thereof, or a combination thereof. The antibody can bind or react with the antigen, which is described in more detail below.

[00188] The antibody may comprise a heavy chain and a light chain complementarity determining region (“CDR”) set, respectively interposed between a heavy chain and a light chain framework (“FR”) set which provide support to the CDRs and define the spatial relationship of the CDRs relative to each other. The CDR set may contain three hypervariable regions of a heavy or light chain V region. Proceeding from the N-terminus of a heavy or light chain, these regions are denoted as “CDR1,” “CDR2,” and “CDR3,” respectively. An antigen-binding site, therefore, may include six CDRs, comprising the CDR set from each of a heavy and a light chain V region.

[00189] The proteolytic enzyme papain preferentially cleaves IgG molecules to yield several fragments, two of which (the F(ab) fragments) each comprise a covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including the F(ab')₂ fragment, which comprises both antigen-binding sites. Accordingly, the antibody can be the Fab or F(ab')₂. The Fab can include the heavy chain polypeptide and the light chain polypeptide. The heavy chain polypeptide of the Fab can include the VH region and the CH1 region. The light chain of the Fab can include the VL region and CL region.

[00190] The antibody can be an immunoglobulin (Ig). The Ig can be, for example, IgA, IgM, IgD, IgE, and IgG. The immunoglobulin can include the heavy chain polypeptide and the light chain polypeptide. The heavy chain polypeptide of the immunoglobulin can include a VH region, a CH1 region, a hinge region, a CH2 region, and a CH3 region. The light chain polypeptide of the immunoglobulin can include a VL region and CL region.

[00191] The antibody can be a polyclonal or monoclonal antibody. The antibody can be a chimeric antibody, a single chain antibody, an affinity matured antibody, a human antibody, a humanized antibody, or a fully human antibody. The humanized antibody can be an antibody from a non-human species that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule.

5. Antigen

[00192] The synthetic antibody is directed to the antigen or fragment or variant thereof. The antigen can be a nucleic acid sequence, an amino acid sequence, or a combination thereof. The nucleic acid sequence can be DNA, RNA, cDNA, a variant thereof, a fragment thereof, or a combination thereof. The amino acid sequence can be a protein, a peptide, a variant thereof, a fragment thereof, or a combination thereof.

[00193] The antigen can be from any number of organisms, for example, a virus, a parasite, a bacterium, a fungus, or a mammal. The antigen can be associated with an autoimmune disease, allergy, or asthma. In other embodiments, the antigen can be associated with cancer, herpes, influenza, hepatitis B, hepatitis C, human papilloma virus (HPV), or human immunodeficiency virus (HIV).

[00194] In some embodiments, the antigen is foreign. In some embodiments, the antigen is a self-antigen.

a. Foreign Antigens

[00195] In some embodiments, the antigen is foreign. A foreign antigen is any non-self substance (i.e., originates external to the subject) that, when introduced into the body, is capable of stimulating an immune response.

(1) Viral Antigens

[00196] The foreign antigen can be a viral antigen, or fragment thereof, or variant thereof. The viral antigen can be from a virus from one of the following families: *Adenoviridae*, *Arenaviridae*, *Bunyaviridae*, *Caliciviridae*, *Coronaviridae*, *Filoviridae*, *Hepadnaviridae*, *Herpesviridae*, *Orthomyxoviridae*, *Papovaviridae*, *Paramyxoviridae*, *Parvoviridae*, *Picornaviridae*, *Poxviridae*, *Reoviridae*, *Retroviridae*, *Rhabdoviridae*, or *Togaviridae*. The viral antigen can be from human immunodeficiency virus (HIV), Chikungunya virus (CHIKV), dengue fever virus, papilloma viruses, for example, human papilloma virus (HPV), polio virus, hepatitis viruses, for example, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV), smallpox virus (*Variola major* and *minor*), vaccinia virus, influenza virus, rhinoviruses, equine encephalitis viruses, rubella virus, yellow fever virus, Norwalk virus, hepatitis A virus, human T-cell leukemia virus (HTLV-I), hairy cell leukemia virus (HTLV-II), California encephalitis virus, Hanta virus (hemorrhagic fever), rabies virus, Ebola fever virus, Marburg virus, measles virus, mumps virus, respiratory syncytial virus (RSV), herpes

simplex 1 (oral herpes), herpes simplex 2 (genital herpes), herpes zoster (varicella-zoster, a.k.a., chickenpox), cytomegalovirus (CMV), for example human CMV, Epstein-Barr virus (EBV), flavivirus, foot and mouth disease virus, lassa virus, arenavirus, or cancer causing virus.

(a) Human Immunodeficiency Virus (HIV) Antigen

[00197] The viral antigen may be from Human Immunodeficiency Virus (HIV) virus. In some embodiments, the HIV antigen can be a subtype A envelope protein, subtype B envelope protein, subtype C envelope protein, subtype D envelope protein, subtype B Nef-Rev protein, Gag subtype A, B, C, or D protein, MPol protein, a nucleic acid or amino acid sequences of Env A, Env B, Env C, Env D, B Nef-Rev, Gag, or any combination thereof.

[00198] A synthetic antibody specific for HIV can include a Fab fragment comprising the amino acid sequence of SEQ ID NO:48, which is encoded by the nucleic acid sequence of SEQ ID NO:3, and the amino acid sequence of SEQ ID NO:49, which is encoded by the nucleic acid sequence of SEQ ID NO:4. The synthetic antibody can comprise the amino acid sequence of SEQ ID NO:46, which is encoded by the nucleic acid sequence of SEQ ID NO:6, and the amino acid sequence of SEQ ID NO:47, which is encoded by the nucleic acid sequence of SEQ ID NO:7. The Fab fragment comprise the amino acid sequence of SEQ ID NO:51, which is encoded by the nucleic acid sequence of SEQ ID NO:50. The Fab can comprise the amino acid sequence of SEQ ID NO:53, which is encoded by the nucleic acid sequence of SEQ ID NO:52.

[00199] A synthetic antibody specific for HIV can include an Ig comprising the amino acid sequence of SEQ ID NO:5. The Ig can comprise the amino acid sequence of SEQ ID NO:1, which is encoded by the nucleic acid sequence of SEQ ID NO:62. The Ig can comprise the amino acid sequence of SEQ ID NO:2, which is encoded by the nucleic acid sequence of SEQ ID NO:63. The Ig can comprise the amino acid sequence of SEQ ID NO:55, which is encoded by the nucleic acid sequence of SEQ ID NO:54, and the amino acid sequence of SEQ ID NO:57, which is encoded by the nucleic acid sequence SEQ ID NO:56.

(b) Chikungunya Virus

[00200] The viral antigen may be from Chikungunya virus. Chikungunya virus belongs to the alphavirus genus of the Togaviridae family. Chikungunya virus is transmitted to humans by the bite of infected mosquitoes, such as the genus *Aedes*.

[00201] A synthetic antibody specific for CHIKV can include a Fab fragment comprising the amino acid sequence of SEQ ID NO:59, which is encoded by the nucleic acid sequence of SEQ ID NO:58, and the amino acid sequence of SEQ ID NO:61, which is encoded by the nucleic acid sequence of SEQ ID NO:60.

(c) Dengue Virus

[00202] The viral antigen may be from Dengue virus. The Dengue virus antigen may be one of three proteins or polypeptides (C, prM, and E) that form the virus particle. The Dengue virus antigen may be one of seven other proteins or polypeptides (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) which are involved in replication of the virus. The Dengue virus may be one of five strains or serotypes of the virus, including DENV-1, DENV-2, DENV-3 and DENV-4. The antigen may be any combination of a plurality of Dengue virus antigens.

[00203] A synthetic antibody specific for Dengue virus can include a Ig comprising the amino acid sequence of SEQ ID NO:45, which is encoded by the nucleic acid sequence of SEQ ID NO:44.

(d) Hepatitis Antigen

[00204] The viral antigen may include a hepatitis virus antigen (i.e., hepatitis antigen), or a fragment thereof, or a variant thereof. The hepatitis antigen can be an antigen or immunogen from one or more of hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and/or hepatitis E virus (HEV).

[00205] The hepatitis antigen can be an antigen from HAV. The hepatitis antigen can be a HAV capsid protein, a HAV non-structural protein, a fragment thereof, a variant thereof, or a combination thereof.

[00206] The hepatitis antigen can be an antigen from HCV. The hepatitis antigen can be a HCV nucleocapsid protein (i.e., core protein), a HCV envelope protein (e.g., E1 and E2), a HCV non-structural protein (e.g., NS1, NS2, NS3, NS4a, NS4b, NS5a, and NS5b), a fragment thereof, a variant thereof, or a combination thereof.

[00207] The hepatitis antigen can be an antigen from HDV. The hepatitis antigen can be a HDV delta antigen, fragment thereof, or variant thereof.

[00208] The hepatitis antigen can be an antigen from HEV. The hepatitis antigen can be a HEV capsid protein, fragment thereof, or variant thereof.

[00209] The hepatitis antigen can be an antigen from HBV. The hepatitis antigen can be a HBV core protein, a HBV surface protein, a HBV DNA polymerase, a HBV protein encoded

by gene X, fragment thereof, variant thereof, or combination thereof. The hepatitis antigen can be a HBV genotype A core protein, a HBV genotype B core protein, a HBV genotype C core protein, a HBV genotype D core protein, a HBV genotype E core protein, a HBV genotype F core protein, a HBV genotype G core protein, a HBV genotype H core protein, a HBV genotype A surface protein, a HBV genotype B surface protein, a HBV genotype C surface protein, a HBV genotype D surface protein, a HBV genotype E surface protein, a HBV genotype F surface protein, a HBV genotype G surface protein, a HBV genotype H surface protein, fragment thereof, variant thereof, or combination thereof.

[00210] In some embodiments, the hepatitis antigen can be an antigen from HBV genotype A, HBV genotype B, HBV genotype C, HBV genotype D, HBV genotype E, HBV genotype F, HBV genotype G, or HBV genotype H.

(e) Human Papilloma Virus (HPV) Antigen

[00211] The viral antigen may comprise an antigen from HPV. The HPV antigen can be from HPV types 16, 18, 31, 33, 35, 45, 52, and 58 which cause cervical cancer, rectal cancer, and/or other cancers. The HPV antigen can be from HPV types 6 and 11, which cause genital warts, and are known to be causes of head and neck cancer.

[00212] The HPV antigens can be the HPV E6 or E7 domains from each HPV type. For example, for HPV type 16 (HPV16), the HPV16 antigen can include the HPV16 E6 antigen, the HPV16 E7 antigen, fragments, variants, or combinations thereof. Similarly, the HPV antigen can be HPV 6 E6 and/or E7, HPV 11 E6 and/or E7, HPV 18 E6 and/or E7, HPV 31 E6 and/or E7, HPV 33 E6 and/or E7, HPV 52 E6 and/or E7, or HPV 58 E6 and/or E7, fragments, variants, or combinations thereof.

(f) RSV Antigen

[00213] The viral antigen may comprise a RSV antigen. The RSV antigen can be a human RSV fusion protein (also referred to herein as “RSV F,” “RSV F protein,” and “F protein”), or fragment or variant thereof. The human RSV fusion protein can be conserved between RSV subtypes A and B. The RSV antigen can be a RSV F protein, or fragment or variant thereof, from the RSV Long strain (GenBank AAX23994.1). The RSV antigen can be a RSV F protein from the RSV A2 strain (GenBank AAB59858.1), or a fragment or variant thereof. The RSV antigen can be a monomer, a dimer, or trimer of the RSV F protein, or a fragment or variant thereof.

[00214] The RSV F protein can be in a prefusion form or a postfusion form. The postfusion form of RSV F elicits high titer neutralizing antibodies in immunized animals and protects the animals from RSV challenge.

[00215] The RSV antigen can also be human RSV attachment glycoprotein (also referred to herein as “RSV G,” “RSV G protein,” and “G protein”), or fragment or variant thereof. The human RSV G protein differs between RSV subtypes A and B. The antigen can be RSV G protein, or fragment or variant thereof, from the RSV Long strain (GenBank AAX23993). The RSV antigen can be RSV G protein from the RSV subtype B isolate H5601, the RSV subtype B isolate H1068, the RSV subtype B isolate H5598, the RSV subtype B isolate H1123, or a fragment or variant thereof.

[00216] In other embodiments, the RSV antigen can be human RSV non-structural protein 1 (“NS1 protein”), or fragment or variant thereof. For example, the RSV antigen can be RSV NS1 protein, or fragment or variant thereof, from the RSV Long strain (GenBank AAX23987.1). The RSV antigen human can also be RSV non-structural protein 2 (“NS2 protein”), or fragment or variant thereof. For example, the RSV antigen can be RSV NS2 protein, or fragment or variant thereof, from the RSV Long strain (GenBank AAX23988.1). The RSV antigen can further be human RSV nucleocapsid (“N”) protein, or fragment or variant thereof. For example, the RSV antigen can be RSV N protein, or fragment or variant thereof, from the RSV Long strain (GenBank AAX23989.1). The RSV antigen can be human RSV Phosphoprotein (“P”) protein, or fragment or variant thereof. For example, the RSV antigen can be RSV P protein, or fragment or variant thereof, from the RSV Long strain (GenBank AAX23990.1). The RSV antigen also can be human RSV Matrix protein (“M”) protein, or fragment or variant thereof. For example, the RSV antigen can be RSV M protein, or fragment or variant thereof, from the RSV Long strain (GenBank AAX23991.1).

[00217] In still other embodiments, the RSV antigen can be human RSV small hydrophobic (“SH”) protein, or fragment or variant thereof. For example, the RSV antigen can be RSV SH protein, or fragment or variant thereof, from the RSV Long strain (GenBank AAX23992.1). The RSV antigen can also be human RSV Matrix protein2-1 (“M2-1”) protein, or fragment or variant thereof. For example, the RSV antigen can be RSV M2-1 protein, or fragment or variant thereof, from the RSV Long strain (GenBank AAX23995.1). The RSV antigen can further be human RSV Matrix protein 2-2 (“M2-2”) protein, or fragment or variant thereof. For example, the RSV antigen can be RSV M2-2 protein, or fragment or variant thereof, from the RSV Long strain (GenBank AAX23997.1). The RSV antigen human can be RSV Polymerase L (“L”) protein, or fragment or variant thereof. For

example, the RSV antigen can be RSV L protein, or fragment or variant thereof, from the RSV Long strain (GenBank AAX23996.1).

[00218] In further embodiments, the RSV antigen can have an optimized amino acid sequence of NS1, NS2, N, P, M, SH, M2-1, M2-2, or L protein. The RSV antigen can be a human RSV protein or recombinant antigen, such as any one of the proteins encoded by the human RSV genome.

[00219] In other embodiments, the RSV antigen can be, but is not limited to, the RSV F protein from the RSV Long strain, the RSV G protein from the RSV Long strain, the optimized amino acid RSV G amino acid sequence, the human RSV genome of the RSV Long strain, the optimized amino acid RSV F amino acid sequence, the RSV NS1 protein from the RSV Long strain, the RSV NS2 protein from the RSV Long strain, the RSV N protein from the RSV Long strain, the RSV P protein from the RSV Long strain, the RSV M protein from the RSV Long strain, the RSV SH protein from the RSV Long strain, the RSV M2-1 protein from the RSV Long strain, the RSV M2-2 protein from the RSV Long strain, the RSV L protein from the RSV Long strain, the RSV G protein from the RSV subtype B isolate H5601, the RSV G protein from the RSV subtype B isolate H1068, the RSV G protein from the RSV subtype B isolate H5598, the RSV G protein from the RSV subtype B isolate H1123, or fragment thereof, or variant thereof.

(g) Influenza Antigen

[00220] The viral antigen may comprise an antigen from influenza virus. The influenza antigens are those capable of eliciting an immune response in a mammal against one or more influenza serotypes. The antigen can comprise the full length translation product HA0, subunit HA1, subunit HA2, a variant thereof, a fragment thereof or a combination thereof. The influenza hemagglutinin antigen can be derived from multiple strains of influenza A serotype H1, serotype H2, a hybrid sequence derived from different sets of multiple strains of influenza A serotype H1, or derived from multiple strains of influenza B. The influenza hemagglutinin antigen can be from influenza B.

[00221] The influenza antigen can also contain at least one antigenic epitope that can be effective against particular influenza immunogens against which an immune response can be induced. The antigen may provide an entire repertoire of immunogenic sites and epitopes present in an intact influenza virus. The antigen may be derived from hemagglutinin antigen sequences from a plurality of influenza A virus strains of one serotype such as a plurality of influenza A virus strains of serotype H1 or of serotype H2. The antigen may be a hybrid

hemagglutinin antigen sequence derived from combining two different hemagglutinin antigen sequences or portions thereof. Each of two different hemagglutinin antigen sequences may be derived from a different set of a plurality of influenza A virus strains of one serotype such as a plurality of influenza A virus strains of serotype H1. The antigen may be a hemagglutinin antigen sequence derived from hemagglutinin antigen sequences from a plurality of influenza B virus strains.

[00222] In some embodiments, the influenza antigen can be H1 HA, H2 HA, H3 HA, H5 HA, or a BHA antigen.

(h) Ebola Virus

[00223] The viral antigen may be from Ebola virus. Ebola virus disease (EVD) or Ebola hemorrhagic fever (EHF) includes any of four of the five known ebola viruses including Bundibugyo virus (BDBV), Ebola virus (EBOV), Sudan virus (SUDV), and Taï Forest virus (TAFV, also referred to as Côte d'Ivoire Ebola virus (Ivory Coast Ebolavirus, CIEBOV).

(2) Bacterial Antigens

[00224] The foreign antigen can be a bacterial antigen or fragment or variant thereof. The bacterium can be from any one of the following phyla: Acidobacteria, Actinobacteria, Aquificae, Bacteroidetes, Caldiseica, Chlamydiae, Chlorobi, Chloroflexi, Chrysiogenetes, Cyanobacteria, Deferribacteres, Deinococcus-Thermus, Dictyoglomi, Elusimicrobia, Fibrobacteres, Firmicutes, Fusobacteria, Gemmatimonadetes, Lentisphaerae, Nitrospira, Planctomycetes, Proteobacteria, Spirochaetes, Synergistetes, Tenericutes, Thermodesulfobacteria, Thermotogae, and Verrucomicrobia.

[00225] The bacterium can be a gram positive bacterium or a gram negative bacterium. The bacterium can be an aerobic bacterium or an anerobic bacterium. The bacterium can be an autotrophic bacterium or a heterotrophic bacterium. The bacterium can be a mesophile, a neutrophile, an extremophile, an acidophile, an alkaliphile, a thermophile, a psychrophile, an halophile, or an osmophile.

[00226] The bacterium can be an anthrax bacterium, an antibiotic resistant bacterium, a disease causing bacterium, a food poisoning bacterium, an infectious bacterium, Salmonella bacterium, Staphylococcus bacterium, Streptococcus bacterium, or tetanus bacterium. The bacterium can be a mycobacteria, *Clostridium tetani*, *Yersinia pestis*, *Bacillus anthracis*,

methicillin-resistant *Staphylococcus aureus* (MRSA), or *Clostridium difficile*. The bacterium can be *Mycobacterium tuberculosis*.

(a) *Mycobacterium tuberculosis* Antigens

[00227] The bacterial antigen may be a *Mycobacterium tuberculosis* antigen (i.e., TB antigen or TB immunogen), or fragment thereof, or variant thereof. The TB antigen can be from the Ag85 family of TB antigens, for example, Ag85A and Ag85B. The TB antigen can be from the Esx family of TB antigens, for example, EsxA, EsxB, EsxC, EsxD, EsxE, EsxF, EsxH, EsxO, EsxQ, EsxR, EsxS, EsxT, EsxU, EsxV, and EsxW.

(3) Parasitic Antigens

[00228] The foreign antigen can be a parasite antigen or fragment or variant thereof. The parasite can be a protozoa, helminth, or ectoparasite. The helminth (i.e., worm) can be a flatworm (e.g., flukes and tapeworms), a thorny-headed worm, or a round worm (e.g., pinworms). The ectoparasite can be lice, fleas, ticks, and mites.

[00229] The parasite can be any parasite causing any one of the following diseases: Acanthamoeba keratitis, Amoebiasis, Ascariasis, Babesiosis, Balantidiasis, Baylisascariasis, Chagas disease, Clonorchiasis, Cochliomyia, Cryptosporidiosis, Diphyllbothriasis, Dracunculiasis, Echinococcosis, Elephantiasis, Enterobiasis, Fascioliasis, Fasciolopsiasis, Filariasis, Giardiasis, Gnathostomiasis, Hymenolepiasis, Isosporiasis, Katayama fever, Leishmaniasis, Lyme disease, Malaria, Metagonimiasis, Myiasis, Onchocerciasis, Pediculosis, Scabies, Schistosomiasis, Sleeping sickness, Strongyloidiasis, Taeniasis, Toxocariasis, Toxoplasmosis, Trichinosis, and Trichuriasis.

[00230] The parasite can be Acanthamoeba, Anisakis, *Ascaris lumbricoides*, Botfly, *Balantidium coli*, Bedbug, *Cestoda* (tapeworm), Chiggers, *Cochliomyia hominivorax*, Entamoeba histolytica, *Fasciola hepatica*, *Giardia lamblia*, Hookworm, *Leishmania*, *Linguatula serrata*, Liver fluke, Loa loa, *Paragonimus* - lung fluke, Pinworm, *Plasmodium falciparum*, Schistosoma, *Strongyloides stercoralis*, Mite, Tapeworm, *Toxoplasma gondii*, *Trypanosoma*, Whipworm, or *Wuchereria bancrofti*.

(a) Malaria Antigen

[00231] The foreign antigen may be a malaria antigen (i.e., PF antigen or PF immunogen), or fragment thereof, or variant thereof. The antigen can be from a parasite causing malaria. The malaria causing parasite can be *Plasmodium falciparum*. The *Plasmodium falciparum* antigen can include the circumsporozoite (CS) antigen.

[00232] In some embodiments, the malaria antigen can be one of *P. falciparum* immunogens CS; LSA1; TRAP; CelTOS; and Ama1. The immunogens may be full length or immunogenic fragments of full length proteins.

[00233] In other embodiments, the malaria antigen can be TRAP, which is also referred to as SSP2. In still other embodiments, the malaria antigen can be CelTOS, which is also referred to as Ag2 and is a highly conserved *Plasmodium* antigen. In further embodiments, the malaria antigen can be Ama1, which is a highly conserved *Plasmodium* antigen. In some embodiments, the malaria antigen can be a CS antigen.

[00234] In other embodiments, the malaria antigen can be a fusion protein comprising a combination of two or more of the PF proteins set forth herein. For example, fusion proteins may comprise two or more of CS immunogen, ConLSA1 immunogen, ConTRAP immunogen, ConCelTOS immunogen, and ConAma1 immunogen linked directly adjacent to each other or linked with a spacer or one or more amino acids in between. In some embodiments, the fusion protein comprises two PF immunogens; in some embodiments the fusion protein comprises three PF immunogens, in some embodiments the fusion protein comprises four PF immunogens, and in some embodiments the fusion protein comprises five PF immunogens. Fusion proteins with two PF immunogens may comprise: CS and LSA1; CS and TRAP; CS and CelTOS; CS and Ama1; LSA1 and TRAP; LSA1 and CelTOS; LSA1 and Ama1; TRAP and CelTOS; TRAP and Ama1; or CelTOS and Ama1. Fusion proteins with three PF immunogens may comprise: CS, LSA1 and TRAP; CS, LSA1 and CelTOS; CS, LSA1 and Ama1; LSA1, TRAP and CelTOS; LSA1, TRAP and Ama1; or TRAP, CelTOS and Ama1. Fusion proteins with four PF immunogens may comprise: CS, LSA1, TRAP and CelTOS; CS, LSA1, TRAP and Ama1; CS, LSA1, CelTOS and Ama1; CS, TRAP, CelTOS and Ama1; or LSA1, TRAP, CelTOS and Ama1. Fusion proteins with five PF immunogens may comprise CS or CS-alt, LSA1, TRAP, CelTOS and Ama1.

(4) Fungal Antigens

[00235] The foreign antigen can be a fungal antigen or fragment or variant thereof. The fungus can be *Aspergillus* species, *Blastomyces dermatitidis*, *Candida* yeasts (e.g., *Candida albicans*), *Coccidioides*, *Cryptococcus neoformans*, *Cryptococcus gattii*, *dermatophyte*, *Fusarium* species, *Histoplasma capsulatum*, *Mucoromycotina*, *Pneumocystis jirovecii*, *Sporothrix schenckii*, *Exserohilum*, or *Cladosporium*.

b. Self Antigens

[00236] In some embodiments, the antigen is a self antigen. A self antigen may be a constituent of the subject's own body that is capable of stimulating an immune response. In some embodiments, a self antigen does not provoke an immune response unless the subject is in a disease state, e.g., an autoimmune disease.

[00237] Self antigens may include, but are not limited to, cytokines, antibodies against viruses such as those listed above including HIV and Dengue, antigens affecting cancer progression or development, and cell surface receptors or transmembrane proteins.

(1) WT-1

[00238] The self-antigen antigen can be Wilm's tumor suppressor gene 1 (WT1), a fragment thereof, a variant thereof, or a combination thereof. WT1 is a transcription factor containing at the N-terminus, a proline/glutamine-rich DNA-binding domain and at the C-terminus, four zinc finger motifs. WT1 plays a role in the normal development of the urogenital system and interacts with numerous factors, for example, p53, a known tumor suppressor and the serine protease HtrA2, which cleaves WT1 at multiple sites after treatment with a cytotoxic drug. Mutation of WT1 can lead to tumor or cancer formation, for example, Wilm's tumor or tumors expressing WT1.

(2) EGFR

[00239] The self-antigen may include an epidermal growth factor receptor (EGFR) or a fragment or variation thereof. EGFR (also referred to as ErbB-1 and HER1) is the cell-surface receptor for members of the epidermal growth factor family (EGF-family) of extracellular protein ligands. EGFR is a member of the ErbB family of receptors, which includes four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3), and Her 4 (ErbB-4). Mutations affecting EGFR expression or activity could result in cancer.

[00240] The antigen may include an ErbB-2 antigen. Erb-2 (human epidermal growth factor receptor 2) is also known as Neu, HER2, CD340 (cluster of differentiation 340), or p185 and is encoded by the ERBB2 gene. Amplification or over-expression of this gene has been shown to play a role in the development and progression of certain aggressive types of breast cancer. In approximately 25-30% of women with breast cancer, a genetic alteration occurs in the ERBB2 gene, resulting in the production of an increased amount of HER2 on

the surface of tumor cells. This overexpression of HER2 promotes rapid cell division and thus, HER2 marks tumor cells.

[00241] A synthetic antibody specific for HER2 can include a Fab fragment comprising an amino acid sequence of SEQ ID NO:41, which is encoded by the nucleic acid sequence of SEQ ID NO:40, and an amino acid sequence of SEQ ID NO:43, which is encoded by the nucleic acid sequence of SEQ ID NO:42.

(3) Cocaine

[00242] The self-antigen may be a cocaine receptor antigen. Cocaine receptors include dopamine transporters.

(4) PD-1

[00243] The self-antigen may include programmed death 1 (PD-1). Programmed death 1 (PD-1) and its ligands, PD-L1 and PD-L2, deliver inhibitory signals that regulate the balance between T cell activation, tolerance, and immunopathology. PD-1 is a 288 amino acid cell surface protein molecule including an extracellular IgV domain followed by a transmembrane region and an intracellular tail.

(5) 4-1BB

[00244] The self-antigen may include 4-1BB ligand. 4-1BB ligand is a type 2 transmembrane glycoprotein belonging to the TNF superfamily. 4-1BB ligand may be expressed on activated T Lymphocytes. 4-1BB is an activation-induced T-cell costimulatory molecule. Signaling via 4-1BB upregulates survival genes, enhances cell division, induces cytokine production, and prevents activation-induced cell death in T cells.

(6) CTLA4

[00245] The self-antigen may include CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4), also known as CD152 (Cluster of differentiation 152). CTLA-4 is a protein receptor found on the surface of T cells, which lead the cellular immune attack on antigens. The antigen may be a fragment of CTLA-4, such as an extracellular V domain, a transmembrane domain, and a cytoplasmic tail, or combination thereof.

(7) IL-6

[00246] The self-antigen may include interleukin 6 (IL-6). IL-6 stimulates the inflammatory and auto-immune processes in many diseases including, but not limited to, diabetes, atherosclerosis, depression, Alzheimer's Disease, systemic lupus erythematosus, multiple myeloma, cancer, Behçet's disease, and rheumatoid arthritis.

(8) MCP-1

[00247] The self-antigen may include monocyte chemotactic protein-1 (MCP-1). MCP-1 is also referred to as chemokine (C-C motif) ligand 2 (CCL2) or small inducible cytokine A2. MCP-1 is a cytokine that belongs to the CC chemokine family. MCP-1 recruits monocytes, memory T cells, and dendritic cells to the sites of inflammation produced by either tissue injury or infection.

(9) Amyloid beta

[00248] The self-antigen may include amyloid beta (A β) or a fragment or a variant thereof. The A β antigen can comprise an A β (X-Y) peptide, wherein the amino acid sequence from amino acid position X to amino acid Y of the human sequence A β protein including both X and Y, in particular to the amino acid sequence from amino acid position X to amino acid position Y of the amino acid sequence

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIV (corresponding to amino acid positions 1 to 47; the human query sequence) or variants thereof. The A β antigen can comprise an A β polypeptide of A β (X-Y) polypeptide wherein X can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, or 32 and Y can be 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, or 15. The A β polypeptide can comprise a fragment that is at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, or at least 46 amino acids.

(10) IP-10

[00249] The self-antigen may include interferon (IFN)-gamma-induced protein 10 (IP-10). IP-10 is also known as small-inducible cytokine B10 or C-X-C motif chemokine 10

(CXCL10). CXCL10 is secreted by several cell types, such as monocytes, endothelial cells and fibroblasts, in response to IFN- γ .

[00250]

(11) PSMA

[00251] The self-antigen may include prostate-specific membrane antigen (PSMA). PSMA is also known as glutamate carboxypeptidase II (GCPII), N-acetyl-L-aspartyl-L-glutamate peptidase I (NAALADase I), NAAG peptidase, or folate hydrolase (FOLH). PSMA is an integral membrane protein highly expressed by prostate cancer cells.

c. Other Antigens

[00252] In some embodiments, the antigen is an antigen other than the foreign antigen and/or the self-antigen.

(a) HIV-1 VRC01

[00253] The other antigen can be HIV-1 VRC01. HIV-1 VRC01 is a neutralizing CD4-binding site-antibody for HIV. HIV-1 VRC01 contacts portions of HIV-1 including within the gp120 loop D, the CD4 binding loop, and the V5 region of HIV-1.

(b) HIV-1 PG9

[00254] The other antigen can be HIV-1 PG9. HIV-1 PG9 is the founder member of an expanding family of glycan-dependent human antibodies that preferentially bind the HIV (HIV-1) envelope (Env) glycoprotein (gp) trimer and broadly neutralize the virus.

(c) HIV-1 4E10

[00255] The other antigen can be HIV-1 4E10. HIV-1 4E10 is a neutralizing anti-HIV antibody. HIV-1 4E10 is directed against linear epitopes mapped to the membrane-proximal external region (MPER) of HIV-1, which is located at the C terminus of the gp41 ectodomain.

(d) DV-SF1

[00256] The other antigen can be DV-SF1. DV-SF1 is a neutralizing antibody that binds the envelope protein of the four Dengue virus serotypes.

(e) DV-SF2

[00257] The other antigen can be DV-SF2. DV-SF2 is a neutralizing antibody that binds an epitope of the Dengue virus. DV-SF2 can be specific for the DENV4 serotype.

(f) DV-SF3

[00258] The other antigen can be DV-SF3. DV-SF3 is a neutralizing antibody that binds the EDIII A strand of the Dengue virus envelope protein.

6. Excipients and Other Components of the Composition

[00259] The composition may further comprise a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient can be functional molecules such as vehicles, carriers, or diluents. The pharmaceutically acceptable excipient can be a transfection facilitating agent, which can include surface active agents, such as immune-stimulating complexes (ISCOMS), Freund's incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs, vesicles such as squalene and squalene, hyaluronic acid, lipids, liposomes, calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents.

[00260] The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. The transfection facilitating agent is poly-L-glutamate, and the poly-L-glutamate may be present in the composition at a concentration less than 6 mg/ml. The transfection facilitating agent may also include surface active agents such as immune-stimulating complexes (ISCOMS), Freund's incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic acid may also be used administered in conjunction with the composition. The composition may also include a transfection facilitating agent such as lipids, liposomes, including lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture (see for example W09324640), calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents. The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. Concentration of the transfection agent in the vaccine is less than 4 mg/ml, less than 2 mg/ml, less than 1 mg/ml, less than 0.750 mg/ml, less than 0.500 mg/ml, less than 0.250 mg/ml, less than 0.100 mg/ml, less than 0.050 mg/ml, or less than 0.010 mg/ml.

[00261] The composition may further comprise a genetic facilitator agent as described in U.S. Serial No. 021,579 filed April 1, 1994, which is fully incorporated by reference.

[00262] The composition may comprise DNA at quantities of from about 1 nanogram to 100 milligrams; about 1 microgram to about 10 milligrams; or preferably about 0.1 microgram to about 10 milligrams; or more preferably about 1 milligram to about 2 milligram. In some preferred embodiments, composition according to the present invention comprises about 5 nanogram to about 1000 micrograms of DNA. In some preferred embodiments, composition can contain about 10 nanograms to about 800 micrograms of DNA. In some preferred embodiments, the composition can contain about 0.1 to about 500 micrograms of DNA. In some preferred embodiments, the composition can contain about 1 to about 350 micrograms of DNA. In some preferred embodiments, the composition can contain about 25 to about 250 micrograms, from about 100 to about 200 microgram, from about 1 nanogram to 100 milligrams; from about 1 microgram to about 10 milligrams; from about 0.1 microgram to about 10 milligrams; from about 1 milligram to about 2 milligram, from about 5 nanogram to about 1000 micrograms, from about 10 nanograms to about 800 micrograms, from about 0.1 to about 500 micrograms, from about 1 to about 350 micrograms, from about 25 to about 250 micrograms, from about 100 to about 200 microgram of DNA.

[00263] The composition can be formulated according to the mode of administration to be used. An injectable pharmaceutical composition can be sterile, pyrogen free and particulate free. An isotonic formulation or solution can be used. Additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol, and lactose. The composition can comprise a vasoconstriction agent. The isotonic solutions can include phosphate buffered saline. The composition can further comprise stabilizers including gelatin and albumin. The stabilizers can allow the formulation to be stable at room or ambient temperature for extended periods of time, including LGS or polycations or polyanions.

7. Method of Generating the Synthetic Antibody

[00264] The present invention also relates a method of generating the synthetic antibody. The method can include administering the composition to the subject in need thereof by using the method of delivery described in more detail below. Accordingly, the synthetic antibody is generated in the subject or in vivo upon administration of the composition to the subject.

[00265] The method can also include introducing the composition into one or more cells, and therefore, the synthetic antibody can be generated or produced in the one or more cells. The method can further include introducing the composition into one or more tissues, for

example, but not limited to, skin and muscle, and therefore, the synthetic antibody can be generated or produced in the one or more tissues.

8. Method of Identifying or Screening for the Antibody

[00266] The present invention further relates to a method of identifying or screening for the antibody described above, which is reactive to or binds the antigen described above. The method of identifying or screening for the antibody can use the antigen in methodologies known in those skilled in art to identify or screen for the antibody. Such methodologies can include, but are not limited to, selection of the antibody from a library (e.g., phage display) and immunization of an animal followed by isolation and/or purification of the antibody. See for example methods available in Rajan, S., and Sidhu, S., Methods in Enzymology, vol 502, Chapter One "Simplified Synthetic Antibody Libraries (2012)", which is incorporated herein in its entirety.

9. Method of Delivery of the Composition

[00267] The present invention also relates to a method of delivering the composition to the subject in need thereof. The method of delivery can include, administering the composition to the subject. Administration can include, but is not limited to, DNA injection with and without in vivo electroporation, liposome mediated delivery, and nanoparticle facilitated delivery.

[00268] The mammal receiving delivery of the composition may be human, primate, non-human primate, cow, cattle, sheep, goat, antelope, bison, water buffalo, bison, bovids, deer, hedgehogs, elephants, llama, alpaca, mice, rats, and chicken.

[00269] The composition may be administered by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intranasal intrathecal, and intraarticular or combinations thereof. For veterinary use, the composition may be administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can readily determine the dosing regimen and route of administration that is most appropriate for a particular animal. The composition may be administered by traditional syringes, needleless injection devices, "microprojectile bombardment gone guns", or other physical methods such as electroporation ("EP"), "hydrodynamic method", or ultrasound.

a. Electroporation

[00270] Administration of the composition via electroporation may be accomplished using electroporation devices that can be configured to deliver to a desired tissue of a mammal, a pulse of energy effective to cause reversible pores to form in cell membranes, and preferable the pulse of energy is a constant current similar to a preset current input by a user. The electroporation device may comprise an electroporation component and an electrode assembly or handle assembly. The electroporation component may include and incorporate one or more of the various elements of the electroporation devices, including: controller, current waveform generator, impedance tester, waveform logger, input element, status reporting element, communication port, memory component, power source, and power switch. The electroporation may be accomplished using an in vivo electroporation device, for example CELLECTRA EP system (VGX Pharmaceuticals, Blue Bell, PA) or Elgen electroporator (Genetronics, San Diego, CA) to facilitate transfection of cells by the plasmid.

[00271] The electroporation component may function as one element of the electroporation devices, and the other elements are separate elements (or components) in communication with the electroporation component. The electroporation component may function as more than one element of the electroporation devices, which may be in communication with still other elements of the electroporation devices separate from the electroporation component. The elements of the electroporation devices existing as parts of one electromechanical or mechanical device may not limited as the elements can function as one device or as separate elements in communication with one another. The electroporation component may be capable of delivering the pulse of energy that produces the constant current in the desired tissue, and includes a feedback mechanism. The electrode assembly may include an electrode array having a plurality of electrodes in a spatial arrangement, wherein the electrode assembly receives the pulse of energy from the electroporation component and delivers same to the desired tissue through the electrodes. At least one of the plurality of electrodes is neutral during delivery of the pulse of energy and measures impedance in the desired tissue and communicates the impedance to the electroporation component. The feedback mechanism may receive the measured impedance and can adjust the pulse of energy delivered by the electroporation component to maintain the constant current.

[00272] A plurality of electrodes may deliver the pulse of energy in a decentralized pattern. The plurality of electrodes may deliver the pulse of energy in the decentralized pattern through the control of the electrodes under a programmed sequence, and the programmed sequence is input by a user to the electroporation component. The programmed sequence

may comprise a plurality of pulses delivered in sequence, wherein each pulse of the plurality of pulses is delivered by at least two active electrodes with one neutral electrode that measures impedance, and wherein a subsequent pulse of the plurality of pulses is delivered by a different one of at least two active electrodes with one neutral electrode that measures impedance.

[00273] The feedback mechanism may be performed by either hardware or software. The feedback mechanism may be performed by an analog closed-loop circuit. The feedback occurs every 50 μ s, 20 μ s, 10 μ s or 1 μ s, but is preferably a real-time feedback or instantaneous (i.e., substantially instantaneous as determined by available techniques for determining response time). The neutral electrode may measure the impedance in the desired tissue and communicates the impedance to the feedback mechanism, and the feedback mechanism responds to the impedance and adjusts the pulse of energy to maintain the constant current at a value similar to the preset current. The feedback mechanism may maintain the constant current continuously and instantaneously during the delivery of the pulse of energy.

[00274] Examples of electroporation devices and electroporation methods that may facilitate delivery of the composition of the present invention, include those described in U.S. Patent No. 7,245,963 by Draghia-Akli, et al., U.S. Patent Pub. 2005/0052630 submitted by Smith, et al., the contents of which are hereby incorporated by reference in their entirety. Other electroporation devices and electroporation methods that may be used for facilitating delivery of the composition include those provided in co-pending and co-owned U.S. Patent Application, Serial No. 11/874072, filed October 17, 2007, which claims the benefit under 35 USC 119(e) to U.S. Provisional Applications Ser. Nos. 60/852,149, filed October 17, 2006, and 60/978,982, filed October 10, 2007, all of which are hereby incorporated in their entirety.

[00275] U.S. Patent No. 7,245,963 by Draghia-Akli, et al. describes modular electrode systems and their use for facilitating the introduction of a biomolecule into cells of a selected tissue in a body or plant. The modular electrode systems may comprise a plurality of needle electrodes; a hypodermic needle; an electrical connector that provides a conductive link from a programmable constant-current pulse controller to the plurality of needle electrodes; and a power source. An operator can grasp the plurality of needle electrodes that are mounted on a support structure and firmly insert them into the selected tissue in a body or plant. The biomolecules are then delivered via the hypodermic needle into the selected tissue. The programmable constant-current pulse controller is activated and constant-current electrical pulse is applied to the plurality of needle electrodes. The applied constant-current electrical

pulse facilitates the introduction of the biomolecule into the cell between the plurality of electrodes. The entire content of U.S. Patent No. 7,245,963 is hereby incorporated by reference.

[00276] U.S. Patent Pub. 2005/0052630 submitted by Smith, et al. describes an electroporation device which may be used to effectively facilitate the introduction of a biomolecule into cells of a selected tissue in a body or plant. The electroporation device comprises an electro-kinetic device ("EKD device") whose operation is specified by software or firmware. The EKD device produces a series of programmable constant-current pulse patterns between electrodes in an array based on user control and input of the pulse parameters, and allows the storage and acquisition of current waveform data. The electroporation device also comprises a replaceable electrode disk having an array of needle electrodes, a central injection channel for an injection needle, and a removable guide disk. The entire content of U.S. Patent Pub. 2005/0052630 is hereby incorporated by reference.

[00277] The electrode arrays and methods described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/0052630 may be adapted for deep penetration into not only tissues such as muscle, but also other tissues or organs. Because of the configuration of the electrode array, the injection needle (to deliver the biomolecule of choice) is also inserted completely into the target organ, and the injection is administered perpendicular to the target issue, in the area that is pre-delineated by the electrodes. The electrodes described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/005263 are preferably 20 mm long and 21 gauge.

[00278] Additionally, contemplated in some embodiments that incorporate electroporation devices and uses thereof, there are electroporation devices that are those described in the following patents: US Patent 5,273,525 issued December 28, 1993, US Patents 6,110,161 issued August 29, 2000, 6,261,281 issued July 17, 2001, and 6,958,060 issued October 25, 2005, and US patent 6,939,862 issued September 6, 2005. Furthermore, patents covering subject matter provided in US patent 6,697,669 issued February 24, 2004, which concerns delivery of DNA using any of a variety of devices, and US patent 7,328,064 issued February 5, 2008, drawn to method of injecting DNA are contemplated herein. The above-patents are incorporated by reference in their entirety.

10. Method of Treatment

[00279] Also provided herein is a method of treating, protecting against, and/or preventing disease in a subject in need thereof by generating the synthetic antibody in the subject. The

method can include administering the composition to the subject. Administration of the composition to the subject can be done using the method of delivery described above.

[00280] Upon generation of the synthetic antibody in the subject, the synthetic antibody can bind to or react with the antigen. Such binding can neutralize the antigen, block recognition of the antigen by another molecule, for example, a protein or nucleic acid, and elicit or induce an immune response to the antigen, thereby treating, protecting against, and/or preventing the disease associated with the antigen in the subject.

[00281] The composition dose can be between 1 µg to 10 mg active component/kg body weight/time, and can be 20 µg to 10 mg component/kg body weight/time. The composition can be administered every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days. The number of composition doses for effective treatment can be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00282] In one method of treatment, the synthetic antibodies, or functional fragments thereof, can be administered to a subject in need of treatment against an infection, whether viral or bacterial, or cancerous cells. The administration of the synthetic antibodies described herein can provide, upon expression in vivo, functional antibodies that can rapidly present itself in the diseased area of the body and mount a neutralizing response to the target (which it was designed to bind, and preferably neutralize). This rapid presence can be important for disease pathology that is rather rapid and/or in individuals that do not have an existing memory immunity. Some particular cases where rapid neutralization is critical for the subject that is infected is in tropic diseases such as dengue, chikungunya and ebola. Such infections require rapid neutralization from the instant of infection with the virus. Example 5 and Figures 6A and 6B display the rapid generation of antibodies using the expression constructs generated with the described methods. Figure 6A shows that within a day of administration of the plasmid DNA constructs antibody is expressed; whereas in Figure 6B, administration of the protein/antigen results in antibody expression in about 8 days.

[00283] This method of treatment can be alone, or it can be combined with normal vaccinations with an antigen, which would then cause the subject to generate a host immune response against the target. A combination vaccine would provide the benefit of a two phase immune response against the intended target: 1) a first rapid response as provided by the nucleotide sequences encoding synthetic antibodies, and functional fragments thereof, and 2) a second host immune response triggered by a traditional vaccine (which can include a DNA vaccine or synthetic immunogen), which would have a lag period until the host can mount its own immune response against the target.

[00284] The present invention has multiple aspects, illustrated by the following non-limiting examples.

11. Examples

[00285] The present invention is further illustrated in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

Example 1

[00286] A high expression system for *in vivo* immunoglobulin (Ig) generation was constructed. In particular, Ig heavy and light chain sequences were modified in order to improve *in vivo* expression of the fully assembled Ig molecule, which included 2 heavy and 2 light chain polypeptides. Constructs of gp120IgG-heavy and light chain molecules were created and inserted separately in the pVAX1 vector (Life Technologies, Carlsbad, CA). This antibody has defined properties that allow it to be used for characterization studies as described below. Several modifications were included when creating the constructs to optimize expression of the Ig *in vivo*. Optimization included codon optimization and the introduction of a kozak sequence (GCC ACC). The nucleic acid sequences of the optimized constructs for the heavy and light chains of the Ig are set forth in SEQ ID NO:6 and SEQ ID NO:7, respectively (FIGS. 1 and 2, respectively). In FIGS. 1 and 2, underlining and double underlining mark the BamHI (GGA TCC) and XhoI (CTC GAG) restriction enzymes sites used to clone the constructs into the pVAX1 vector while bold marks the start (ATG) and stop (TGA TAA) codons. SEQ ID NO:6 encodes the amino acid sequence set forth in SEQ ID NO:46, i.e., the amino acid sequence of the IgG heavy chain (FIG. 42). SEQ ID NO:7 encodes the amino acid sequence set forth in SEQ ID NO:47, i.e., the amino acid sequence of the IgG light chain (FIG. 43).

[00287] Cells were transfected with either native Ig constructs (i.e., not optimized) or constructs containing SEQ ID NOS:6 and 7 (i.e., optimized). After transfection, IgG secretion was measured from the transfected cells and the kinetics of IgG synthesis are shown in FIG. 3. As shown in FIG. 3, both the non-optimized and optimized constructs expressed the heavy and light chains of the Ig to form IgG, but the optimized constructs resulted in quicker accumulation of IgG antibody. Cells transfected with the plasmid containing SEQ ID NOS:6 and 7 (i.e., optimized Ig sequences) showed greater production of fully assembled Ig molecules than did cells transfected with the plasmid containing non-optimized Ig sequences. Accordingly, the optimization or modification of the constructs substantially increased Ig expression. In other words, the constructs containing SEQ ID NOS:6 and 7 provided substantially higher expression of Ig as compared to the native constructs because of the optimization or modification used to create SEQ ID NOS:6 and 7. These data also demonstrated that the heavy and light chains of an Ig can be efficiently assembled in vivo from a plasmid system.

[00288] To further examine the constructs containing SEQ ID NOS:6 and 7, mice were administered plasmid containing the sequences set forth in SEQ ID NOS:6 and 7. In particular, the plasmid was administered using electroporation. After administration, induction of immune response (i.e., IgG level) in the immunized mice was evaluated by Western Blot (i.e., sera from the mice was used to detect the gp120 antigen). As shown in FIG. 4, mice administered the plasmid containing SEQ ID NOS:6 and 7 resulted in strong antibody production because binding of the antibody was observed in the Western blot analysis. Only one administration was required to observe this antibody production.

[00289] In summary, these data indicated that nucleic acid sequences encoding Ig heavy and light chains, when included in an expression vector such as pVAX1, resulted in the expression of assembled IgG (i.e., heavy and light chains came together to form an antibody that bound its antigen) in transfected cells and mice administered the expression vector. These data further indicated that optimization or modification of the nucleic acid sequences encoding the Ig heavy and light chains significantly increased Ig production.

Example 2

Materials and Methods for Examples 3-7

[00290] *Cells and Reagents.* 293T and TZM-B1 cells were maintained in Dulbecco's Modified Eagle's medium (DMEM; Gibco-Invitrogen, CA) supplemented with 10% fetal

bovine serum (FBS) and antibiotics and passaged upon confluence. Recombinant HIV-1 p24 and gp120 Env (rgp120) proteins were acquired from Protein Science Inc. and peroxidase-conjugated streptavidin from Jackson Laboratory. Cell lines and other reagents listed were obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH.

[00291] *Animals and Protein and Plasmid Administration and Delivery.* Female BALB/c mice (8 weeks of age) were purchased from Taconic Farms (Germantown, NY). For these administrations, 25 µg of plasmid DNA in 50µl volume (pVax1 or pHIV-1Env-Fab) was injected intramuscularly (IM) followed by EP mediated enhanced delivery by the MID-EP system (CELLECTRA®; Inovio Pharmaceuticals, Blue Bell, PA). Pulsing parameters for delivery were: 3 pulses of 0.5 Amp constant current, 1 second apart and 52 ms in length. Each animal received a single administration of either experimental or control plasmid formulations. For the protein immunization analysis, HIV-1 recombinant gp120 (rgp120) from the JRFL strain (purchased from Immune Technology Corp, NY) was used. In the protein immunization study, a single 25 µg dose of the rgp120 was mixed with TiterMax adjuvant and injected subcutaneously. Sera from the pHIV-1 Env Fab or rgp120-administered mice were collected at different time points depending on the particular analysis.

[00292] *Construction of HIV-1Env-Fab Plasmid DNA.* The HIV-1 Env-Fab sequences (VH and VL) from the anti-Env VRC01 human mAb were generated by use of synthetic oligonucleotides with several modifications. The heavy chain (VH-CH1) is encoded by the nucleic acid sequence set forth in SEQ ID NO:3, and the light chain (VL-CL) is encoded by the nucleic sequence set forth in SEQ ID NO:4 (FIGS. 9 and 10, respectively). In FIGS. 9 and 10, underlining and double underlining mark the HindIII (AAG CTT) and XhoI (CTC GAG) restriction enzyme sites used to clone the encoding nucleic acid sequences into pVAX1 while bold marks the start (ATG) and stop (TGA or TAA) codons. SEQ ID NO:3 encodes the amino acid sequence set forth in SEQ ID NO:48, i.e., the amino acid sequence of the VH-CH1 of HIV-1 Env-Fab (FIG. 44). SEQ ID NO:4 encodes the amino acid sequence set forth in SEQ ID NO:49, i.e., the amino acid sequence of the VL-CL of HIV-1 Env-Fab (FIG. 45).

[00293] An efficient IgE leader sequence (SEQ ID NO:65 nucleotide encoding SEQ ID NO:66 protein) was incorporated into the Env antigen gene sequences in order to improve expression. The resulting modified and enhanced HIV-1Env-Fab DNA immunogens were codon-and RNA-optimized, followed by cloning into the pVax1 expression vector by

GenScript (Piscataway, NJ), with subsequent large-scale production of these constructs. The VH and VL genes (SEQ ID NOs:3 and 4, respectively) were inserted between the BamHI and XhoI restriction sites. Purified plasmid DNA was then formulated in water for subsequent administration into mice. As a negative control plasmid, pIgG-E1M2, which generates an “irrelevant”/control Ig, was used.

[00294] *HIV-1 Env-Fab Expression and Immunoblot Analysis.* The 293T cell line was utilized for expression analysis using the non-liposomal FuGENE6 transfection reagent (Promega, WI), by methods as recommended by the manufacturer. Briefly, cells were seeded at 50-70% confluence ($1-3 \times 10^5$ cells/2 mL per well in 35 mm culture dish) 24 hours before subsequent transfection with 5 μ g of the pVax1 control or pHIV-1 Env-Fab. Supernatants were collected at various time points up to 70 hours and assessed for levels of specific Fab molecules by standard ELISA methods. Supernatants from pVax1 transfected cells were used as a negative control. In addition, 293T cells were transfected with a gene for the HIV gp160 Env protein.

[00295] Further confirmation of recognition of native HIV-1 Env protein by the generated Fab was performed by immunoblot analysis. For this study, rgp120, described above, underwent electrophoresis on 12% SDS-PAGE. The gel was blotted onto a nitrocellulose membrane (Millipore, Bedford, MA) and blocked with 5% w/v nonfat dry milk in PBS-T (0.05%). The nitrocellulose was then subsequently cut into individual strips for analysis. Sera from pHIV-1 Env Fab administered mice, collected 48 hours after administration, were diluted 1:100 in PBS and reacted with individual nitrocellulose strips for 1 hour. Subsequently, strips were washed 4 times with Tris-buffered saline-0.2% Tween, reacted with a peroxidase-coupled antiserum against mouse IgG (Jackson Laboratories, ME), and incubated with diaminobenzidine substrate (Sigma, St. Louis, MO), allowing for the visualization of proper binding of the generated HIV-1 Env Fab to gp120.

[00296] *Ig Binding Analysis – ELISA.* Confirmation of binding of DNA plasmid generated Fab or anti-rgp120 antibody to rgp120 by ELISA was evaluated. Ig binding assays were carried out with sera from individual animals administered either pHIV-1 Env Fab, pVax1 or rgp120 protein. Again, for this basic Ig immunoassay analysis, sera samples were collected 48 hours after the single DNA plasmid administration. Briefly, 96-well high-binding polystyrene plates (Corning, NY) plates were coated overnight at 4°C with clade B HIV MN rgp120 (2 μ g /mL), diluted in PBS. The following day, plates were washed with PBS-T (PBS, 0.05% Tween 20), blocked for 1 hour with 3% BSA in PBS-T, and incubated with 1:100 dilutions of serum from immunized and naïve mice for 1 hour at 37°C. Bound IgG was

detected using goat anti-mouse IgG-HRP (Research Diagnostics, NJ) at a dilution of 1:5,000. Bound enzyme were detected by the addition of the chromogen substrate solution TMB (R&D Systems), and read at 450 nm on a Biotek EL312e Bio-Kinetics reader. All sera samples were tested in duplicate. An additional immunoassay analysis was performed which quantified the Fab concentrations in sera from pHIV-1 Env Fab administered mice using a commercial IgG1 quantitation ELISA kit. This analysis was performed by manufacturer's specifications.

[00297] *Flow Cytometric Analysis (FACS).* For flow cytometry analyses (FACS), 293T cells were transfected with either a consensus clade A Env plasmid (pCon-Env-A) or an optimized clade A plasmid (pOpt-Env-A) expressing an Env from a primary viral isolate (Q23Env17). Transfection was performed by standard methods. After confirmation of transfection, cells were washed with ice-cold buffer A (PBS/0.1% BSA/0.01% NaN₃) and incubated for 20 min at 4°C with a 1:100 dilution of primary Ig (either purified VRC01 or sera from mice injected with either pHIV-1 Env Fab or control pIgG-E1M2 plasmid, collected 48 hours after plasmid administration). This was followed by washing and incubation for another 20 min with 50 µl of a 1:100 diluted fluorescent-labeled secondary Igs conjugated to phycoerythrin (PE). Cells were then washed and immediately analyzed on a flow cytometer (Becton Dickinson FACS). All incubations and washes were performed at 4°C with ice-cold buffer A. Cells were gated on singlets and live cells. To assess GFP expression GFP-positive cells was performed with a FACS-LSR instrument using CellQuest software (BD Bioscience). Data were analyzed with Flow Jo software.

[00298] *Single-Cycle HIV-1 Neutralization Assay.* Fab mediated HIV-1 neutralization analysis was measured with a TZM-BI (HeLa cell derived) based assay in which a reduction in luciferase gene expression as used as an endpoint for neutralization, following a single round of infection with Env-pseudotyped virus in the presence or absence of experimental or control sera. The TZM-BI cells were engineered to express CD4 and CCR5 and contained reporter genes for firefly luciferase. In this assay, sera from mice administered pVax1 only or pHIV-1Env Fab were diluted 1:50 in wells followed by addition of pseudotyped HIV-1 Bal26, Q23Env17, SF162S or ZM53M cell free virus, at a multiplicity of infection (MOI) of 0.01. Both Bal26 and SF162S are clade B tier 1 viruses, with this tier status indicating that the viruses had high or above average sensitivity to neutralization. Q23Env17 and ZM53M are clade A, Tier 1 and clade C, Tier 2 viruses, respectively. Tier 2 status indicated that the virus had average or moderate sensitivity to neutralization. Subsequently in this assay, 10⁴ TZM-BL cells were added to each well, incubated for 48 hours, lysed and followed by

subsequent addition of 100 µl of Bright-Glo substrate (Luciferase Assay System, Promega, WI), followed by luciferase quantitation using a luminometer. The readout of this assay was RLU (relative light units). The percentages of RLU reduction were calculated as $(1 - (\text{mean RLU of experimental samples} - \text{controls}) / \text{mean RLU from controls-no addition control wells})) \times 100$. HIV-1 neutralization was then expressed as percent decrease in RLU, which was indicative of the percent inhibition of infection.

Example 3

Generation of anti-HIV-1 Env-Fab Expressing Constructs

[00299] The cDNAs for both the VH and VL-Ig (immunoglobulin) chains coding sequences for the anti-HIV-1 Envelope broadly neutralizing human mAb VRC01 were obtained from the VRC (Vaccine Research Center, NIH) through the NIH AIDS Research and Reference Reagent Program and subsequently cloned into a pVax1 vector. Several modifications, as indicated in Example 2 above, were incorporated into the expression vectors in order to maximize and optimize the production of biologically active Ig molecules. Specifically, these modifications included codon and RNA optimization and stabilization, enhanced leader sequence utilization, plasmid production at high concentrations and facilitated in vivo plasmid delivery through EP. The constructs generated were placed under the control of an immediate early promoter from the human cytomegalovirus (CMV), which is important for proper and efficient expression in mammalian cells and tissues. The schematic maps of the construct used in this study are indicated in FIGS. 5A and 5B.

[00300] Additionally, anti-HIV-1 Env Fab was prepared from pHIV-Env-Fab and used to stain cells transfected with a plasmid encoding HIV Env. pVAX1 was used as a control. As shown in FIG. 11, immunofluorescence staining demonstrated that the vector pHIV-Env-Fab allowed for the preparation of anti-HIV-1 Env Fab because the anti-HIV-1 Env Fab stained the cells transfected with the plasmid encoding HIV Env. Accordingly, the anti-HIV-1 Env Fab was specific for binding to the HIV Env glycoprotein.

Example 4

Ig Production by Transfected Cells

[00301] To evaluate the expression of pHIV-1Env-Fab, the constructs were transfected into 293T cells. An ELISA immunoassay, using a consensus HIV-1 clade B gp120 protein,

confirmed the presence of the anti-HIV-1 Env-Fab in the supernatant from the transfected 293 T cells as early as 24 hours post transfection (FIG. 5C). High OD450nm values (i.e. ranging from approximately 0.5 to 0.8) were detected in cell extracts from 24 to 72 hours post transfection and subsequently reached a peak and plateau at 48 hours. These results confirmed the specificity of the anti-HIV-1 Env Fab for the HIV Env glycoprotein. Statistical analysis of the data presented in FIG. 5C was as follows: OD450nm values for sera from pHIV-1 Env-Fab injected mice were significant ($p < 0.05$, student t test) compared to pVax1 control from the 22 through 72 hour time points measurements.

Example 5

In Vivo Characterization of HIV-1 Env Fab

[00302] To demonstrate in vivo Fab production from the DNA plasmids, mice were administered the pHIV-1 Env Fab by the intramuscular route followed by enhanced delivery through EP. A single injection of the DNA plasmids was delivered and sera was collected at 12 hours and at days 1, 2, 3, 4 7 and 10 following administration. Sera (at a dilution of 1:100 dilution) were then subsequently evaluated for Ig/Fab levels by ELISA analysis, as shown in FIG. 6A. Data in FIG. 6A are presented (from individual mice in both the pVax1 and HIV-1 Env-Fab groups) as OD450nm, which was proportional to the level of Ig/Fab. These data demonstrated that the relative levels of Fab after single administration of pHIV-1 Env-Fab became detectable on day 1 and subsequently increased over time. For comparative purposes, a single administration / immunization of rgp120, as described above in Example 2, was made into Balb/C mice with subsequent sera collection and analysis (at 1:100 dilution) over time by ELISA in order to determine the extent and longevity of specific anti-gp120 antibody levels. FIG. 6B show the results.

[00303] In this protein delivery study, antigen specific Ig levels over background were only detectable 10 days after immunization. This was in contrast to the Fab levels elicited by pHIV-1 Env Fab administration (FIG. 6A) where OD450nm values attained at least 0.1 OD450nm units by day 1 post administration and plateaued at day 10 at levels between 0.28 and 0.35 OD units. Therefore, the delivery of pHIV-1 Env Fab resulted in a more rapid generation of specific Fab than conventional protein immunization. This finding underscored the potential clinical utility of this DNA plasmid delivery method for generation of biologically active Ig.

[00304] Additional analyses were performed to ensure the quality as well as quantity of the recombinant Fab produced by the DNA delivery technology. Specifically, immunoblot analysis was performed using electrophoresed and blotted recombinant HIV-1 gp120 protein and probed with sera from pHIV-1Env-Fab mice 48 hours post administration (FIG. 6C). The blot indicated a band appropriate for the molecular weight of gp120 protein confirming that it was functional and able to bind to gp120. Likewise, human Fab quantitation, by ELISA, was performed and presented as a function of time (i.e. days) after plasmid administration (FIG. 6D). The results indicate that the levels of Fab generated peaked at 2-3µg/ml. These results demonstrated the correct polypeptide assembly of the VH and VL chains of the generated VRC01 based Fab, as well as the ability to recognize and bind specifically to the HIV-1 Env protein.

[00305] Statistical analyses of the presented data in FIG. 6 are as follows. For data summarized in FIG. 6A, OD450nm values for the sera from the pHIV-1 Env-Fab injected mice were statistically elevated ($p < 0.05$, student t test) compared to the sera from pVax1 injected mice from the days 1 through 10 measurement time points. For data summarized in FIG. 6B, OD450nm values from the rpg120 group were significantly elevated ($p < 0.05$, student t test) compared to PBS control from the day 10 through 14 time point measurements. For data summarized in FIG. 6D, OD450nm values from pHIV-1 Env-Fab injected mice were significantly elevated ($p < 0.05$, student t test) from the day 2 through 10 time point measurements.

Example 6

Binding of Fab/Igs to Cells Expressing Different HIV-1 Env Proteins: FACS Based Analysis

[00306] Sera from the mice administered pHIV-1Env-Fab were also used to test binding of the generated Fab to different HIV- Env proteins transiently expressed by 293T cells. The native form of the VRC01-mAb was used as a positive control, to ensure proper expression and detection of the Env proteins on the surface of the cells. As indicated earlier, the “irrelevant/unrelated” Ig (Ig-E1M2) was used as a negative control. As demonstrated in FIGS. 7A and 7B, there was essentially only background staining by different Igs/Fabs to pVax1 (i.e. lacking the Env insert) transfected cells. However, for both the purified VRC01 mAb and sera from pHIV-1Env-Fab administered mice there was significant positive staining of transfected cells expressing either the consensus clade A Env plasmid (pCon-Env-A) as

well as an optimized clade C plasmid (pOpt-Env-A) expressing and Env from the primary HIV-1 isolate pQ23Env17. Moreover, sera from pIg-E1M2 administered mice failed to demonstrate staining of any of the HIV1 Env transfected cells above background levels. FACS analysis indicating these results are provided in FIG. 7A. A representative graph showing the data from the FACS analysis (i.e., FIG. 7A) for this experiment was provided in FIG. 7B.

[00307] Statistical analyses of data presented in FIG. 7B are as follows. There was no significant difference ($p < 0.05$, student t test) in specific binding between native VRC01 antibody and sera from pHIV-1 Env-Fab injected mice to the envelope glycoprotein generated by pCon-Env-A. However, binding of VRC01 antibody to the envelope glycoprotein generated by pOpt-Env-A was significantly higher ($p < 0.05$, student t test) than binding by sera from pHIV-1 Env-Fab injected mice.

Example 7

HIV Neutralizing Activity of Ig Produced by pHIV-1 Env Fab

[00308] Sera from mice administered pHIV-1Env-Fab were used to test binding of the HIV-Env Fab to HIV-1 Env proteins expressed in transiently transfected 293T cells. Sera was obtained from the mice 6 days after administration of pHIV-1Env-Fab. Specifically, cells were transfected with a plasmid from which HIV-1 Env from a Clade A, B or C strain was expressed. The clade A, B, and C strains were 92RW020, SF162, and ZM197. As shown in FIG. 12, sera from mice administered pHIV-1Env-Fab bound the HIV-1 Env from the clade A, B, and C HIV-1 strains, thereby indicating that the sera contained an antibody (i.e., HIV-Env Fab) that was cross-reactive with HIV-1 Env from multiple subtypes of HIV-1.

[00309] In order to assess the potential HIV-1 neutralizing activity of the HIV-Env Fab produced in this study, a luminescence based neutralization assay based using TZM-B1 target cells was performed. The TZM-B1 target cells were infected with the 4 different pseudotyped HIV viral isolates in the absence or presence of the experimental sera and control, as described in Example 2 above.

[00310] FIG. 8 depicts the neutralization curves for sera from pHIV-1 Env Fab injected mice against the HIV pseudotyped viruses. Specifically tested were the HIV-1 tier 1 viruses Bal26 and SF162S (both clade B), as well as Q23Env (clade A). In addition, sera were also tested against the HIV-1 clade C tier 2 virus ZM53M. The data are presented as percent

neutralization/inhibition of HIV infection. The hatched horizontal lines in the graphs indicated the 50% neutralization/inhibition level in the assay. A positive neutralization control mAb (data not shown) was utilized in this study to confirm the utility and validity of this assay method. Briefly, the positive control neutralizing mAb was able to inhibit infection of the all four of the viral pseudotypes by at least 50%.

[00311] Sera from the pHIV-1 Env Fab administered mice demonstrated an increase in HIV neutralizing activity over time following plasmid administration, with percent neutralization reaching at 50% by Day 2 for Bal25, Q23Env17 and SF162S. As well plateau percent neutralization for these 3 viruses was approximately 62, 60 and 70%, respectively. For the ZM53M, the 50% neutralization threshold was not reached until 3 days and plateau neutralization did not exceed 50%. This less robust neutralization profile, compared to the other 3 tested, was likely reflective of it being a less neutralizable Tier 2 virus. In sum, the Fab generated in this study was able to effectively neutralize a range of HIV isolates. Statistical analyses of data presented in FIG. 8 are as follows. Based on Kruskal-Wallis non-parametric analysis, only HIV neutralization levels for the ZM53M Clade C virus (FIG. 8D), induced by sera from pHIV-1 Env-Fab injected mice, was significantly different from the other viruses tested (FIGS. 8A, 8B, and 8C). This difference was in time (days) required to achieve 50% neutralization as well as in the maximally attained level of neutralization.

[00312] In summary of Examples 3-7, the sera concentration of VRC01 Fab in pHIV-1 Env Fab administered mice peaked at 2-3 μ g/mL at day 12 post-injection. This range was comparable to a number of monoclonal antibodies currently licensed by the FDA, indicating that our antibody approach produced significant and biologically relevant levels of antibodies in this small animal model. In particular, Ustekinumab (trade name: Stelara) and Golimumab (Simponi), two antibodies indicated for use against autoimmune diseases such as plaque psoriasis and arthritis, have mean \pm SD serum concentrations of 0.31 \pm 0.33 μ g/mL and 1.8 \pm 1.1 μ g/mL, respectively. Furthermore, the TNF inhibitor Adalimumab (Humira) has a mean rough serum concentration of around 6 μ g/mL. In this regard, the data described in Examples 4-8 demonstrated that delivery of DNA encoding the antibody to the organism resulted in the being assembled in vivo such that significant and biologically relevant levels of the antibody were present in the organism.

[00313] These data also demonstrated the ability to more rapidly produce Fabs in vivo, after a single EP enhanced administration of pHIV-1Env Fab, compared to Igs produced by conventional protein administration (FIGS. 6A and 6B). In addition, the ability to generate functional protective Ig-like molecules against difficult vaccine targets was addressed. To

date, inducing HIV-1 neutralizing antibodies following active vaccination has been incredibly difficult, and during primary infection, neutralizing antibodies do not develop until years after transmission. With this DNA plasmid approach, neutralization titers were observed within 1-2 days post delivery with peak neutralizing Fab sera concentrations ($3.31 \pm 0.13 \mu\text{g/mL}$) occurring one-week post-administration (FIG. 6D). This level of Ig was relatively similar to the $8.3 \mu\text{g/mL}$ concentration that has been demonstrated to provide complete protection from infection in a recent study. These data demonstrated the rapid induction of biologically active Ig fragments.

[00314] These data also showed the neutralizing antibody titer and the responses against HIV-1 primary isolates that were elicited by HIV-1 Env-Fab DNA administration. Sera were tested against a panel of different viral tier 1, and 2 viral isolates that represent examples from clades A, B and C. The results indicated generation of potent neutralizing activity against these viruses (FIG. 8).

[00315] Accordingly, this DNA plasmid-based method generated specific and biologically active Fab or Ig molecules in vivo, bypassed the need to use conventional antigen-based vaccination for antibody generation, and obviated the need to generate and purify Igs made in vitro.

Example 8

Construction of a Plasmid Encoding a Human Ig Antibody

[00316] As described above, a Fab was generated from the VRC01 antibody, namely HIV-Env Fab, which was generated in vivo upon administration of the encoding nucleic acid to the subject. To further extend these studies, nucleic acid sequence was created that encoded an IgG1 antibody derived from the VRC01 antibody. As shown in the schematic in FIG. 13, this nucleic acid sequence encoded IgG heavy and light chains separated by a furin cleavage site and a nucleic acid sequence encoding P2A peptide sequence. The P2A peptide sequence increases the efficiency of cleavage by the protease, thereby resulting in discrete polypeptides after cleavage.

[00317] The IgG heavy chain included the variable heavy (VH), constant heavy 1 (CH1), hinge, constant heavy 2 (CH2), and constant heavy 3 (CH3) regions. The IgG light chain included the variable light (VL) and constant light (CL) regions. This construct was placed under the control of a cytomegalovirus (CMV) promoter, for example, in the expression vector pVAX1. This construct resulted in the production of fully assembled IgG antibody (as

shown in FIG. 14) that was reactive gp120 (i.e., the antigen recognized by the VRC01 antibody). This fully assembled IgG is referred to herein as VRC01 IgG. The amino acid sequence of the VRC01 IgG (before cleavage by furin) is shown in FIG. 15 and is set forth in SEQ ID NO:5, which is encoded by the nucleic acid sequence encoding SEQ ID NO:64 (see FIG 62).

[00318] In particular, the amino acid sequence of the VRC01 IgG (before cleavage by furin; SEQ ID NO:5 and FIG. 15, which is encoded by nucleotide sequence SEQ ID NO:64) has the following structure: an immunoglobulin E1 (IgE1) signal peptide, variable heavy region (VH), constant heavy region 1 (CH1), hinge region, constant heavy region 2 (CH2), constant heavy region 3 (CH3), furin cleavage site, GSG linker, P2A peptide, IgE1 signal peptide, variable light region (VL), and constant light region (CL, specifically kappa). The sequence of each portion of the structure (all which are contained within SEQ ID NO:15 in the order described above and shown in FIG. 13) is provided below.

[00319] IgE1 Signal Peptide of VRC-1 IgG - MDWTWILFLVAAATRVHS (SEQ ID NO:8).

[00320] Variable Heavy Region of VRC01 IgG -

QVQLVQSGGQMKKPGESMRISCASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRG
GAVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGNCDYNWDFEH
WGRGTPVIVSSPSTKG (SEQ ID NO:9).

[00321] Constant Heavy region 1 (CH1) of VRC01 IgG -

PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSC (SEQ ID NO:10).

[00322] Hinge Region of VRC01 IgG EPKSCDKT HTCPCP (SEQ ID NO:11).

[00323] Constant Heavy Region 2 (CH2) of VRC01 IgG -

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK (SEQ
ID NO:12).

[00324] Constant Heavy Region 3 (CH3) of VRC01 IgG -

GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID
NO:13)

[00325] Furin Cleavage Site of VRC01 IgG - RGRKRRS (SEQ ID NO:14).

[00326] GSG Linker and P2A Peptide of VRC01 IgG - GSGATNFSLLKQAGDVEENPGP
(SEQ ID NO:15).

[00327] IgE1 Signal Peptide of VRC01 IgG - MDWTWILFLVAAATRVHS (SEQ ID NO:8).

[00328] Variable Light Region (VL) of VRC01 IgG -
EIVLTQSPGTLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDR
FSGSRWGPDYNTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKR (SEQ ID NO:16).

[00329] Constant Light Region (CL, kappa) of VRC01 IgG -
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGEC (SEQ ID NO:17).

Example 9

HIV-1 VRC01 IgG Encoded by Two Plasmids

[00330] As described above in Examples 2-8, a Fab (each chain expressed from a separate plasmid) was generated from the VRC01 antibody, namely HIV-Env Fab, and an IgG (expressed from a single plasmid) was generated from the VRC01 antibody, namely VRC01 IgG. To further extend these studies, an IgG was generated from the VRC01 antibody, in which the heavy chain (i.e., variable heavy region (VH), constant heavy region 1 (CH1), hinge region, constant heavy region 2 (CH2), and constant heavy region 3 (CH3)) and the light chain (i.e., variable light region (VL) and constant light region (CL)) were encoded by separate constructs (FIGS. 50 and 51). This IgG is referred to herein as HIV-1 VRC01 IgG.

[00331] Each construct also included a leader sequence for optimizing secretion of the antibody once generated in vivo. Each construct was cloned into the BamHI and XhoI sites of the pVAX1 vector, thereby placing the construct under the control of a cytomegalovirus (CMV) promoter (FIGS. 50 and 51). Accordingly, to form or generate the VRC01 IgG in vivo a mixture of plasmids has to be administered to the subject, namely a plasmid containing the construct encoding the heavy chain and a plasmid containing the construct encoding the light chain.

[00332] Additionally, each construct was further optimized. Optimization included addition of a kozak sequence (GCC ACC) and codon optimization. The nucleic acid sequence encoding the IgG1 heavy chain of the HIV-1 VRC01 IgG is set forth in SEQ ID NO:54 and FIG. 52. In FIG. 52, underlining and double underling mark the BamHI (GGA TCC) and XhoI (CTC GAG) restriction enzyme sites used to clone the nucleic acid sequence into the pVAX1 vector while bold marks the start (ATG) and stop (TGA TAA) codons. SEQ

ID NO:54 encodes the amino acid sequence set forth in SEQ ID NO:55 and FIG. 53, i.e., the amino acid sequence of the IgG1 heavy chain of the HIV-1 VRC01 IgG.

[00333] The nucleic acid sequence encoding the IgG light chain of the HIV-1 VRC01 IgG is set forth in SEQ ID NO:56 and FIG. 54. In FIG. 54, underlining and double underlining mark the BamHI (GGA TCC) and XhoI (CTC GAG) restriction enzyme sites used to clone the nucleic acid sequence into the pVAX1 vector while bold marks the start (ATG) and stop (TGA TAA) codons. SEQ ID NO:56 encodes the amino acid sequence set forth in SEQ ID NO:57 and FIG. 55, i.e., the amino acid sequence of the IgG light chain of the HIV-1 VRC01 IgG.

Example 10

HIV-1 Env-PG9 Ig

[00334] In addition to VRC01 IgG, another construct was created that encoded IgG that was reactive to HIV-1 Env. This construct was HIV-1 Env-PG9, which was optimized and cloned into an expression vector (FIGS. 16A and 16B). Optimization included introduction of a kozak sequence (e.g., GCC ACC), a leader sequence, and codon optimization. Creation of the expression vector containing the nucleic acid sequence encoding HIV-1 Env-PG9 Ig was confirmed by restriction enzyme digestion as shown in FIG. 16C. In FIG. 16C, lane 1 was undigested expression vector, lane 2 was the expression vector digested with BamHI and XhoI, and lane M was the Marker.

[00335] The nucleic acid sequence encoding HIV-1 Env-PG9 Ig is set forth in SEQ ID NO:63 and FIG. 61. In FIG. 61, underlining and double underlining mark the BamHI (GGA TCC) and XhoI (CTC GAG) restriction enzyme sites used to clone the nucleic acid sequence into the pVAX1 vector while bold marks the start (ATG) and stop (TGA TAA) codons. SEQ ID NO:63 encodes the amino acid sequence set forth in SEQ ID NO:2 and FIG. 18, i.e., the amino acid sequence of HIV-1 ENv-PG9 Ig (before cleavage by furin).

[00336] In this amino acid sequence, a signal peptide is linked by peptide bond to each of the heavy and light chains to improve secretion of the antibody generated in vivo. Additionally, a nucleic acid sequence encoding the P2A peptide is located between the nucleic acid sequences encoding the heavy and light chains to allow for more efficient cleavage of the translated polypeptide into separate polypeptides containing the heavy or light chain.

[00337] In particular, the amino acid sequence of the HIV-1 Env-PG9 Ig (before cleavage by furin; SEQ ID NO:2 and FIG. 18) has the following structure: human IgG heavy chain signal peptide, variable heavy region (VH), constant heavy region 1 (CH1), hinge region, constant heavy region 2 (CH2), constant heavy region 3 (CH3), furin cleavage site, GSG linker, P2A peptide, human lambda light chain signal peptide, variable light region (VL), and constant light region (CL, specifically lambda). The sequence of each portion of the structure (all which are contained within SEQ ID NO:2 in the order described above) is provided below.

[00338] Human IgG Heavy Chain Signal Peptide of HIV-1 Env-PG9 Ig –
MDWTWRILFLVAAATGTHA (SEQ ID NO:18).

[00339] Variable Heavy Region of HIV-1 Env-PG9 Ig –
EFGLSWVFLVAFLRGVQCQRLVESGGGVVQPGSSRLSCAASGFDFSRQGMHWVR
QAPGQGLEWVAFIKYDGSEKYHADSVWGRLSISRDNKDTLYLQMNSLRVEDTATY
FCVREAGGPDYRNGYNYDFYDGYNYHYMDVWVGKGTTVTVSS (SEQ ID NO:19).

[00340] Constant Heavy region 1 (CH1) of HIV-1 Env-PG9 Ig –
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV (SEQ ID NO:20).

[00341] Hinge Region of HIV-1 Env-PG9 Ig – EPKSCDKTHTCPPCP (SEQ ID NO:21).

[00342] Constant Heavy Region 2 (CH2) of HIV-1 Env-PG9 Ig –
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK (SEQ
ID NO:22).

[00343] Constant Heavy Region 3 (CH3) of HIV-1 Env-PG9 Ig –
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPV
LDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID
NO:23).

[00344] Furin Cleavage Site of HIV-1 Env-PG9 Ig – RGRKRRS (SEQ ID NO:24).

[00345] GSG Linker and P2A Peptide of HIV-1 Env-PG9 Ig –
GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:25).

[00346] Human Lambda Light Chain Signal Peptide of HIV-1 Env-PG9 Ig –
MAWTPLFLFLTCCPGGSNS (SEQ ID NO:26).

[00347] Variable Light Region (VL) of HIV-1 Env-PG9 Ig –
QSALTQPASVSGSPGQSITISCNGTSDNDVGGYESVSWYQQHPGKAPKVVIYDVSKRP

SGVSNRFSGSKSGNTASLTISGLQAEDEGDYYCKSLTSTRRRRVFGTGTKLTVL (SEQ ID NO:27).

[00348] Constant Light Region (CL, lambda) of HIV-1 Env-PG9 Ig –
GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTT
PSKQSNKYYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:28).

Example 11

HIV-1 PG9 Single Chain Fab (scFab)

[00349] In addition to HIV-1 Env-PG9 Ig described above, a single chain Fab (i.e., VH/CH1 and VL/CL encoded by a nucleic sequence that is transcribed into a single transcript and translated into a single polypeptide) was created based upon the PG9 antibody (referred to herein as HIV-1 PG9 scFab). The nucleic acid sequence encoding HIV-1 PG9 scFab is set forth in SEQ ID NO:50 and FIG. 46. In FIG. 46, underlining and double underlining mark the BamHI (GGA TCC) and XhoI (CTC GAG) that were used to clone this nucleic acid sequence into the pVAX1 vector while bold marks the start (ATG) and stop (TGA TAA) codons. The nucleic acid sequence set forth in SEQ ID NO:50 was an optimized nucleic acid sequence, i.e., inclusion of a kozak sequence (GCC ACC), codon optimization, and leader sequence. The leader sequence was located at the 5' end of the construct, i.e., preceding the single chain Fab, and thus, the signal peptide encoded by the linker sequence was linked by a peptide bond to the amino terminus of the single chain Fab. The nucleic acid sequence set forth in SEQ ID NO:50 also included a linker sequence that was positioned between the nucleic acid sequence encoding the VH/CH1 and the nucleic acid sequence encoding the VL/CL. Accordingly, in the polypeptide encoded by SEQ ID NO:50, the amino acid sequence encoded by the linker sequence kept the VH/CH1 and VL/CL together. SEQ ID NO:50 encoded the amino acid sequence set forth in SEQ ID NO:51 and FIG. 47, i.e., the amino acid sequence of the HIV-1 PG9 scFab.

Example 12

HIV-1 Env-4E10 Ig

[00350] In addition to VRC01 IgG and HIV-1 Env-PG9 Ig, another construct was created that encoded IgG that was reactive to HIV-1 Env. This construct was HIV-1 Env-4E10,

which was optimized and cloned into an expression vector (FIGS. 17A and 17B).

Optimization included introduction of a kozak sequence (e.g., GCC ACC), a leader sequence, and codon optimization. Creation of the expression vector containing the nucleic acid sequence encoding HIV-1 Env-4E10 Ig was confirmed by restriction enzyme digestion as shown in FIG. 17C. In FIG. 17C, lane 1 was undigested expression vector, lane 2 was the expression vector digested with BamHI and XhoI, and lane M was the Marker.

[00351] The nucleic acid sequence encoding HIV-1 Env-4E10 Ig is set forth in SEQ ID NO:62 and FIG. 60. In FIG. 60, underlining and double underlining mark the BamHI (GGA TCC) and XhoI (CTC GAG) restriction enzyme sites used to clone the nucleic acid sequence into the pVAX1 vector while bold marks the start (ATG) and stop (TGA TAA) codons. SEQ ID NO:62 encodes the amino acid sequence set forth in SEQ ID NO:1 and FIG. 19, i.e., the amino acid sequence of HIV-1 ENv-4E10 Ig (before cleavage by furin).

[00352] In this amino acid sequence, a signal peptide is linked by peptide bond to each of the heavy and light chains to improve secretion of the antibody generated in vivo. Additionally, a nucleic acid sequence encoding the P2A peptide is located between the nucleic acid sequences encoding the heavy and light chains to allow for more efficient cleavage of the translated polypeptide into separate polypeptides containing the heavy or light chain.

[00353] In particular, the amino acid sequence of the HIV-1 Env-4E10 Ig (before cleavage by furin; SEQ ID NO:1 and FIG. 19) has the following structure: human IgG heavy chain signal peptide, variable heavy region (VH), constant heavy region 1 (CH1), hinge region, constant heavy region 2 (CH2), constant heavy region 3 (CH3), furin cleavage site, GSG linker, P2A peptide, human kappa light chain signal peptide, variable light region (VL), and constant light region (CL, specifically kappa). The sequence of each portion of the structure (all which are contained within SEQ ID NO:1 in the order described above) is provided below.

[00354] Human IgG Heavy Chain Signal Peptide of HIV-1 Env-4E10 Ig –
MDWTWRILFLVAAATGTHA (SEQ ID NO:29).

[00355] Variable Heavy Region of HIV-1 Env-4E10 Ig –
QVQLVQSGAEVKRPGSSVTVSCKASGGSFSTYALSWVRQAPGRGLEWMGGVIPLLT
ITNYAPRFQGRITITADRSTSTAYLELNSLRPEDTAVYYCAREGTTGWGWL GKPIGAF
AHWGQGTLVTVSS (SEQ ID NO:30).

- [00356] Constant Heavy region 1 (CH1) of HIV-1 Env-4E10 Ig –
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKV (SEQ ID NO:31).
- [00357] Hinge Region of HIV-1 Env-4E10 Ig – EPKSCDKTHTCPPCP (SEQ ID NO:32).
- [00358] Constant Heavy Region 2 (CH2) of HIV-1 Env-4E10 Ig –
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK (SEQ
ID NO:33).
- [00359] Constant Heavy Region 3 (CH3) of HIV-1 Env-4E10 Ig –
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPV
LDSGSGFLYSLKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID
NO:34).
- [00360] Furin Cleavage Site of HIV-1 Env-4E10 Ig – RGRKRRS (SEQ ID NO:35).
- [00361] GSG Linker and P2A Peptide of HIV-1 Env-4E10 Ig –
GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:36).
- [00362] Human Kappa Light Chain Signal Peptide of HIV-1 Env-4E10 Ig –
MVLQTQVFISLLLWISGAYG (SEQ ID NO:37).
- [00363] Variable Light Region (VL) of HIV-1 Env-4E10 Ig –
EIVLTQSPGTQSLSPGERATLSCRASQSVGNNKLAWYQQRPGQAPRLLIYGASSRPSG
VADRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGQSLSTFGQGKVE (SEQ ID
NO:38).
- [00364] Constant Light Region (CL, kappa) of HIV-1 Env-4E10 Ig –
KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV
TEQDSKDYSLSSLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGE (SEQ ID
NO:39).

Example 13

HIV-1 4E10 ScFab

[00365] In addition to HIV-1 Env-PG9 Ig described above, a single chain Fab (i.e., VH/CH1 and VL/CL encoded by a nucleic acid sequence that is transcribed into a single transcript and translated into a single polypeptide) was created based upon the 4E10 antibody (referred to herein as HIV-1 4E10 scFab). The nucleic acid sequence encoding HIV-1 4E10 scFab is set forth in SEQ ID NO:52 and FIG. 48. In FIG. 48, underlining and double underlining

mark the BamHI (GGA TCC) and XhoI (CTC GAG) that were used to clone this nucleic acid sequence into the pVAX1 vector while bold marks the start (ATG) and stop (TGA TAA) codons. The nucleic acid sequence set forth in SEQ ID NO:52 was an optimized nucleic acid sequence, i.e., inclusion of a kozak sequence (GCC ACC), codon optimization, and leader sequence. The leader sequence was located at the 5' end of the construct, i.e., preceding the single chain Fab, and thus, the signal peptide encoded by the linker sequence was linked by a peptide bond to the amino terminus of the single chain Fab. The nucleic acid sequence set forth in SEQ ID NO:52 also included a linker sequence that was positioned between the nucleic acid sequence encoding the VH/CH1 and the nucleic acid sequence encoding the VL/CL. Accordingly, in the polypeptide encoded by SEQ ID NO:52, the amino acid sequence encoded by the linker sequence kept the VH/CH1 and VL/CL together. SEQ ID NO:52 encoded the amino acid sequence set forth in SEQ ID NO:53 and FIG. 49, i.e., the amino acid sequence of the HIV-1 4E10 scFab.

Example 14

CHIKV-Env-Fab

[00366] As described above, an Fab reactive to HIV-1 Env was assembled or generated in vivo upon delivery of the nucleic acid sequences encoding the heavy (VH-CH1) and light (VL-CL) chains of HIV-1 Env Fab to the cell or mouse. To determine if Fabs reactive to other antigens could be generated in vivo upon delivery of encoding nucleic acid sequences to the cell or subject, constructs were created that encoded the heavy (VH-CH1) and light (VL-CL, lambda type) chains of an antibody reactive to an envelope protein (Env) of the Chikungunya virus (CHIKV). Each construct included a leader sequence and a kozak sequence as shown in FIGS. 20A, 20B, and 21. The constructs encoding the VH-CH1 and VL-CL were cloned into an expression vector and thus, placed under the control of the cytomegalovirus (CMV) promoter (FIG. 21). The expression vectors containing the constructs encoding the VH-CH1 and VL-CL were known as CHIKV-H and CHIKV-L, respectively. Together, a mixture of the CHIKV-H and CHIKV-L vectors was known as pCHIKV-Env-Fab and this generated CHIKV-Env-Fab in vivo (i.e., upon introduction into a cell or subject). In other words, both vectors were required to generate the CHIKV-Env-Fab in vivo as described in more detail below.

[00367] The constructs were also optimized for expression. In particular, a leader sequence was included in each construct to increase the efficiency of secretion of the CHIKV-Env-Fab

upon generation of the CHIKV-Env-Fab in vivo. Each construct was also codon optimized and included a kozak sequence (GCC ACC). The nucleic acid sequence encoding the heavy chain (VH-CH1) of the CHIKV-Env-Fab is set forth in SEQ ID NO:58 and FIG. 56. In FIG. 56, underlining and double underling mark the BamHI (GGA TCC) and XhoI (CTC GAG) restriction enzyme sites used to clone the nucleic acid sequence into the pVAX1 vector while bold marks the start (ATG) and stop (TGA TAA) codons. SEQ ID NO:58 encodes the amino acid sequence set forth in SEQ ID NO:59 and FIG. 57, i.e., the amino acid sequence of the heavy chain (VH-CH1) of the CHIKV-Env-Fab.

[00368] The nucleic acid sequence encoding the light chain (VL-CL) of the CHIKV-Env-Fab is set forth in SEQ ID NO:60 and FIG. 58. In FIG. 58, underlining and double underling mark the BamHI (GGA TCC) and XhoI (CTC GAG) restriction enzyme sites used to clone the nucleic acid sequence into the pVAX1 vector while bold marks the start (ATG) and stop (TGA TAA) codons. SEQ ID NO:60 encodes the amino acid sequence set forth in SEQ ID NO:61 and FIG. 59, i.e., the amino acid sequence of the light chain (VL-CL) of the CHIKV-Env-Fab.

[00369] To measure the temporal kinetics of CHIKV-Env-Fab generation in vivo, cells were transfected with pVAX1, CHIKV-H, CHIKV-L, or pCHIKV-Env-Fab. After transfection, ELISA was used to measure the level of CHIKV-Env-Fab generation over time. As shown in FIG. 22, cells transfected with pVAX1, CHIKV-H, or CHIKV-L did not produce antibody that was reactive with the CHIKV Env antigen. In contrast, cells transfected with pCHIKV-Env-Fab produced antibody (i.e., CHIKV-Env-Fab, also known as CHIKV-Fab) that was reactive to the CHIKV Env antigen. Accordingly, these data indicated that delivery of nucleic acid sequences encoding the heavy (VH-CH1) and light (VL-CL) of the CHIKV-Env-Fab resulted in the generation of a Fab that bound or was reactive to the CHIKV-Env antigen.

[00370] Additionally, CHIKV-Env-Fab was used in a Western blot of lysates obtained from cells transfected with pCHIKV-Env, which is a plasmid that encodes the CHIKV-Env antigen. As shown in the FIG. 23, the CHIKV-Env antigen was detected via the CHIKV-Env-Fab, indicating that this Fab bound to the antigen.

[00371] To further examine the generation or assembly of CHIKV-Env-Fab in vivo, mice were administered pCHIKV-Env-Fab (i.e., 12.5 μ g CHIKV-H and 12.5 μ g CHIKV-L). Additionally, a second, third, and fourth group of mice were administered 25 μ g pVAX1, CHIKV-H, and CHIKV-L, respectively, and served as controls. Specifically, the plasmids

were administered to the respective groups of mice on day 0 after obtaining a pre-bleed sample. Bleeds were taken on day 1, day 2, day 3, day 5, day 7, and day 10 (FIG. 24). ELISA measurements were performed on these bleeds to determine the levels of antibody reactive to the CHIKV-Env antigen. As shown in FIG. 25, mice administered pCHIKV-Env-Fab resulted in the generation of antibody (i.e., CHIKV-Env-Fab) that was reactive to the CHIKV-Env antigen. Mice administered pVAX1, CHIKV-H or CHIKV-L did not generate antibodies having significant reactivity with the CHIKV-Env antigen. Accordingly, these data further demonstrated that upon delivery of nucleic acid sequences encoding the heavy (VH-CH1) and light (VL-CL) chains of the CHIKV-Env-Fab, this Fab was generated in vivo (i.e., in the mice) and was reactive to its antigen (i.e., CHIKV-Env), thereby demonstrating that the Fab was correctly assembled in vivo.

[00372] To determine if the CHIKV-Env-Fab could protect against CHIKV infection, C57BL/6 mice (2-3 weeks of age; about 20-25 grams in weight) were administered on day 0 pCHIKV-Env-Fab (50 µg) or pVAX1. 6 hours after administration of pCHIKV-Env-Fab, each mouse was inoculated with 7 log 10 PFU in a total volume of 25 µl by an intranasal route. Each subsequent day, body weight was determined for each mouse and a mouse was sacrificed if weight loss was more than 30%.

[00373] As shown in FIG. 26, about 75% of the mice administered pCHIKV-Env-Fab survived CHIKV infection as of day 14 of study while by day 14, all of mice that were administered pVAX1 were dead. Additionally, mice administered pCHIKV-Env-Fab were associated with lower levels of the cytokines TNF- α and IL-6 as compared to the mice administered pVAX1 (FIGS. 27 and 28). TNF- α and IL-6 levels were measured in sera obtained from the mice. These surviving mice exhibited no signs of pathology, body weight loss, and had lower levels of the cytokines TNF- α and IL-6. Accordingly, these data indicated that the pCHIKV-Env-Fab administration protected the mice from CHIKV infection and promoted survival of CHIKV infection. In other words, in vivo generation of CHIKV-Env-Fab in the mice protected against and promoted survival of CHIKV infection.

Example 15

Anti-Her-2 Fab

[00374] As described above, an Fab (i.e., VH/CH1 and VL/CL) reactive to HIV-1 Env or CHIKV Env was assembled or generated in vivo upon delivery of the nucleic acid sequences encoding the heavy (VH-CH1) and light (VL-CL) chains of the HIV-1Env Fab or CHIKV

Env-Fab to the cell or mouse. To determine if Fabs reactive to a self antigen (i.e., an antigen endogenous to the subject being administered the nucleic acid sequences encoding the Fab) could be generated in vivo upon delivery of encoding nucleic acid sequences to the cell or subject, constructs were created that encoded the heavy (VH-CH1) and light (VL-CL, kappa type) chains of an antibody reactive to human epidermal growth factor receptor 2 (Her-2; also known as Erb2). Each construct included a leader sequence and a kozak sequence (GCC ACC), which preceded the nucleic acid sequence encoding the VH-CH1 or VL-CL of the anti-Her-2 Fab as shown in FIGS. 28, 30, and 31. Accordingly, these constructs were optimized due to the introduction of the leader sequence and kozak sequence, and were further optimized for codon usage.

[00375] The constructs encoding the VH-CH1 and VL-CL were cloned into the pVAX1 expression vector, namely between the BamHI and XhoI restriction sites and thus, were placed under the control of the cytomegalovirus (CMV) promoter. In particular, the constructs encoding the VH-CH1 and VL-CL were cloned into two separate pVAX1 vectors, and thus, the resulting two plasmids were required to generate the anti-Her-2 Fab in vivo.

[00376] The nucleic acid sequence encoding the VH-CH1 of the anti-Her-2 Fab is set forth in SEQ ID NO:40 and FIG. 32. In FIG. 32, underlining and double underlining mark the BamHI (GGA TCC) and XhoI (CTC GAG) restriction enzyme sites, respectively, used to clone the nucleic acid sequence into the pVAX1 vector while bold marks the start (ATG) and stop (TGA TAA) codons. SEQ ID NO:40 encodes the amino acid sequence set forth in SEQ ID NO:41, i.e., the amino acid sequence of the VH-CH1 of the anti-Her-2 Fab (FIGS. 32 and 33).

[00377] The nucleic acid sequence encoding the VL-CL of the anti-Her-2 Fab is set forth in SEQ ID NO:42 and FIG. 34. In FIG. 34, underlining and double underlining mark the BamHI (GGA TCC) and XhoI (CTC GAG) restriction enzyme sites, respectively, used to clone the nucleic acid sequence into the pVAX1 vector while bold marks the start (ATG) and stop (TGA TAA) codons. SEQ ID NO:42 encodes the amino acid sequence set forth in SEQ ID NO:43, i.e., the amino acid sequence of the VL-CL of the anti-Her-2 Fab (FIGS. 34 and 35).

[00378] To determine whether a mixture of the plasmids encoding the VH-CH1 and VL-CL of the anti-Her-2 Fab generated the anti-Her-2 Fab in vivo, 293T cells were transfected with a mixture of the plasmids encoding the heavy (VH-CH1) and light (VL and CL) of anti-Her-2 Fab or pVAX1. After transfection, total IgG concentration was measured as shown in FIG. 36. In FIG. 36, error bars represented the standard deviation. These data indicated that the

anti-Her-2 Fab was generated in vivo upon introduction of the two plasmids, each encoding the VH-CH1 or VL-CL of anti-Her-2 Fab.

Example 16

Anti-Dengue Virus Human IgG

[00379] A single plasmid system was created to generate an anti-Dengue virus (DENV) human IgG antibody in vivo. Specifically, a construct was generated as shown in the schematic of FIG. 37. Specifically, a leader sequence was placed upstream of the nucleic acid sequence encoding the IgG heavy chain (i.e., variable heavy region (VH), constant heavy region 1 (CH1), hinge region, constant heavy region 2 (CH2), and constant heavy region 3 (CH3)). In turn, a sequence encoding a protease cleavage site was placed downstream of the nucleic acid sequence encoding the IgG heavy chain. A nucleic acid sequence encoding the IgG light chain (i.e., variable light region (VL) and constant light region (CL)) was located after the sequence encoding the protease cleavage site (i.e., furin cleavage site). The signal peptides encoded by this construct were cognate signal peptides, thereby providing proper secretion of the antibody upon expression. Additionally, upon expression a single transcript is translated into a single polypeptide, which is then processed by the protease into the polypeptides corresponding to the heavy and light chains of the anti-DENV human IgG. These heavy and light chain polypeptides then assemble into a functional anti-DENV human IgG, i.e., an antibody that binds its cognate antigen.

[00380] This construct was cloned into the expression vector pVAX1 (namely the BamHI and XhoI sites), thereby placing it under the control of a promoter. This construct encoding the anti-Dengue virus human IgG has the nucleic acid sequence set forth in SEQ ID NO:44 (FIG. 38), which has been optimized for expression. In FIG. 38, underlining and double underlining mark the BamHI (GGA TCC) and XhoI (CTC GAG) restriction enzyme sites used to clone the construct into the pVAX 1 vector while bolds marks the start (ATG) and stop (TGA TAA) codons. Optimization included inclusion of a kozak sequence (GCC ACC) and codon optimization. SEQ ID NO:44 encodes the amino acid sequence set forth in SEQ ID NO:45 and FIG. 39, i.e., the amino acid sequence of the anti-DENV human IgG before cleavage by the protease to separate the heavy and light chains into two separate polypeptides.

[00381] The plasmid containing the nucleic acid sequence encoding the anti-Dengue virus human IgG was administered to mice to determine if the anti-Dengue virus human IgG was

generated in vivo (i.e., in the mice). After administration of the plasmid, sera were obtained from the mice and analyzed via ELISA to determine whether the sera contained antibody that was reactive to the Dengue E protein from four Dengue virus serotypes, namely DENV-1, DENV-2, DENV-3, and DENV-4. As shown in FIG. 40, sera from mice administered the plasmid containing the nucleic acid sequence encoding the anti-DENV human IgG was reactive to the DENV E protein from serotypes DENV-1, -2, -3, and -4. An isotypic antibody was used as a positive control. Accordingly, these data indicated that upon introduction of the plasmid into mice, the nucleic acid sequence encoding the anti-DENV human IgG was transcribed and translated into a polypeptide that was processed to yield polypeptides containing the heavy and light chains of the anti-DENV human IgG. These polypeptides assembled into the anti-DENV human IgG, thereby providing a functional antibody that bound or was reactive to the DENV E protein.

To further examine the generation of anti-DENV human IgG in vivo by administration of a single plasmid, mice were administered via injection the plasmid containing the nucleic acid sequence encoding the anti-DENV human IgG. Specifically, mice were administered 50 µg or 100 µg of the plasmid and 5 mice were in each group. On day 3 and day 6 post-injection, the mice were examined for seroconversion. As shown in FIG. 41, mice from both groups were seropositive for anti-DENV IgG antibodies. In particular, the mice administered 50 µg of the plasmid had about 110 ng/mL of human IgG and the mice administered 100 µg of the plasmid had about 170 ng/mL of human IgG. Accordingly, these data further demonstrated the generation of anti-DENV human IgG in vivo after administration of a plasmid encoding the same. These data also demonstrated that anti-DENV human IgG antibody production occurred in less than 1 week, thereby allowing for rapid production of anti-DENV human IgG.

[00382] It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the invention, which is defined solely by the appended claims and their equivalents.

[00383] Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, compositions, formulations, or methods of use of the invention, may be made without departing from the spirit and scope thereof.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of generating a synthetic antibody in a subject, the method comprising administering to the subject a composition comprising a recombinant nucleic acid sequence encoding an antibody or fragment thereof, wherein the recombinant nucleic acid sequence is expressed in the subject to generate the synthetic antibody.
2. The method of claim 1, wherein the antibody comprises a heavy chain polypeptide, or fragment thereof, and a light chain polypeptide, or fragment thereof.
3. The method of claim 2, wherein the heavy chain polypeptide, or fragment thereof, is encoded by a first nucleic acid sequence and the light chain polypeptide, or fragment thereof, is encoded by a second nucleic acid sequence.
4. The method of claim 3, wherein the recombinant nucleic acid sequence comprises the first nucleic acid sequence and the second nucleic acid sequence.
5. The method of claim 4, wherein the recombinant nucleic acid sequence further comprises a promoter for expressing the first nucleic acid sequence and the second nucleic acid sequence as a single transcript in the subject.
6. The method of claim 5, wherein the promoter is a cytomegalovirus (CMV) promoter.
7. The method of claim 5, wherein the recombinant nucleic acid sequence further comprises a third nucleic acid sequence encoding a protease cleavage site, wherein the third nucleic acid sequence is located between the first nucleic acid sequence and second nucleic acid sequence.
8. The method of claim 7, wherein the protease of the subject recognizes and cleaves the protease cleavage site.
9. The method of claim 8, wherein the recombinant nucleic acid sequence is expressed in the subject to generate an antibody polypeptide sequence, wherein the antibody polypeptide sequence comprises the heavy chain polypeptide, or fragment thereof, the protease cleavage site, and the light chain polypeptide, or fragment thereof, wherein the protease produced by the subject recognizes and cleaves the protease cleavage site of the antibody polypeptide sequence thereby generating a cleaved heavy chain polypeptide and a cleaved light chain polypeptide, wherein the synthetic antibody is generated by the cleaved heavy chain polypeptide and the cleaved light chain polypeptide.
10. The method of claim 4, wherein the recombinant nucleic acid sequence comprises a first promoter for expressing the first nucleic acid sequence as a first transcript and a second promoter for expressing the second nucleic acid sequence as a second transcript, wherein the first

transcript is translated to a first polypeptide and the second transcript is translated into a second polypeptide, wherein the synthetic antibody is generated by the first and second polypeptide.

11. The method of claim 10, wherein the first promoter and the second promoter are the same.

12. The method of claim 11, wherein the promoter is a cytomegalovirus (CMV) promoter.

13. The method of claim 2, wherein the heavy chain polypeptide comprises a variable heavy region and a constant heavy region 1.

14. The method of claim 2, wherein the heavy chain polypeptide comprises a variable heavy region, a constant heavy region 1, a hinge region, a constant heavy region 2 and a constant heavy region 3.

15. The method of claim 2, wherein the light chain polypeptide comprises a variable light region and a constant light region.

16. The method according to any one of claims 1 to 15, wherein the recombinant nucleic acid sequence further comprises a Kozak sequence.

17. The method according to any one of claims 1 to 16, wherein the recombinant nucleic acid sequence further comprises an immunoglobulin (Ig) signal peptide.

18. The method of claim 17, wherein the Ig signal peptide comprises an IgE or IgG signal peptide.

19. The method of claim 1, wherein the recombinant nucleic acid sequence comprises a nucleic acid sequence encoding at least one amino acid sequence of SEQ ID NOs:1, 2, 5, 41, 43, 45, 46, 47, 48, 49, 51, 53, 55, 57, 59, and 61.

20. The method of claim 1, wherein the recombinant nucleic acid sequence comprises at least one nucleic acid sequence of SEQ ID NOs:3, 4, 6, 7, 40, 42, 44, 50, 52, 54, 56, 58, 60, 62 and 63.

21. A method of generating a synthetic antibody in a subject, the method comprising administering to the subject a composition comprising a first recombinant nucleic acid sequence encoding a heavy chain polypeptide, or fragment thereof, and a second recombinant nucleic acid sequence encoding a light chain polypeptide, or fragment thereof, wherein the first recombinant nucleic acid sequence is expressed in the subject to generate a first polypeptide and the second recombinant nucleic acid is expressed in the subject to generate a second polypeptide, wherein the synthetic antibody is generated by the first and second polypeptides.

22. The method of claim 21, wherein the first recombinant nucleic acid sequence further comprises a first promoter for expressing the first polypeptide in the subject and wherein the second

recombinant nucleic acid sequence further comprises a second promoter for expressing the second polypeptide in the subject.

23. The method of claim 22, wherein the first promoter and second promoter are the same.

24. The method of claim 23, wherein the promoter is a cytomegalovirus (CMV) promoter.

25. The method according to any one of claims 21 to 24, wherein the heavy chain polypeptide comprises a variable heavy region and a constant heavy region 1.

26. The method according to any one of claims 21 to 24, wherein the heavy chain polypeptide comprises a variable heavy region, a constant heavy region 1, a hinge region, a constant heavy region 2 and a constant heavy region 3.

27. The method according to any one of claims 21 to 26, wherein the light chain polypeptide comprises a variable light region and a constant light region.

28. The method according to any of claims 21 to 27, wherein the first recombinant nucleic acid sequence and the second recombinant nucleic acid sequence further comprise a Kozak sequence.

29. The method according to any one of claims 21 to 27, wherein the first recombinant nucleic acid sequence and the second recombinant nucleic acid sequence further comprise an immunoglobulin (Ig) signal peptide.

30. The method of claim 29, wherein the Ig signal peptide comprises an IgE or IgG signal peptide.

31. A method of preventing or treating a disease in a subject, the method comprising generating a synthetic antibody in a subject according to the method according to any one of claims 1 to 31.

32. The method of claim 31, wherein the synthetic antibody is specific for a foreign antigen.

33. The method of claim 32, wherein the foreign antigen is derived from a virus.

34. The method of claim 33, wherein the virus is Human immunodeficiency virus (HIV), Chikungunya virus (CHIKV) or Dengue virus.

35. The method of claim 34, wherein the virus is HIV.

36. The method of claim 35, wherein the recombinant nucleic acid sequence comprises a nucleic acid sequence encoding at least one amino acid sequence of SEQ ID NOs:1, 2, 5, 46, 47, 48, 49, 51, 53, 55, and 57.

37. The method of claim 35, wherein the recombinant nucleic acid sequence comprises at least one nucleic acid sequence of SEQ ID NOs:3, 4, 6, 7, 50, 52, 55, 56, 62, 63 and 64.
38. The method of claim 34, wherein the virus is CHIKV.
39. The method of claim 38, wherein the recombinant nucleic acid sequence comprises a nucleic acid sequence encoding at least one amino acid sequence of SEQ ID NOs:59 and 61.
40. The method of claim 38, wherein the recombinant nucleic acid sequence comprises at least one nucleic acid sequence of SEQ ID NOs:58 and 60.
41. The method of claim 34, wherein the virus is Dengue virus.
42. The method of claim 41, wherein the recombinant nucleic acid sequence comprises a nucleic acid sequence encoding at least one amino acid sequence of SEQ ID NO:45.
43. The method of claim 41, wherein the recombinant nucleic acid sequence comprises at least one nucleic acid sequence of SEQ ID NO:44.
44. The method of claim 31, wherein the synthetic antibody is specific for a self-antigen.
45. The method of claim 44, wherein the self-antigen is Her2.
46. The method of claim 45, wherein the recombinant nucleic acid sequence comprises a nucleic acid sequence encoding at least one amino acid sequence of SEQ ID NOs:41 and 43.
47. The method of claim 45, wherein the recombinant nucleic acid sequence comprises at least one nucleic acid sequence of SEQ ID NOs: 40 and 42.
48. A product produced by any one of the methods of claims 1-47.
49. The product of claim 48, wherein the product is single DNA plasmid capable of expressing a functional antibody.
50. The product of claim 48, wherein the product is comprised of two distinct DNA plasmids capable of expressing components of a functional antibody that combine in vivo to form a functional antibody.
51. A method of treating a subject from infection by a pathogen, comprising administering a nucleotide sequence encoding a synthetic antibody specific for the pathogen.
52. The method of claim 51, further comprising administering an antigen of the pathogen to generate an immune response in the subject.

Optimized Nucleic Acid Sequence Encoding IgG Heavy Chain

GGATCCGCCACCATGGAAACCGACACTCTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCCCGGCTCAACAGGCGACGGC
GCTCAGGTCCAGCTGGTCCAGTCTGGAGCTGTGATCAAGACCCCTGGCAGCTCCGTCAAAATTTCTTGCAGAGCAAGTG
GCTACAACTTCCGGGACTATAGCATCCACTGGGTGCGGCTGATTCTGATAAGGGATTTGAGTGGATCGGCTGGATCAA
GCCACTGTGGGGCGCTGTGTCCTACGCAAGGCAGCTGCAGGGGCGCGTCTCCATGACACGACAGCTGTCTCAGGACCC
AGACGATCCCGATTGGGGGGTGGCCTACATGGAGTTCAGTGGACTGACTCCCGCAGACACCGCCGAATATTTTTGCGTG
CGGAGAGGCTCCTGCGACTACTGTGGGGATTTCCCATGGCAGTATTGGTGTGAGGAACTGTGGTCGTGGTCTCTAGTG
CATCAACCAAGGGCCCCAGCGTGTTTCTCTGGCCCCATCAAGCAAAAGTACATCAGGAGGAACTGCAGCTCTGGGAT
GTCTGGTGAAGGATTACTTCCCCGAGCCTGTGACCGTCAGCTGGAACCTCCGGAGCACTGACCTCCGGAGTGCACACATT
TCCCGCTGTCCTGCAGTCCTCTGGGCTGTACTCTCTGAGTTCAGTGGTCACAGTGCCTAGCTCCTCTCTGGGCACCCAGA
CATATATCTGCAACGTCAATCATAAGCCAAGTAATACTAAAGTGGACAAGAAAGTCGAACCCAAATCATGTTACCCCT
ATGACGTGCCTGATTATGCTTGATAAACTCGAG (SEQ ID NO:6)

FIG. 1

GGATCCGCCACCA**TGG**GAGACTGATACACTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCTGGCTCAACCGGCGACGGG
GCTCAGGTCCAGATTGTGCTGACCCAGAGCCCTGGCATCCTGTCACTGAGCCCAGGAGAGACCGCAACACTGTTCTGCA
AGGCCTCCCAGGGCGGGAACGCTATGACATGGTACCAGAAACGGAGAGGACAGGTGCCCCGACTGCTGATCTATGACA
CTTCAAGGCGAGCAAGCGGAGTGCCTGATCGATTGTGCGCAGCGGCTCTGGGACAGACTTCTTTCTGACTATTAATAA
GCTGGACAGAGAGGATTTGCTGTGTACTATTGCCAGCAGTTTGAATTCTTTGGACTGGGCAGCGAGCTGGAAGTGCAC
AGGACCGTCGCCGCTCCAAGTGTGTTCATTTTCCCCCTAGCGATGAGCAGCTGAAATCCGGGACAGCCTCTGTGGTCT
GTCTGCTGAACAATTTCTACCCCCGCGAAGCAAAGGTGCAGTGGAAAGTCGACAACGCCCTGCAGAGTGGCAATTCAC
AGGAGAGCGTGACCGAACAGGACTCCAAGGATTCTACATATAGTCTGAGCTCCACTCTGACCCTGTCTAAAGCTGATTA
CGAGAAGCACAAAGTGTATGCATGCGAAGTCACTCATAGGGCCTGTCTAGTCTCTGTACCAAGAGCTTTAACCGAGG
GGAGTGTTACCCATATGACGTCCCCGATTACGCC**TGATAACTCGAG** (SEQ ID NO:7)

FIG. 2

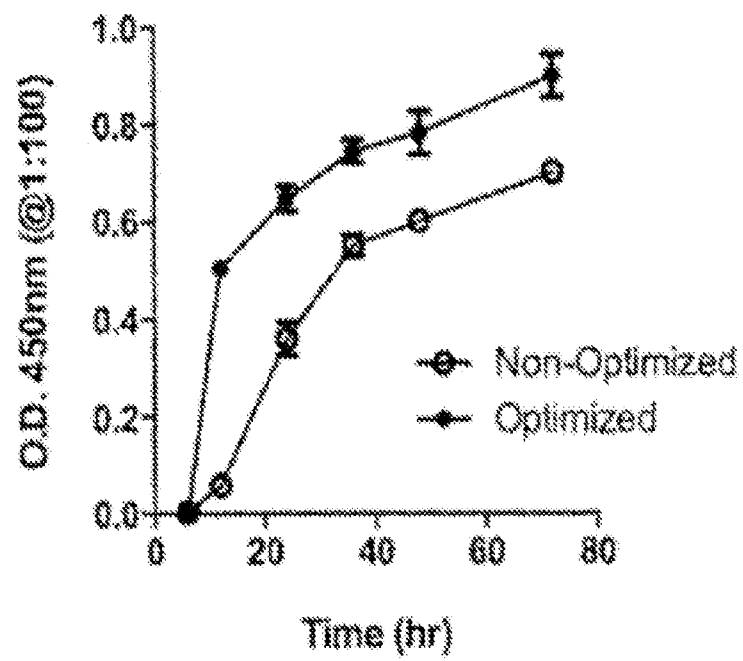


FIG. 3

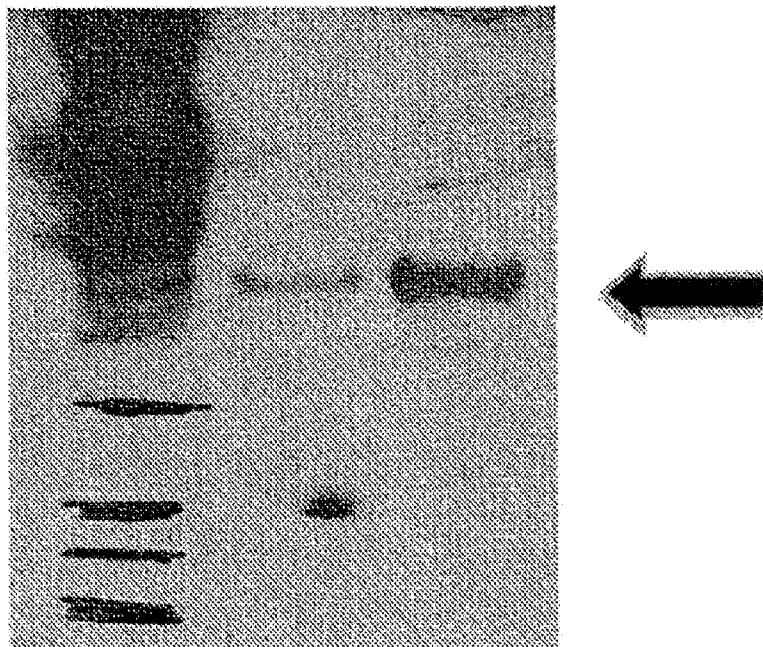


FIG. 4

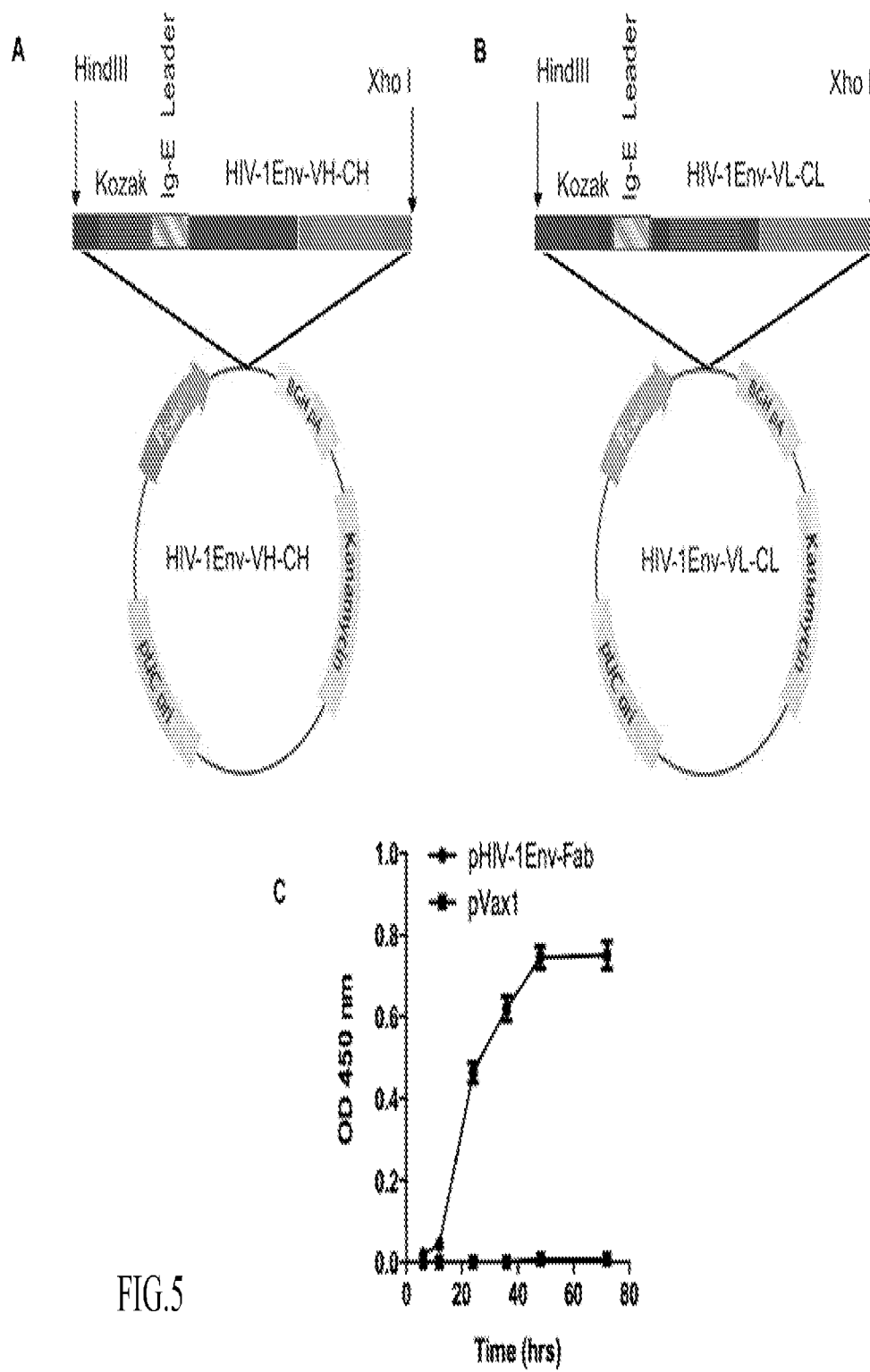


FIG.5

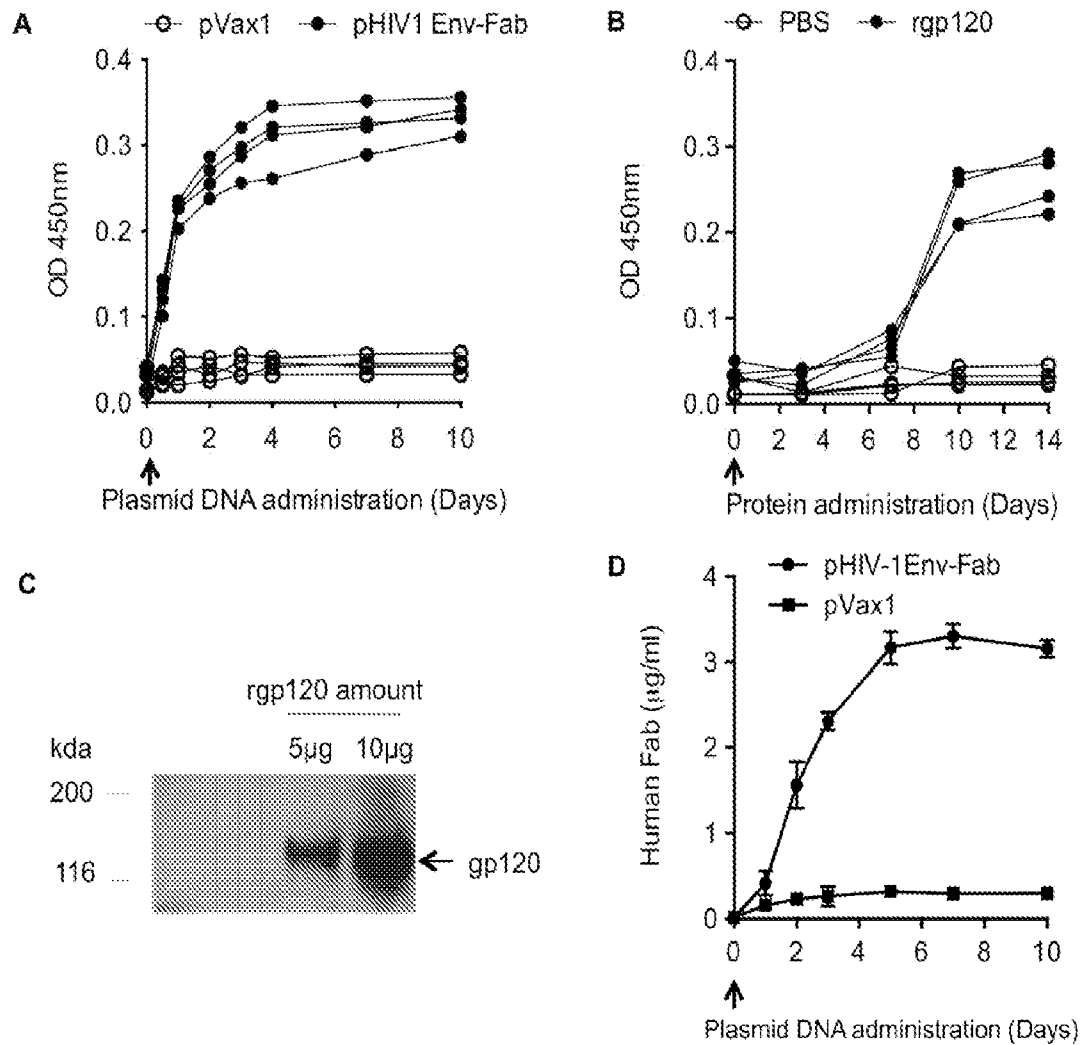


FIG. 6

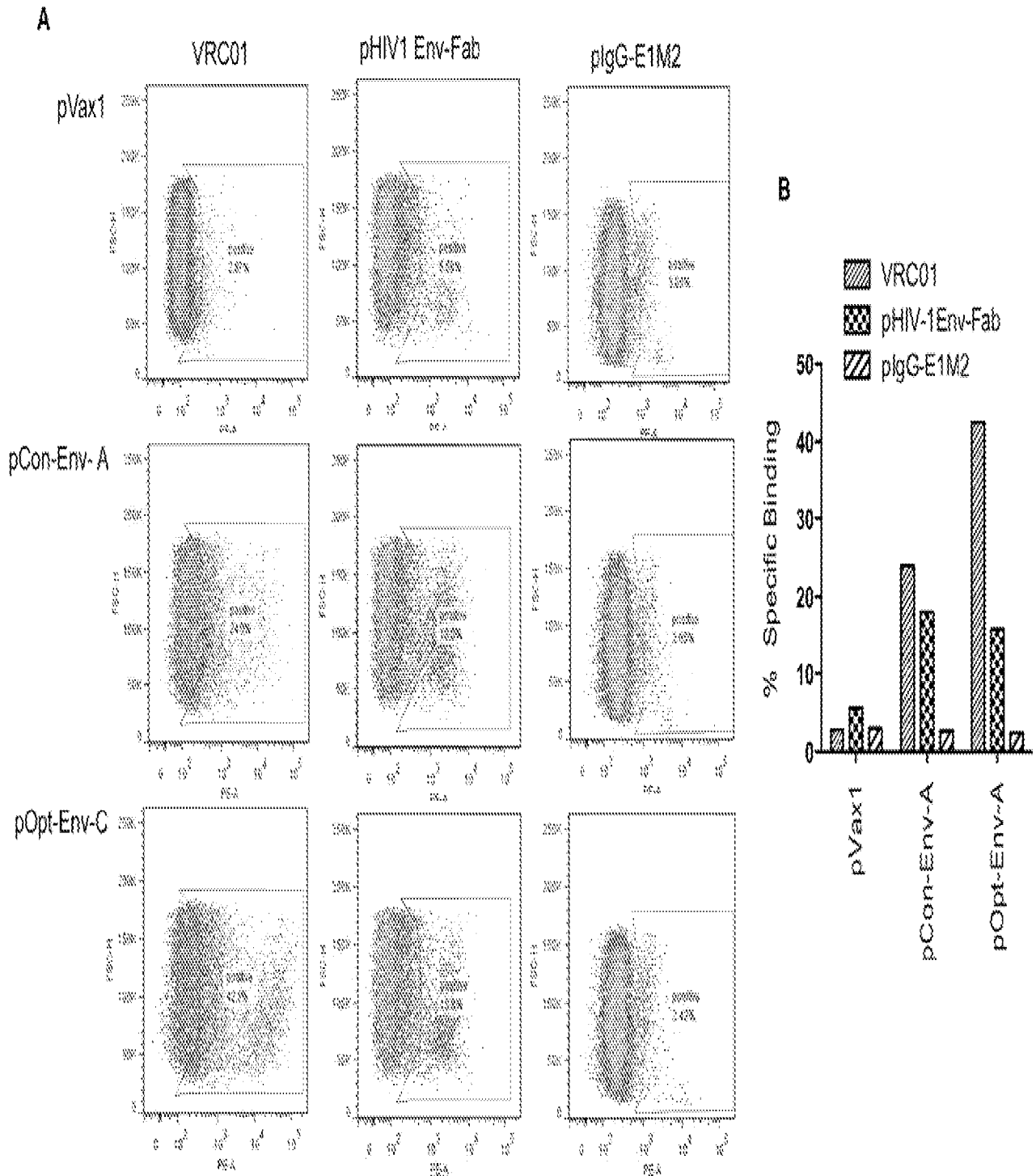


FIG.7

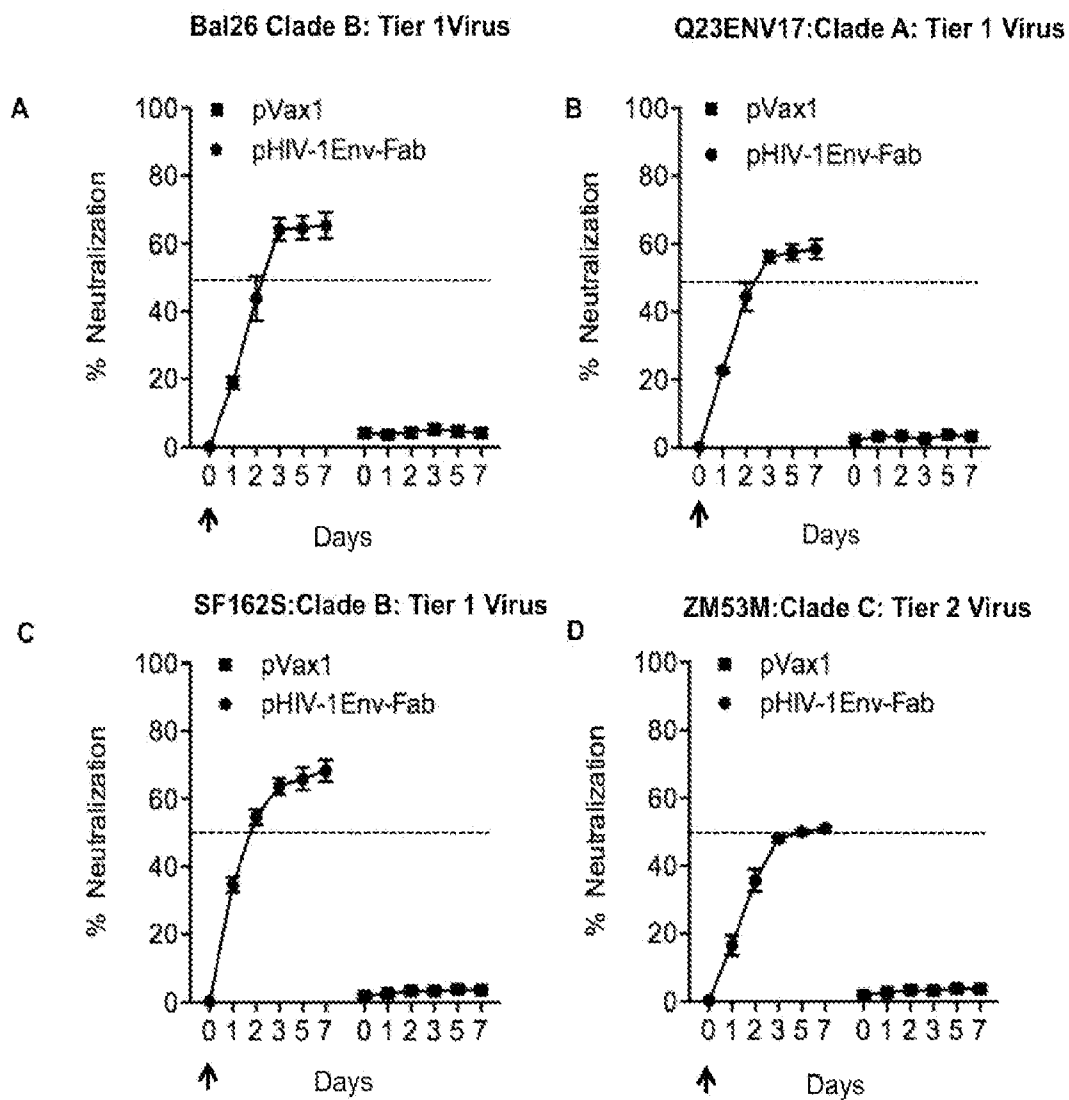


FIG. 8

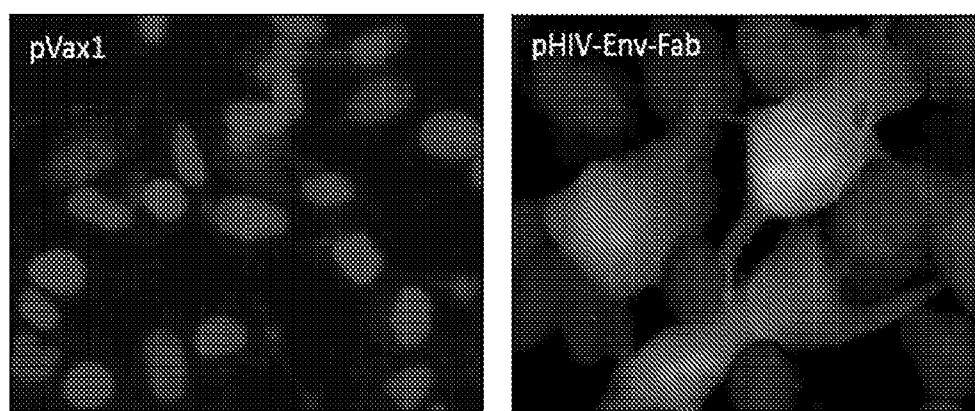
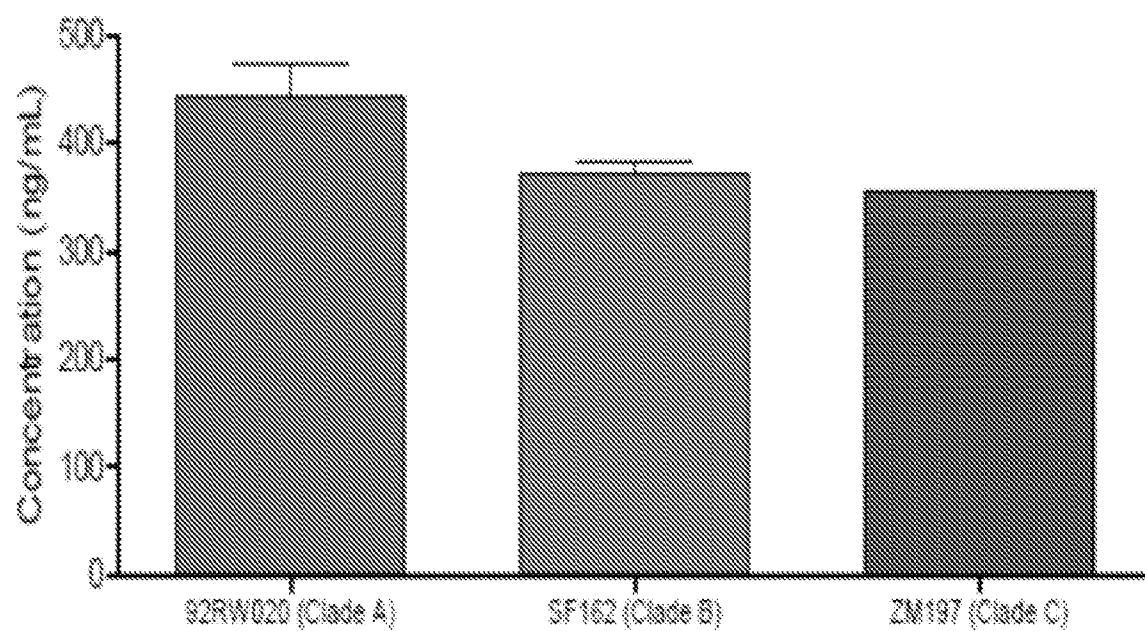
Nucleic Acid Sequence Encoding the Heavy Chain (VH-CH1) of HIV-1 Env Fab

AAGCTTGCCGCCACCATGGAGACTGATACACTGCTGCTGTGGGTGCTGCTGCTGTGG
GTGCCAGGGTCAACCGGAGATGGGGCTCAGGTCCAGCTGGTCCAGAGCGGCGGACA
GATGAAGAAACCCGGCGAGAGCATGAGGATCTCCTGCAGAGCATCTGGATACGAGT
TCATCGACTGTACCCTGAACTGGATTAGGCTGGCTCCTGGAAAGAGACCAGAGTGG
ATGGGGTGGCTGAAACCACGAGGGGGAGCAGTGAATTACGCCCCGGCCCCCTGCAGGG
ACGAGTGACCATGACCAGGGACGTGTACAGCGATACCGCCTTCCTGGAGCTGCGGT
CCCTGACAGTGGACGATACTGCTGTCTACTTCTGCACACGCGGAAAGAACTGTGACT
ATAATTGGGATTTTGAACACTGGGGCCGGGGAACACCCGTGATCGTCAGCTCCCCCA
GTACTAAGGGACCTTCAGTGTTTCCACTGGCCCCCTCTAGTAAATCCACCTCTGGAG
GGACAGCCGCTCTGGGATGCCTGGTGAAAGATTATTTCCCGAACCTGTGACCGTCA
GTTGGAACCTCAGGGGCTCTGACTTCTGGCGTGCACACCTTTCCTGCAGTCCTGCAGT
CAAGCGGGCTGTACAGTCTGTCTCTGTGGTCACTGTGCCTAGTTCAAGCCTGGGCA
CTCAGACCTATATTTGTAACGTGAATCATAAGCCATCCAATACAAAAGTGGACAAA
AAAGCCGAACCCAAATCCTGTTACCCTTATGATGTGCCCGACTACGCCTGACTCGAG
(SEQ ID NO:3)

FIG. 9**Light Chain (VL-CL) of HIV-1 Env Fab**

AAGCTTGCCGCCACCATGGAAACCGATACACTGCTGCTGTGGGTGCTGCTGCTGTGG
GTGCCAGGAAGTACCGGGGATGGGGCTCAGGTCCAGATTGTGCTGACTCAGTCCCCT
GGGACCCTGTCTCTGAGTCCAGGCGAGACAGCTATCATTTTCATGCCGAAGTAGCCAG
TACGGCAGCCTGGCTTGGTATCAGCAGCGACCAGGACAGGCACCACGACTGGTCAT
CTACTCAGGCAGCACAAAGGGCCGCTGGCATCCCCGACAGGTTCTCCGGCAGCAGGT
GGGGGCCTGATTACAACCTGACTATCTCTAATCTGGAGAGTGGGGACTTTGGCGTGT
ACTATTGCCAGCAGTATGAGTTCTTCGGCCAGGGAAGTAAGGTGCAGGTGGACATC
AAAAGAACCGTGGCAGCCCCATCCGTCTTCATTTTTCCCCCTTCTGATGAGCAGCTG
AAGTCAGGCACCGCCAGCGTGGTCTGTCTGCTGAACAATTTCTACCCCCGGGAAGCC
AAGGTGCAGTGGAAGTGGACAACGCTCTGCAGAGTGGAAATTCACAGGAGAGCGT
GACCGAACAGGACTCCAAGGATTCTACATATAGTCTGAGCAGCACCTGACCCTGA
GTAAAGCAGATTACGAGAAGCACAAAGTGTATGCCTGTGAAGTCACACATCAGGGC
CTGAGGAGCCCCGTGACTAAAAGTTTCAACCGAGGAGAGTGCTACCCTTATGATGTG
CCCGACTACGCCTAAAGTCTCGAG (SEQ ID NO:4)

FIG. 10

**FIG. 11****FIG. 12**

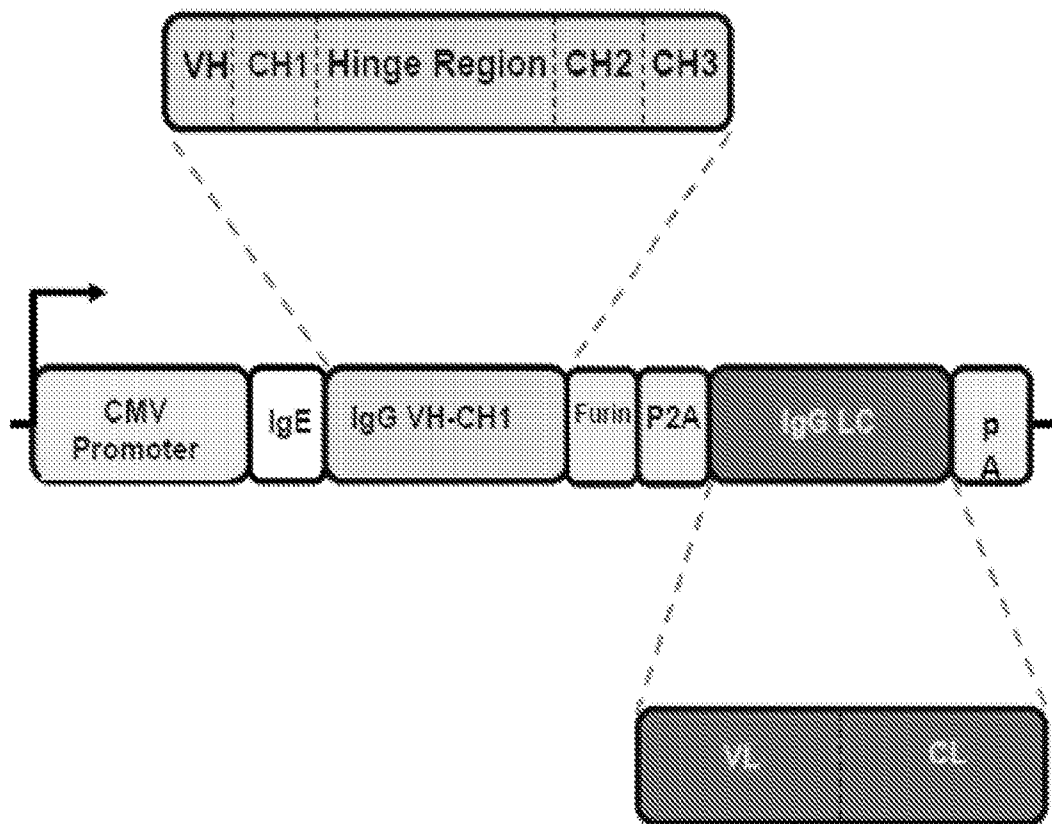


FIG. 13

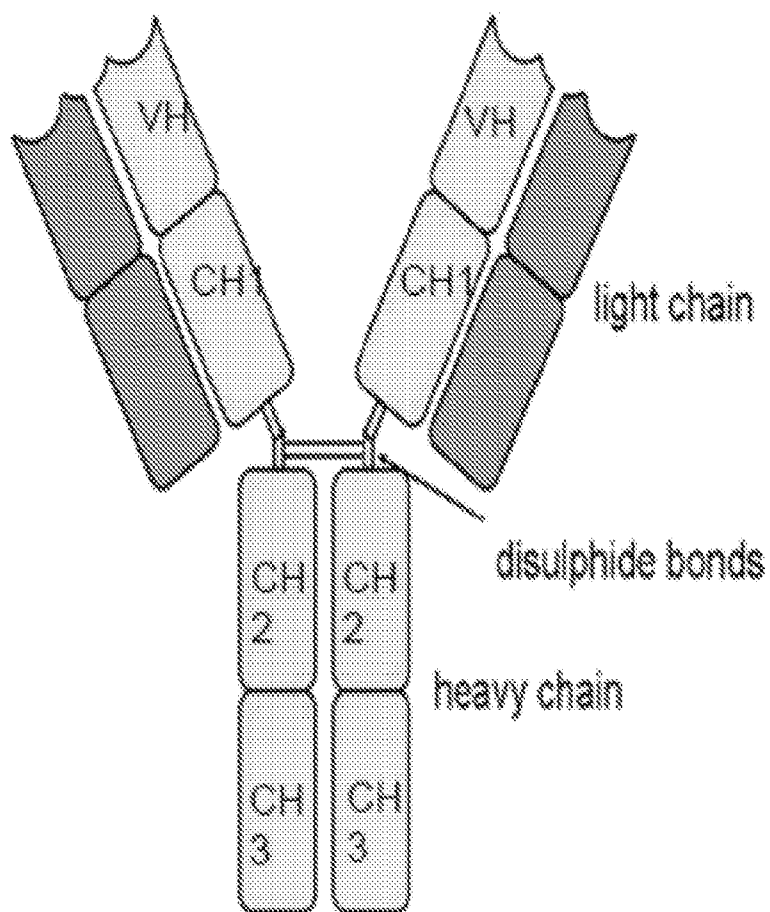


FIG. 14

VRC01 IgG

MDWTWILFLVAAATRVHSQVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLA
PGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCT
RGKNCDYNWDFEHWGRGTPVIVSSPSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKAEPKSCEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTTPVLDSGSEFLYSLKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKRGRKRRSGSGATNFSLLKQAGDVEENPGPMDWTWILFLVAAATRVHSEIVLTQ
SPGTLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGP
DYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYLSSTLTLSKADYEKH
KVYACEVTHQGLRSPVTKSFNRGEC (SEQ ID NO:5)

FIG. 15

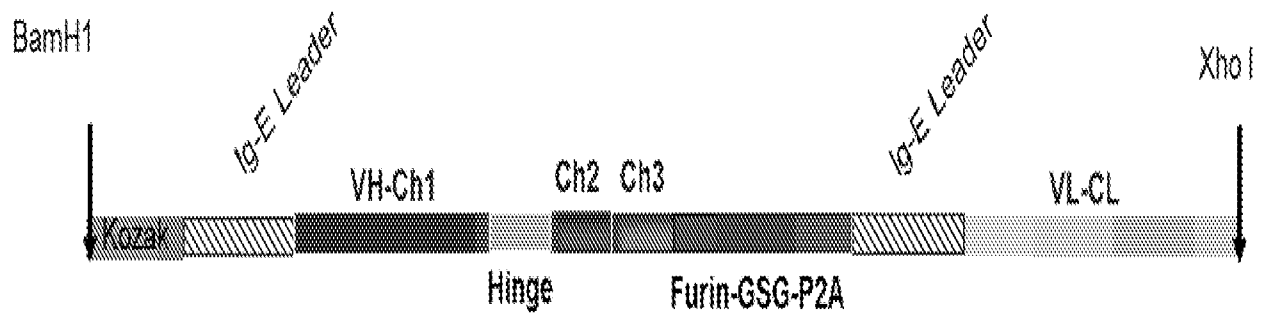


FIG. 16A

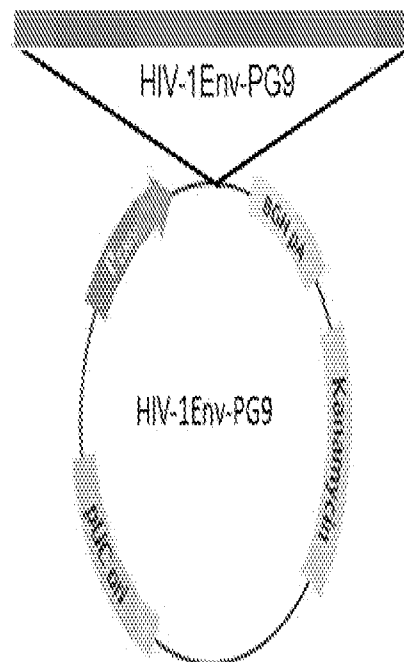
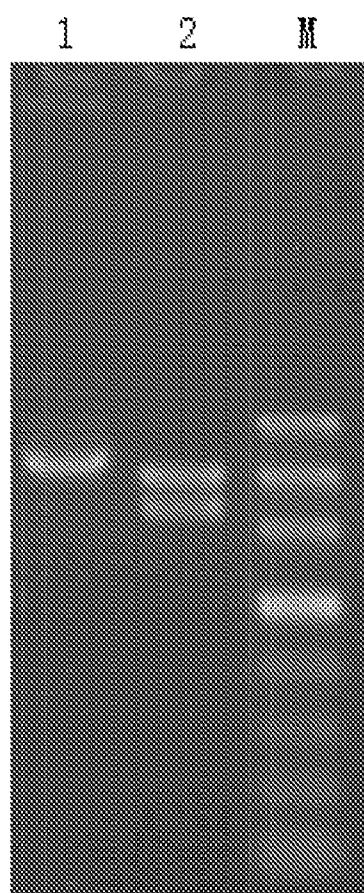


FIG. 16B

**FIG. 16C**

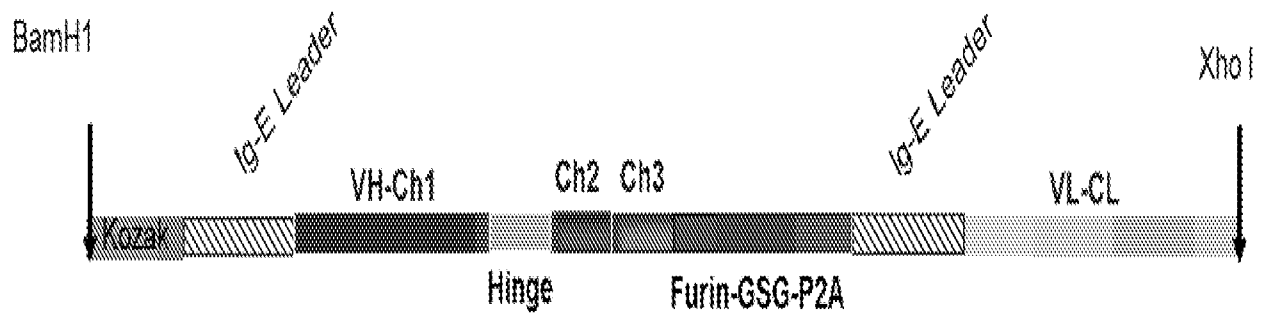


FIG. 17A

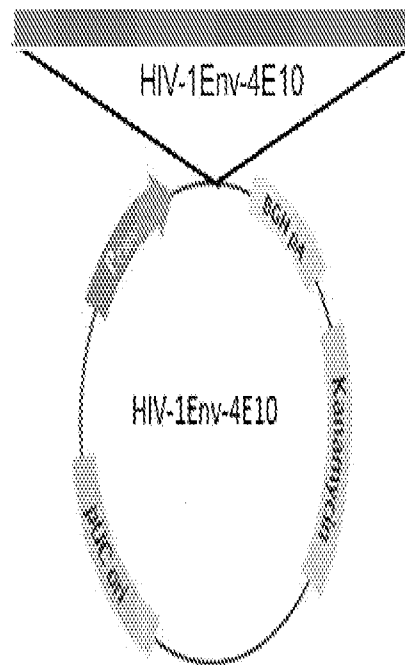


FIG. 17B

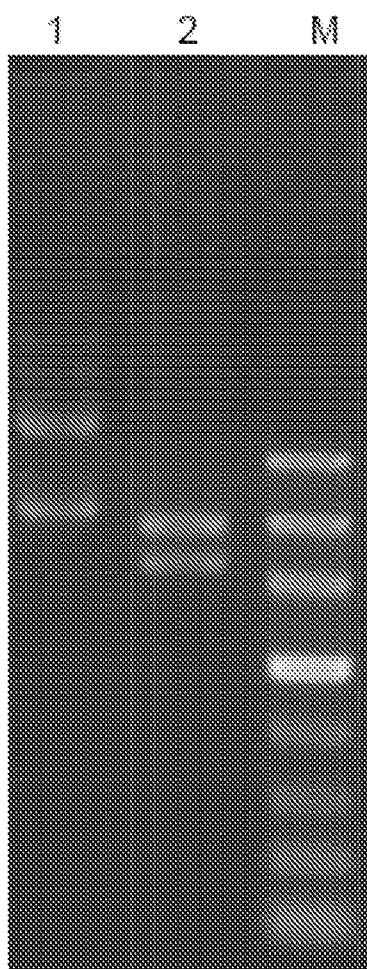


FIG. 17C

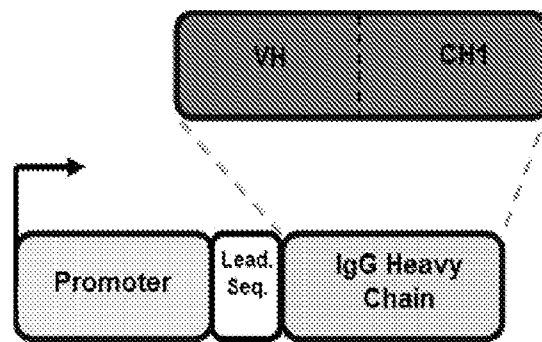
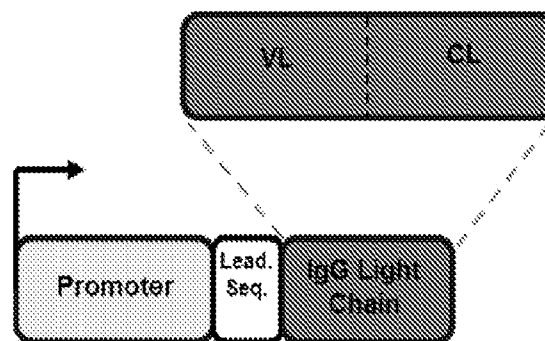
Amino Acid Sequence of HIV-1 Env-PG9 Ig (before protease cleavage)

MDWTWRILFLVAAATGTHAEFGLSWVFLVAFRLRGVQCQRLVESGGGVVQPGSSRLRLSC
AASGFDFSRQGMHWVRQAPGQGLEWVAFIKYDGSEKYHADSVWGRLSISRDNKDTL
YLQMNSLRVEDTATYFCVREAGGPDYRNGYNYDFYDGYNYHYMDVWGKGTTVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLTP
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKRGKRKRRSGSGATNFSLLKQAGD
VEENPGPMAWTPFLFLLTCCPGGSNSQSALTQPASVSGSPGQSITISCNSTNDVGGYE
SVSWYQQHPGKAPKVVIYDVSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEGDYCKS
LTSTRRRVFGTGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAW
KADSSPVKAGVETTTPSKQSNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTV
APTECS (SEQ ID NO:2)

FIG. 18**Amino Acid Sequence of HIV-1 Env-4E10 Ig (before protease cleavage)**

MDWTWRILFLVAAATGTHAQVQLVQSGAEVKRPGSSVTVSCKASGGSFSTYALSWVR
QAPGRGLEWMGGVIPLLTITNYAPRFQGRITITADRSTSTAYLELNSLRPEDTAVYYCAR
EGTTGWGWL GKPIGAFAHWGQGT LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
LSLSPGKRGKRKRRSGSGATNFSLLKQAGDVEENPGPMVLQTQVFISLLLWISGAYGEIVL
TQSPGTQSLSPGERATLSCRASQSVGNKLAWYQQRPGQAPRLLIYGASSRPSGVADRF
SGSGSGTDFTLTISRLEPEDFAVYYCQYQGSLSTFGQGTKVEKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK
ADYEKHKVYACEVTHQGLSSPVTKSFNRGE (SEQ ID NO:1)

FIG. 19

**FIG. 20A****FIG. 20B**

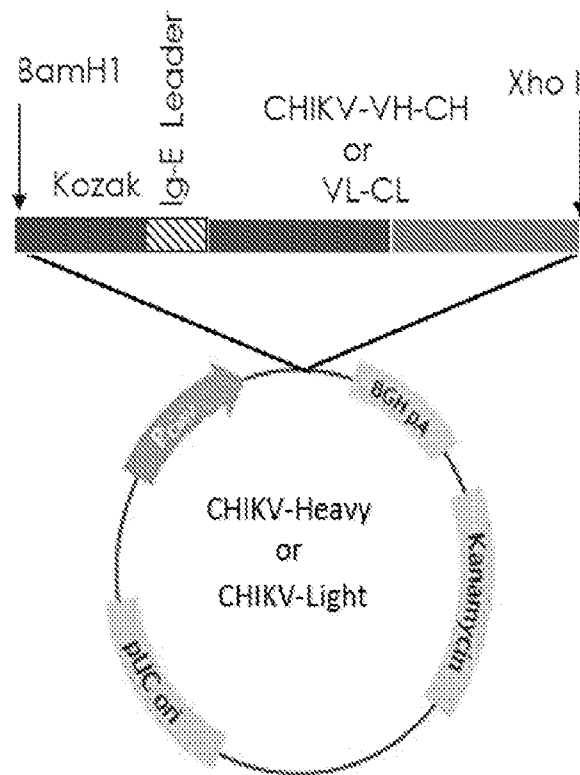


FIG. 21

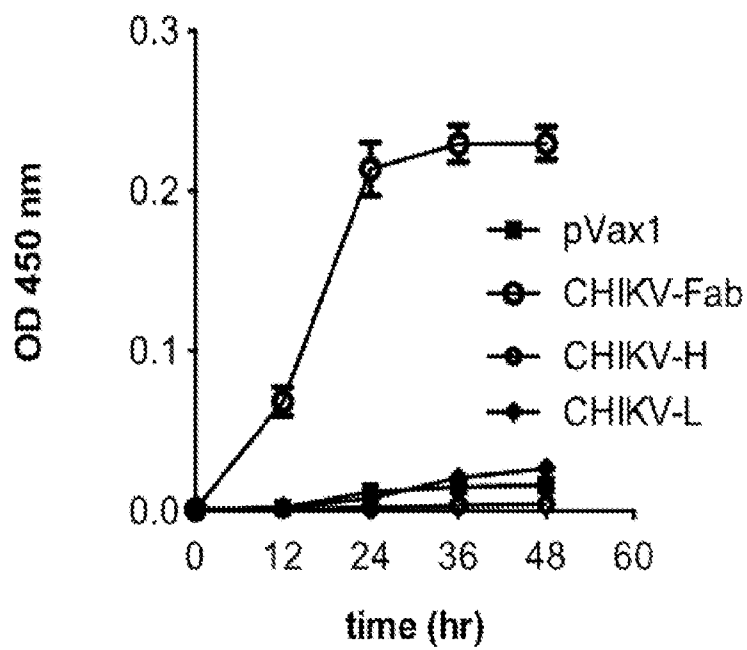


FIG. 22

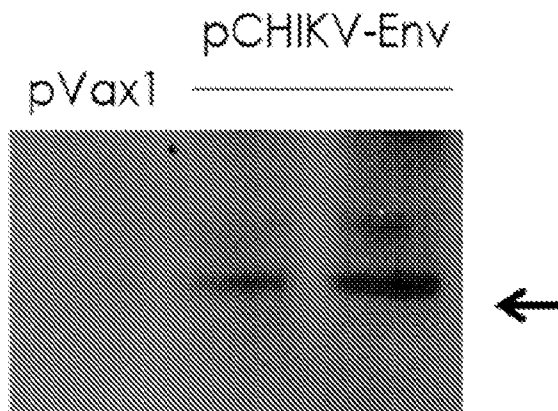


FIG. 23

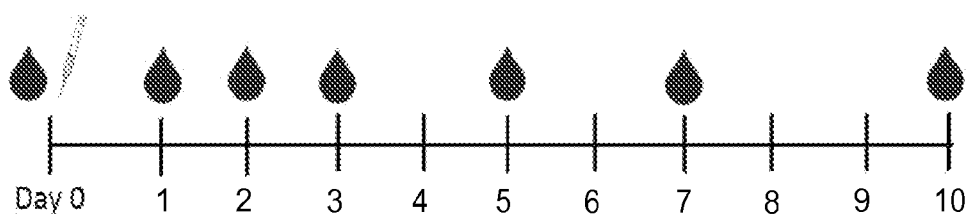


FIG. 24

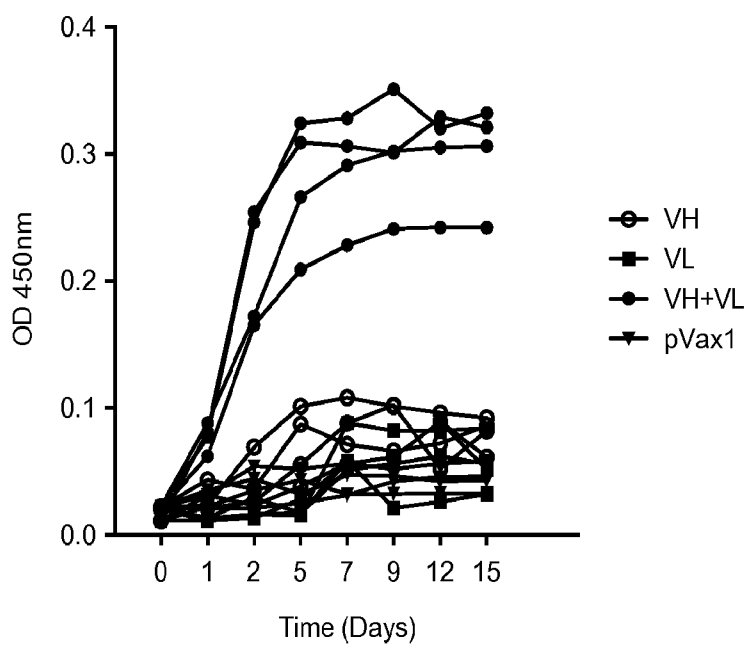


FIG. 25

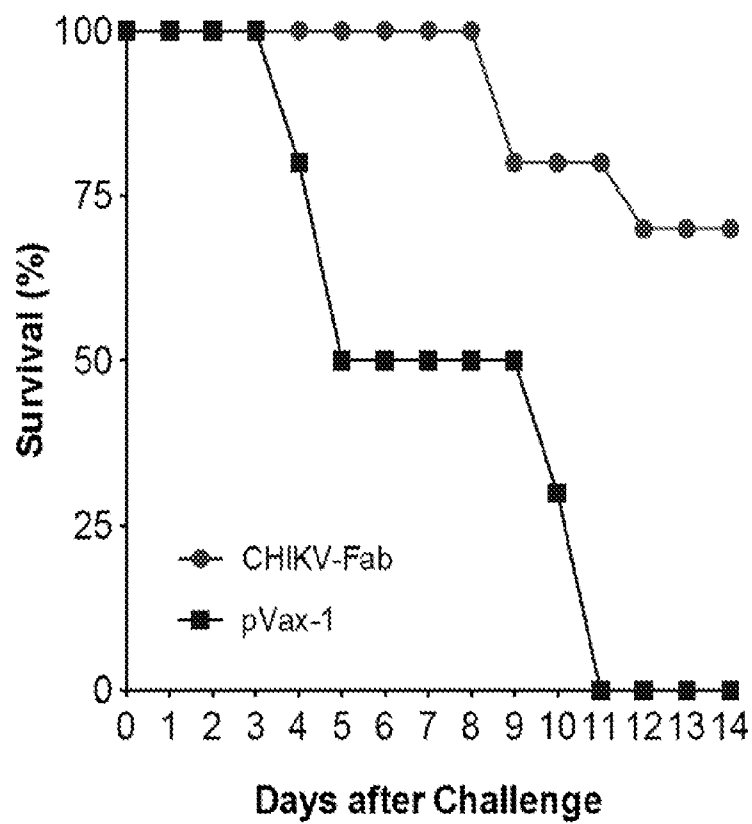


FIG. 26

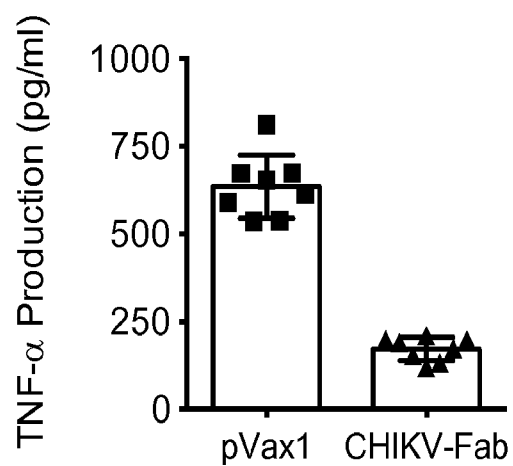
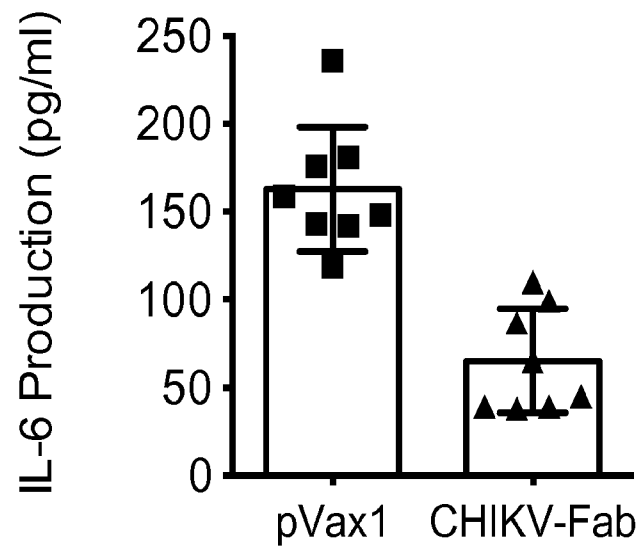


FIG. 27

**FIG. 28**

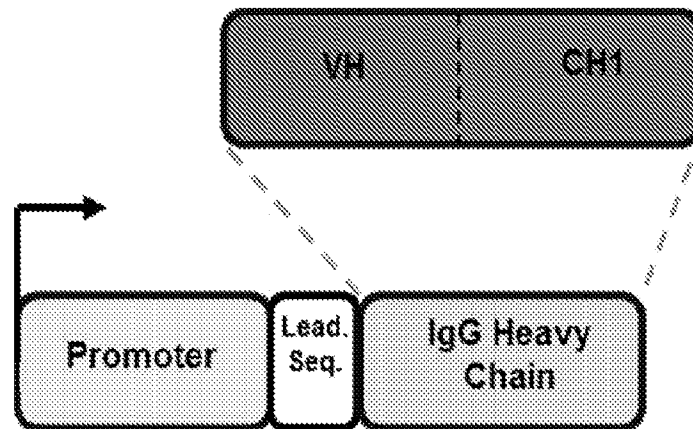


FIG. 29

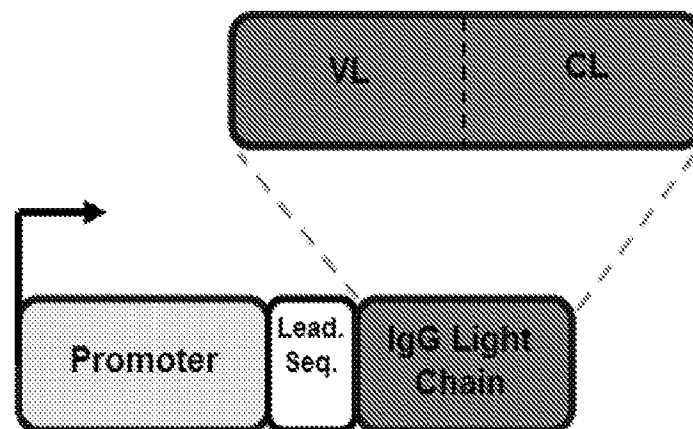
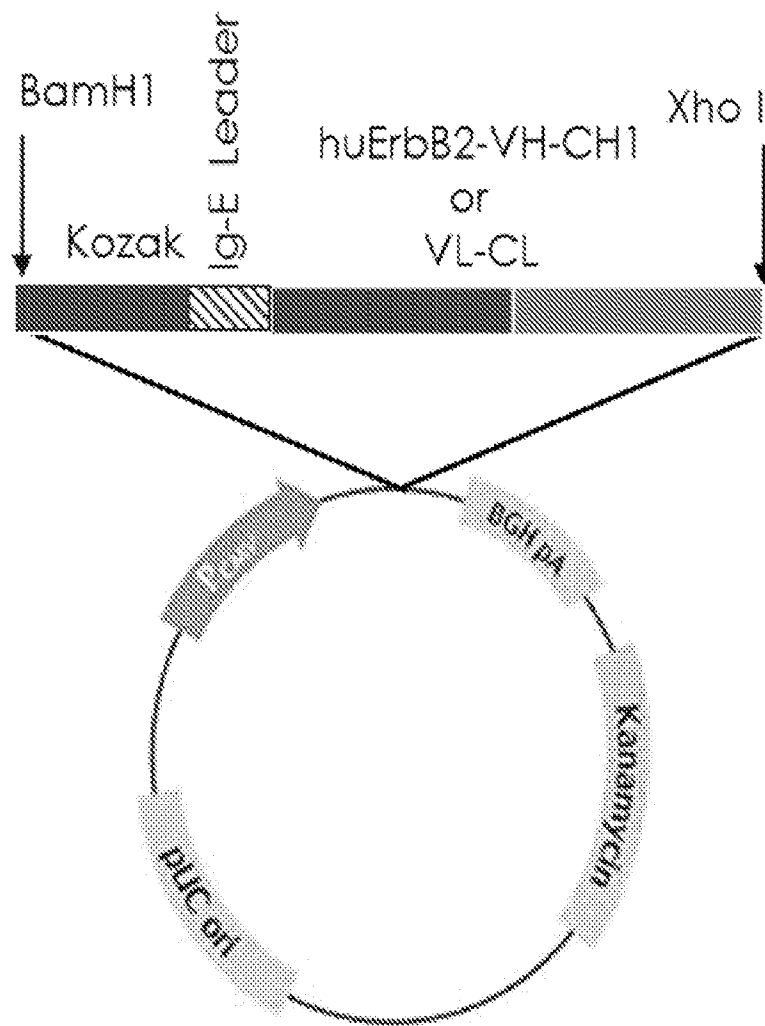


FIG. 30

**FIG. 31**

Nucleic Acid Sequence Encoding the VH-CH1 of anti-Her-2 Fab

GGATCCGCCACCATGGACTGGACATGGATTCTGTTTCTGGTCGCCGCCGCTACAAGAGTGCATTCCGAAGTGCAGCTGG
TCGAGAGTGGAGGGGGACTGGTGCAGCCCCGGCGGATCTCTGCGACTGAGTTGCGCCGCTTCAGGCTTCACCTTTACAGA
CTACACCATGGATTGGGTGAGACAGGCACCTGGCAAGGGACTGGAGTGGGTGGCTGATGTCAACCCAAATAGTGGGGG
CTCAATCTACAACCAGAGGTTCAAGGGCAGGTTACCCCTGAGCGTGGACAGGTCCAAAAACACTCTGTATCTGCAGAT
GAATTCTCTGCGGGCTGAAGATACCGCAGTCTACTATTGCGCCCGCAATCTGGGCCCCAAGCTTCTACTTTGACTATTGG
GGGCAGGGCACACTGGTGACTGTCAGCTCCGCTTCTACAAAGGGACCAAGCGTGTTCCCACTGGCACCCTCTAGTAAAT
CCACCTCTGGAGGGACAGCAGCCCTGGGCTGTCTGGTGAAAGACTATTTCCCCGAGCCTGTGACTGTCAGCTGGAACCTC
CGGAGCACTGACTAGCGGAGTGCACACCTTTCCAGCCGTCCTGCAGTCAAGCGGCCTGTACTCCCTGTCTCTGTGGTC
ACAGTGCCTAGTTCAAGCCTGGGAACTCAGACCTATATTTGTAATGTGAACCATAAACCAAGCAATACAAAGGTGGAC
AAGAAGGTGGAACCAAAATCCTGCTGATAACTCGAG (SEQ ID NO:40)

FIG. 32**Amino Acid Sequence of the VH-CH1 of anti-Her-2 Fab**

MDWTWILFLVAAATRVHSEVQLVESGGGLVQPGGSLRLSCAASGFTFTDYM¹MDWVRQAPGKLEWVADVNPNSGGSIYN
QRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
(SEQ ID NO:41)

FIG. 33

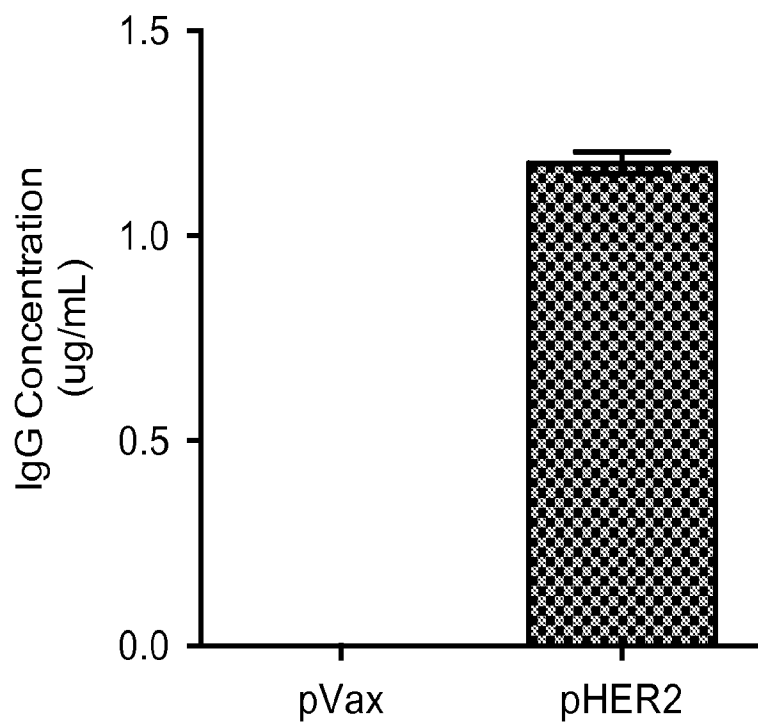
Nucleic Acid Sequence Encoding the VL-CL of anti-Her-2 Fab

GGATCCGCCACCATGGATTGGACTTGGATTCTGTTCTGGTCGCCGCCGCTACCCGCGTGCATTCCGATATTCAGATGA
CTCAGAGCCCCCTCCTCACTGTCAGCCAGCGTGGGCGACCGAGTCACCATCACATGCAAAGCTTCTCAGGATGTGAGTAT
TGGGGTCGCATGGTACCAGCAGAAGCCAGGCAAAGCACCCAAGCTGCTGATCTATTCCGCCTCTTACAGGTATACAGG
AGTGCCCGCAGCAGATTCAGTGGCTCAGGAAGCGGGACTGACTTTACTCTGACCATCAGCTCCCTGCAGCCTGAGGATTTC
GCTACCTACTATTGCCAGCAGTACTATATCTACCCATATACCTTTGGCCAGGGAACAAAAGTGGAGATCAAGCGGACCG
TGGCCGCTCCCTCCGTCTTCATTTTTCCCCCTTCTGACGAACAGCTGAAGAGCGGAACAGCAAGCGTGGTCTGTCTGCT
GAACAATTTCTACCCCTCGCGAGGCCAAAGTGCAGTGGGAAGGTCGATAACGCTCTGCAGTCCGGGAATTCTCAGGAGAG
TGTGACTGAACAGGACTCAAAGATAGCACCTATCCCTGTCTAGTACACTGACTCTGAGCAAGGCAGACTACGAAAA
GCACAAAGTGTATGCCTGTGAGGTCACCCACCAGGGGCTGTCAAGTCCCGTCACCAAGTCCTTCAATAGAGGCGAATG
CTGATAACTCGAG (SEQ ID NO:42)

FIG. 34**Amino Acid Sequence of the VL-CL of anti-Her-2 Fab**

MDWTWILFLVAAATRVHSDIQMTQSPSSLSASVGDRTITCKASQDVSIQVAVYQQKPGKAPKLLIYSASYRYTGVPSTRFSG
SGSGTDFTLTISLQPEDFATYYCQYYIYPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW
KVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:43)

FIG. 35

**FIG. 36**

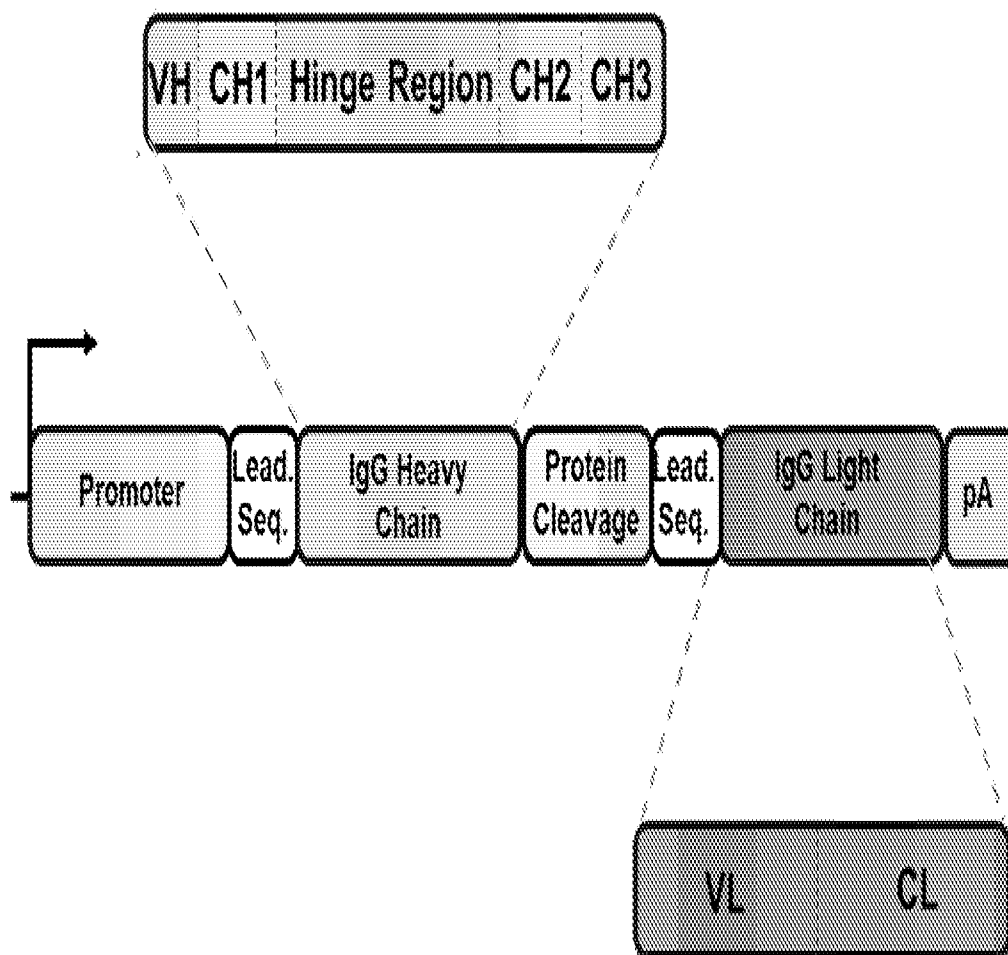


FIG. 37

Nucleic Acid Sequence Encoding anti-DENV Human IgG

GGATCCGCCACCATGGACTGGACTTGGAGGATTCTGTTTCTGGTCGCCGCCGCTACTGGGACTCAGCTCAGGCACATC
TGGTCGAATCTGGAGGAGGAGTGGTCCAGCCTGGCCGATCCCTGCGACTGTCTTGCGCAGCTAGCGCCTTCAACTTCAG
CACAAACGCAATGCACTGGGTGCGACAGGCACCAGGCAAGGGACTGGAGTGGGTGCTGTGATCTCATACGACGGAA
GCCATAAGTACTATGCAGATTCTGTGAAAGGCCGGTTCACCATTTCCAGGGACAATTCTAAGAACACCCTGTATCTGCA
GATGAATAGCCTGCGCGCAGCCGATACCGCAGTGTACTATTGCGCAACTGTGCGCGTGCTGACCTGGCCAGTGAACGC
CGAATACTTTCACCATTTGGGGACAGGGCAGTCTGGTCTCAGTGAGCTCCGCAAGTACTAAGGGACCATCAGTGTTCCCA
CTGGCACCCCTCTAGTAAATCTACTAGTGGCGGGACCGCTGCACTGGGATGTCTGGTGAAGGACTATTTCCCCGAGCCTG
TCACCGTGAGCTGGAATTCGGGAGCCCTGACAAGCGGCGTCCACACTTTTCCCGCTGTGCTGCAGTCAAGCGGACTGTA
CTCCCTGTCTCTGTGGTCACTGTGCCTAGTTCAAGCCTGGGCACCTCAGACCTATATCTGCAATGTGAACCACAAGCCCT
CTAACACCAAAGTCGACAAGAAAGTGGAACCTAAGAGCTGTGATAAAACACATACTTGCCACCTTGTCCAGCACCAG
AGCTGCTGGGAGGACCAAGCGTGTTCTGTTCACCCAAGCCTAAAGACACACTGATGATTAGCCGGACACCTGAAG
TCACTTGCGTGGTCGTGGACGTGTCCACGAGGACCCCGAAGTCAAGTTAATTGGTACGTGGATGGCGTCGAGGTGCA
TAACGCCAAGACCAAACCCCGGGAGGAACAGTACAATAGCACATATAGAGTCGTGTCCGTCTCTGACTGTGCTGCATCA
GGATTGGCTGAATGGGAAGGAGTAAAGTGCAAAGTGTCTAACAAGGCTCTGCCTGCACCAATCGAGAAAACCATTAG
CAAGGCTAAAGGCCAGCCTAGGGAACCACAGGTGTACACACTGCCTCCAAGTCGCGACGAGCTGACCAAGAATCAGGT
CTCCCTGACATGTCTGGTGAAAGGCTTCTATCCATCAGATATCGCCGTGGAGTGGGAAAGCAACGGGCAGCCCGAAAA
CAATTACAAGACCACACCCCCTGTGCTGGACTCTGATGGCAGTTTCTTTCTGTATTCTAAGCTGACCGTGGACAAAAGT
AGATGGCAGCAGGGGAATGTCTTTTCATGTAGCGTGATGCACGAGGCCCTGCACAACCATTACACACAGAAGTCCCTG
TCTCTGAGTCCCGGAAAGAGGGGCCGCAAACGGAGATCAGGGAGCGGAGCTACTAATTTACAGCCTGCTGAAACAGGCCA
GGGGATGTGGAGGAAAACCCCGGACCTATGGCTTGGACCCCACTGTTCTCTGTTCTGCTGACATGCTGTCCCGGGGGCA
GCAATTCTCAGAGTGTCTGACACAGCCACCATCAGTGAGCGGAGCACCAGGACAGAGGGTGACCATCTCCTGCACAG
GCAGCAGCAGCAACATTGGCGCCGGGTACGACGTGCATTGGTATCAGCAGCTGCCCGGCACCGCTCCTAAGCTGCTGA
TCTGTGGCAACAATAACCGCCCATCTGGGGTGCCCGATCGATTCTCCGGCTCTAAAAGTGGGACTTCAGCCAGCCTGGC
TATTACCGGCCTGCAGGCCGAGGACGAAGCTGATTACTATTGCCAGAGCTACGACTCAAGCCTGACCGGAGTCGTGTT
GGAGGAGGAACCAAGCTGACAGTCTGGGACAGCCTAAAGCCGCTCCAAGCGTGACACTGTTTCTCTCCATCCTCTGAG
GAACTGCAGGCAAACAAGGCCACCCTGGTGTGCCTGATTTCCGACTTCTACCCCGGGCAGTCACTGTGGCTTGAAG
GCAGATAGTTCACCTGTCAAAGCCGGAGTGGAGACTACCACACCATCAAAGCAGAGCAATAACAAATACGCAGCCAG
CTCCTATCTGTCCCTGACCCCTGAGCAGTGAAGTCTCACAATCCTATTCTTGCCAGGTCACTCACGAAGGAAGCACT
GTGGAGAAAACCTGTCGCACCAACCGAATGTAGTTGATAACTCGAG (SEQ ID NO:44)

FIG. 38

Amino Acid Sequence of anti-DENV Human IgG (before protease cleavage to separate heavy and light chain polypeptides)

MDWTWRILFLVAAATGTHAQAHLVESGGGVVQPGRSLRLSCAASAFNFSTNAMHWVRQAPGKGLEWVAVISYDGS HKYY
ADSVKGRFTISRDN SKNTLYLQMNSLRAADTAVYYCATVGVLTPVPVNAEYFHHWGQGSLSVSSASTKGPSVFPLAPSSKS
TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE
PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGKRGRKRRSGSGATNF
SLLKQAGDVEENPGPMAWTPFLFLLTCCPGGSNSQSVLTQPPSVSGAPGQRTISCTGSSSNIGAGYDVHWYQQLPGTAPK
LLICGNNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLTGVVFGGGTKLTVLGQPKAAPSVTLFPPSSEEL
QANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTV
APTECS (SEQ ID NO:45)

FIG. 39

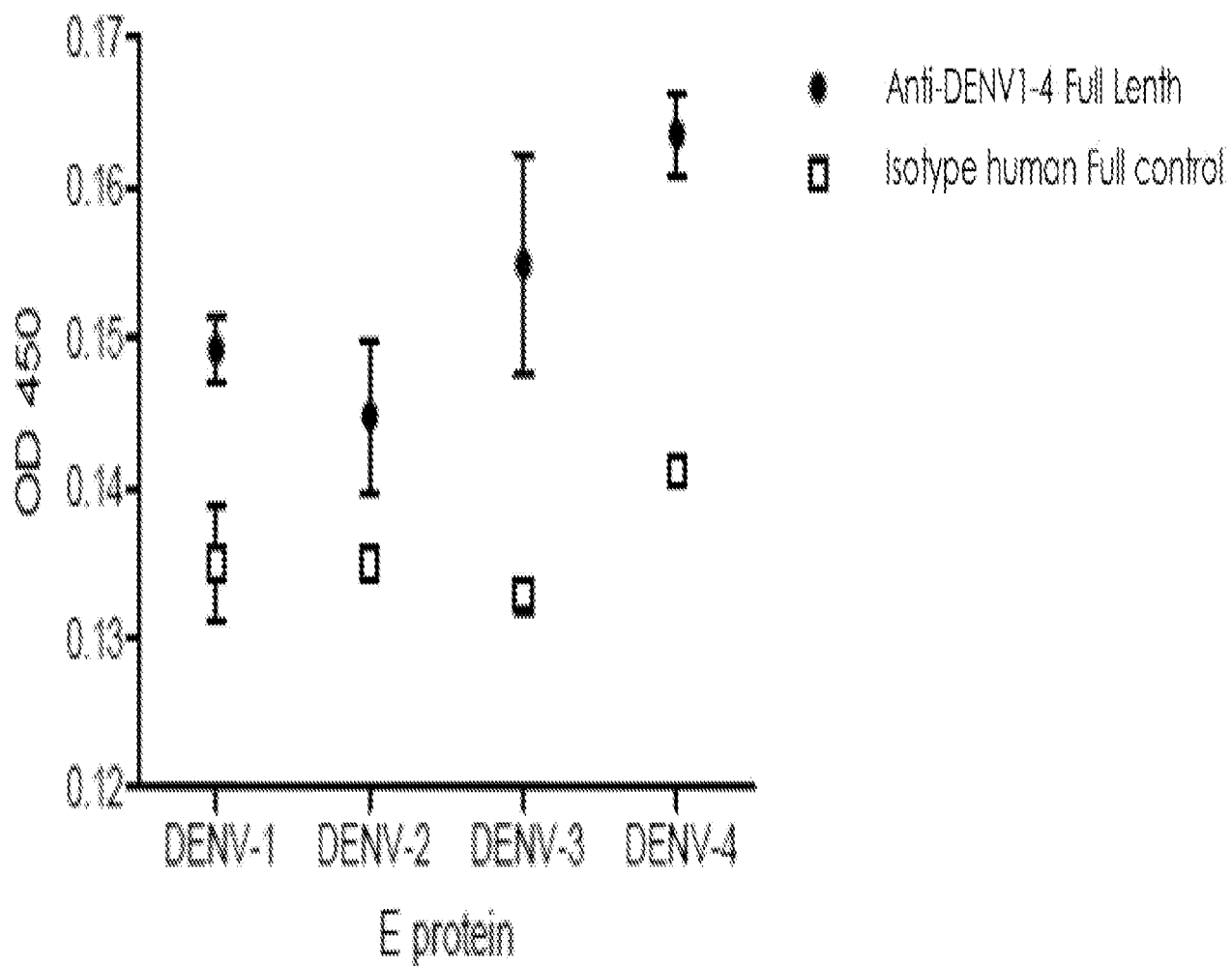


FIG. 40

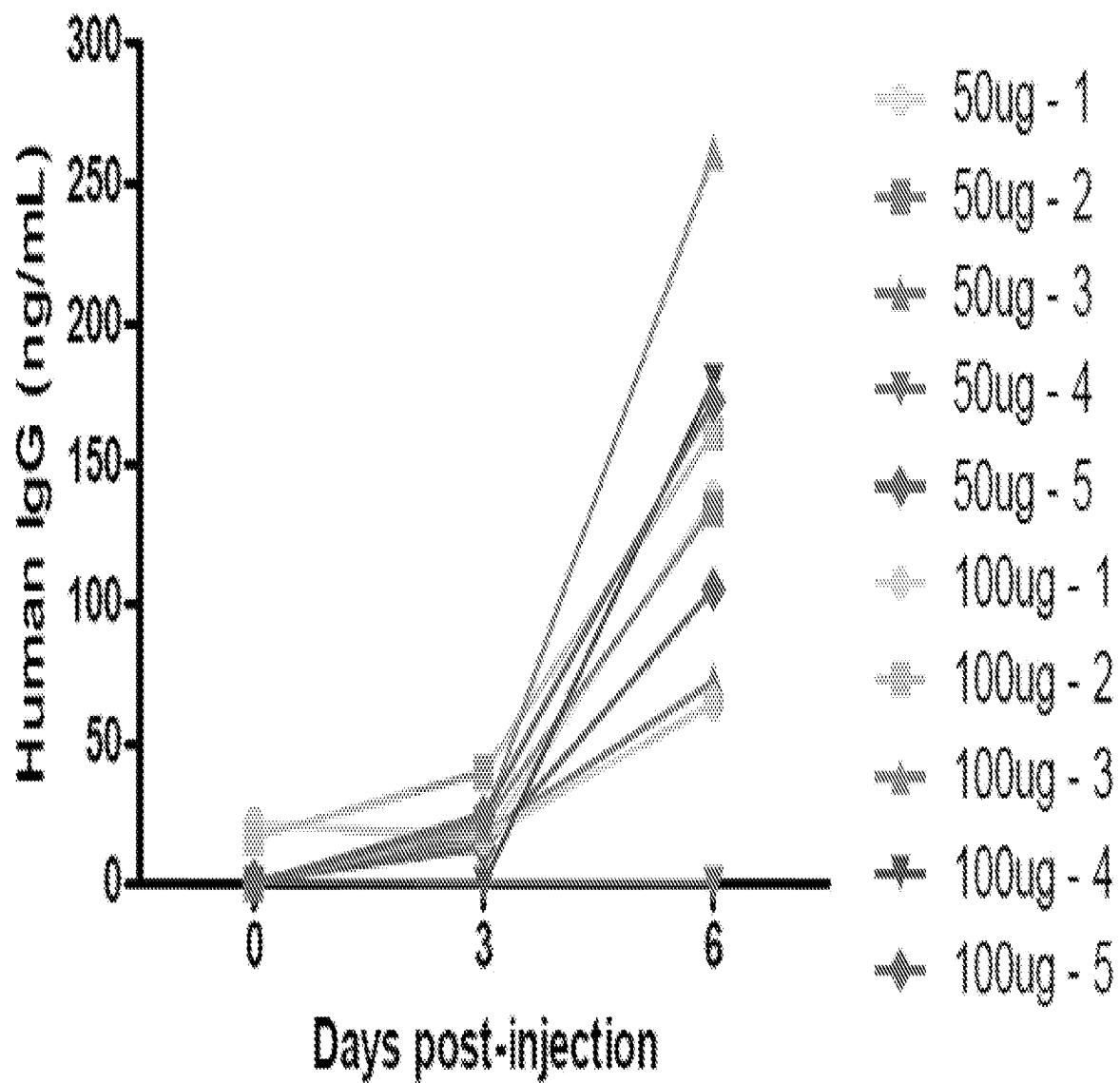


FIG. 41

IgG Heavy Chain

METDTLLLWVLLLWVPGSTGDGAQVQLVQSGAVIKTPGSSVKISCRASGYNFRDYSIHVVRLIPDKGFEWIGWIKPLWGAV
SYARQLQGRVSMTRQLSQDPDDPDWGVAYMEFSGLTPADTAEYFCVRRGSCDYCGDFPWQYWCQGTVVVVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPS
NTKVDKKVEPKSCYPYDVPDYA (SEQ ID NO:46)

FIG. 42**IgG Light Chain**

METDTLLLWVLLLWVPGSTGDGAQVQIVLTQSPGILSLSPGETATLFCKASQGGNAMTWYQKRRGQVPRLLIYDTSRRASG
VPDRFVGSQSGTDFFLTINKLDREDFAVYYCQQFEFFGLGSELEVHRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECYPYDVPDYA
(SEQ ID NO:47)

FIG. 43

Amino Acid Sequence of the Heavy Chain (VH-CH1) of HIV-1 Env Fab

METDTLLLWVLLLWVPGSTGDGAQVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGG
AVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGNCDYNWDFEHWGRGTPVIVSSPSTKGPSVFPLAPSSK
STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKA
EPKSCYPYDVPDYA (SEQ ID NO:48)

FIG. 44**Amino Acid Sequence of the Light Chain (VL-CL) of HIV-1 Env Fab**

METDTLLLWVLLLWVPGSTGDGAQVQIVLTQSPGTLSPGETAIISCRTSQYGS LAWYQQRPGQAPRLVIYSGSTRAAGIPD
RFSGSRWGPDYNTISNLESGDFGVYYCQYEFFGQGTKVQVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGECYPYDVPDYA
(SEQ ID NO:49)

FIG. 45

Nucleic Acid Sequence Encoding HIV-1 PG9 Fab

GGATCCGCCACCATGGCAAGACCCCTGTGCACCCCTGCTGCTGCTGATGGCAACCCTGGCCGGAGCCCTGGCACAGAGC
GCCCTGACCCAGCCCGCAAGCGTCTCCGGCTCACCAGGCCAGAGCATCACTATTAGTTGCAACGGGACTAGCAACGAC
GTGGGAGGCTATGAGAGTGTGAGTGGTACCAGCAGCATCCCGGAAAAGCACAAAAGTGGTCATCTACGATGTCAGT
AAAAGGCCAAGTGGGGTCTCAAATAGGTTCTCAGGGAGTAAATCTGGGAATACAGCATCTCTGACCATCTCCGGACTG
GGCGCAGAAGATGAAGGCGACTACTATTGCAAAAGCCTGACCTCAACCAGACGGCGAGTCTTTGGGACAGGCACCAA
GCTGACAGTCCCTGACAGTCGCTGCCCCCTCCGTCTTCATTTTTCCACCTTCAGATGAGCAGCTGAAATCTGGCACTGCAT
CTGTGGTCTGCCTGCTGAACAATTCTATCCACGAGAGGCCAAGGTGCAGTGGAAAGTGGATAACGCACTGCAGTCCG
GCAATAGTCAGGAAAGCGTGACTGAGCAGGATTCCAAGGACAGTACCTATAGCCTGTCCAGTACACTGACCCTGTCCA
AGGCTGACTACGAAAAACATAAGGTGTATGCATGTGAAGTGAAGTCAACCAGGGACTGAGGTACCAGTCACTAAGTCTT
TTAACAGGGGAGAGTGCGGCGGGGGAGGATCTGGAGGCGGCGGCTCTGGAGGGGGAGGCTCAGGGGGCGGAGGAAG
CGGCGGAGGAGGGTCCGGAGGAGGAGGCAGTCAGAGACTGGTCGAAAGCGGGGGAGGAGTGGTGCAGCCTGGGTCTCT
CACTGAGACTGTCATGCGCTGCCAGTGGCTTTGATTTTTACGACAGGGAATGCATTGGGTCAGGCAGGCACCCGGACA
GGGCTTGAATGGGTGCGCTTCATTAAGTACGACGGAAGCGAGAAGTACCATGCCGACTCAGTGTGGGGAAGGCTGAG
CATCTCAAGGGACAACCTCAAAGGACACCCTGTACCTGCAGATGAATAGCCTGAGAGTGGAAAGATAACGCTACTTATTT
CTGCGTGCGAGAGGCCGGAGGGCCAGATTACCGGAACGGGTACAATTACTATGATTTCTACGACGGCTACTACAATTA
CCATTATATGGATGTCTGGGGCAAAGGAACTACAGTCACCGTGAGCTCCGCAAGTACTAAGGGACCTTCCGTGTTTCCT
CTGGCTCCCAGTTCCAAAAGTACATCCGGAGGAACAGCCGCTCTGGGATGTCTGGTCAAGGACTATTTCCCGAGCCCG
TGACTGTCTCCTGGAACAGCGGGGCTCTGACAAGCGGGGTGCACACCTTTCCTGCCGTGCTGCAGTCCAGTGGGCTGTA
CAGTCTGTCTAGTGTGTCAGTGTGCCAAGCTCAAGTCTGGGGACCCAGACATACATTTGTAATGTGAACCATAAACCC
TCAAACACCAAAGTGGACAAGAAAGTGGAACTAAAAGCTGATAAACTCGAG (SEQ ID NO:50)

FIG. 46

Amino Acid Sequence of HIV-1 PG9 Fab

MARPLCTLLLLMATLAGALAQSAITQPASVSGSPGQSITISCNGTSNDVGGYESVSWYQQHPGKAPKVVIYDVSKRPSGVSN
RFSGSKSGNTASLTISGLGAEDGDYYCKSLTSTRRRVFGTGTKLTVLTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGECGGGGSGGGGS
GGGGSGGGSGGGSGGGGSQRLVESGGGVVQPGSSRLSCAASGFDPSRQGMHWVRQAPGQGLEWVAFIKYDGSEKHYH
ADSVWGRLSISRDNKDTLYLQMNSLRVEDTATYFCVREAGGPDIRNGYNYDFYDGYNYHYMDVWGKGTITVTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV
NHKPSNTKVDKKVEPKS (SEQ ID NO:51)

FIG. 47

Nucleic Acid Sequence Encoding HIV-1 4E10 Fab

GGATCCGCCACCATGGCAAGACCTCTGTGCACTCTGCTGCTGCTGATGGCTACTCTGGCCGGGGCTCTGGCTGAGATTG
TCCTGACCCAGTCCCCTGGCACTCAGTCACTGTCCCCCGGCGAGCGCGCAACTCTGTCCTGCAGAGCAAGCCAGTCCGT
CGGGAACAACAAGCTGGCATGGTACCAGCAGCGCCAGGACAGGCACCCAGGCTGCTGATCTACGGAGCAAGCTCCC
GGCCTAGCGGAGTCGCTGATAGATTCTCCGGAAGCGGCTCCGGGACCGATTTCCTGACCATCTCCAGGCTGGAACC
TGAGGATTTTGCCGTGTATTACTGTCAGCAGTACGGGCAGAGCCTGTCAACTTTCGGCCAGGGAATAAAGTCGAAAA
GAGAACCGTGGCCGCACCAAGCGTCTTTATTTTCCCCCTAGCGATGAACAGCTGAAATCCGGGACTGCTTCCGTGGTC
TGCCTGCTGAATAACTTCTATCCAAGAGAGGCAAAGGTGCAGTGGAAAGTGGACAACGCCCTGCAGAGCGGAAACTCA
CAGGAATCTGTGACAGAGCAGGACTCCAAGGATAGCACATACAGTCTGTCTCAACTCTGACCCTGTCCAAAGCTGAC
TATGAGAAGCATAAAGTCTACGCATGTGAGGTGACCCACCAGGGACTGAGGTCCCCCGTCACTAAGTCCTTCAATAGA
GGCGAGTGCGGGGCGGGGGCAGTGGCGGAGGGGGAAGTGGGGGCGGAGGGAGTGGCGGCGGCGGGAGTGGCGGCG
GCGGCTCAGGGGGCGGCGGCTCCCAGGTCCAGCTGGTCCAGAGCGGAGCCGAGGTCAAGAGACCAGGCTCTTCAGTCA
CCGTGAGCTGCAAAGCCAGCGGAGGCTCCTTTAGCACTTACGCCCTGTCATGGGTGCGGCAGGCCCCAGGCCGAGGCC
TGGAGTGGATGGGCGGCGTGATCCCCCTGCTGACCATTACTAACTATGCCCCCTAGATTTGGAGGCCGGATCACCATCAC
AGCTGACAGATCCACATCCACAGCTTACCTGGAGCTGAACAGTCTGAGGCCCGAGGACACTGCAGTCTACTACTGTGC
ACGAGAAGGCACCACTGGATGGGGGTGGCTGGGGAAGCCCATCGGGGCTTTTGCACATTGGGGCGGAGGGACACTGGT
GACTGTGAGCTCTGCCAGCACTAAAGGGCCAGTGTCTTCCCTCTGGCCCCAAGTTCCAAGAGTACATCAGGGGGCACC
GCGCACTGGGGTGTCTGGTGAAGGATTACTTCCCAGAGCCCGTGACAGTCAGTTGGAACAGCGGCGCTCTGACCAGT
GGGGTGCACACTTTCCCAGCCGTGCTGCAGAGTTCAGGGCTGTACTCCCTGTCTCAGTGGTACTGTGCCCTCAAGCA
GTCTGGGGACTCAGACTTACATTTGTAATGTGAACCATAAACCTCAAATACTAAAGTGGACAAAAAAGTGAACCAA
AGAGCTGATAACTCGAG (SEQ ID NO:52)

FIG. 48

Amino Acid Sequence of HIV-1 4E10 Fab

MARPLCTLLLLMATLAGALAEIVLTQSPGTQSLSPGERATLSCRASQSVGNKLAWYQQRPGQAPRLLIYGASSRPSGVADR
FSGSGSGTDFTLTISRLEPEDFAVYYCQYQGQSLSTFGQGTKVEKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGECGGGGSGGGGSGG
GGSGGGSGGGGSGGGGSQVQLVQSGAEVKRPGSSVTVSCKASGGSFSTYALSWVRQAPGRGLEWMGGVPIPLLITITNYAP
RFGGRITITADRSTSTAYLELNSLRPEDTAVYYCAREGTTGWGWLGPFAFAHWGGGTLVTVSSASTKGPSVFPLAPSSKST
SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KS (SEQ ID NO:53)

FIG. 49

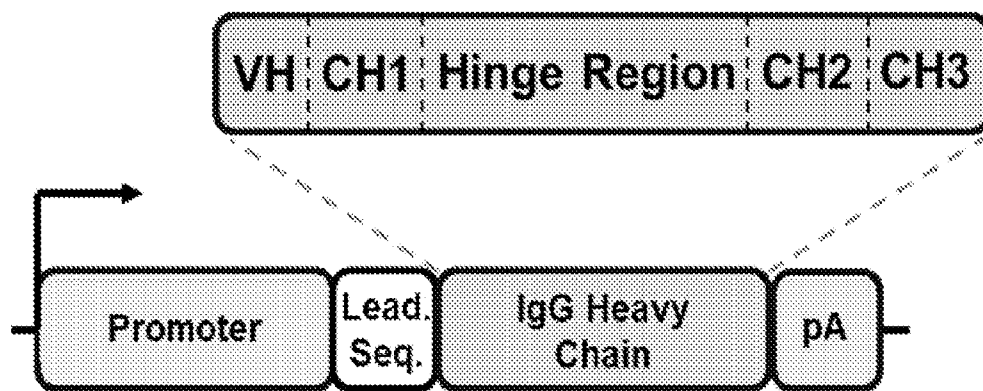


FIG. 50

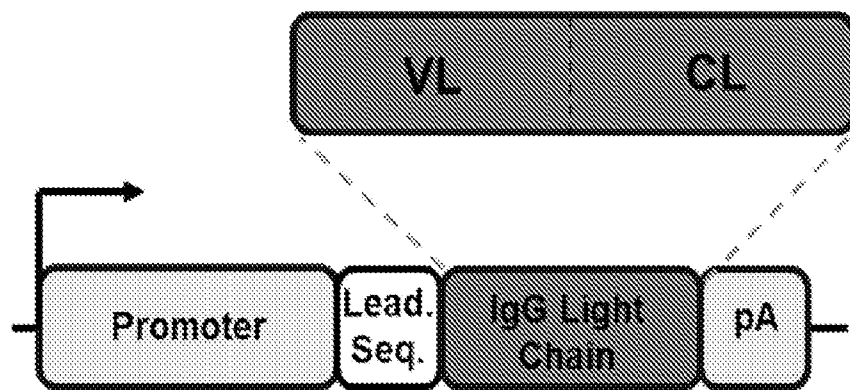


FIG. 51

Nucleic Acid Sequence Encoding the HIV-1 VRC01 IgG1 Heavy Chain (VH/CH1/Hinge/CH2/CH3)

GGATCCGCCACCATGGATTGGACATGGATTCTGTTCTGTCGCCGCCGCAACTAGAGTGCATTACAGGTGCAGCTGG
TGCAGTCAGGCGGGCAGATGAAGAAACCCGGCGAGAGTATGCCAATCTCATGCCGGGCTAGCGGGTACGAATTCATCG
ACTGTACCCTGAACTGGATTAGACTGGCACCTGGGAAGAGGCCAGAGTGGATGGGATGGCTGAAACCTAGAGGCGGG
GCAGTGAATTACGCCAGACCACTGCAGGGCAGGGTCACTATGACCCGCGACGTGTATTCTGATACCGCATTCTGGAG
CTGCCAAGTCTGACAGTCGACGATACTGCCGTGTACTTCTGCACACGGGGCAAGAACTGTGACTATAATTGGGATTTTG
AACTGGGGCAGGGGGACACCTGTCTATTGTGAGCTCCCCAAGTACTAAGGGACCCCTCAGTGTTCCTGGCCCCCTC
TAGTAAAAGTACCTCAGGAGGCACAGCCGCTCTGGGATGCCTGGTGAAGGATTACTTCCTGAGCCAGTCACCGTGAG
TTGGAAGTCAGGCGCCCTGACAAGCGGGTCCATACTTTCCAGCTGTGCTGCAGTCAAGCGGGCTGTACTCCCTGTCC
TCTGTGGTCACAGTGCCAGTTCAAGCCTGGGAACACAGACTTATCTGTAACGTCAATCACAAGCCTAGCAATACTA
AAGTGGACAAGAAAGCCGAGCCTAAGAGCTGCGAACCAAAGTCCTGTGATAAAACCCATACATGCCCTCCCTGTCCAG
CTCCTGAACTGCTGGGCGGCCCATCCGTGTTCTGTTTCCACCCAAGCCCAAAGACACCCTGATGATTAGCAGGACTCC
TGAGGTCACCTGCGTGGTCGTGGACGTGTCCACGAGGACCCCGAAGTCAAGTTTAACTGGTACGTGGATGGCGTCGA
AGTGCATAATGCCAAGACAAAACCCCGGGAGGAACAGTACAACCTCTACCTATAGAGTCGTGAGTGTCTGACAGTGCT
GCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTGTCTAATAAGGCCCTGCCAGCTCCCATCGAGAAAAC
AATTTCCAAGGCAAAAGGCCAGCCAAGGGAACCCAGGTGTACACTCTGCCTCCATCCCGCGACGAGCTGACTAAGAA
CCAGGTCTCTCTGACCTGTCTGGTGAAAGGATTCTATCCAAGCGATATCGCCGTGGAGTGGGAATCCAATGGCCAGCCC
GAGAACAAATTACAAGACCACACCCCTGTGCTGGACAGCGATGGCTCCTTCTTTCTGTATTCAAAGCTGACCGTGGATA
AAAGCCGCTGGCAGCAGGGGAACGTCTTTAGCTGCTCCGTGATGCACGAAGCTCTGCACAATCATTACACCCAGAAGT
CTCTGAGTCTGTACCTGGCAAGTGATAACTCGAG (SEQ ID NO:54)

FIG. 52

Amino Acid Sequence of the HIV-1 VRC01 IgG1 Heavy Chain (VH/CH1/CH2/CH3)

MDWTWILFLVAAATRVHSQVQLVQSGGQMKKPGESMRISCASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYA
RPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGNCDYNWDFEHWGRGTPVIVSSPSTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHNKPSNTKVDKKAEPKSCE
PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:55)

FIG. 53

Nucleic Acid Sequence Encoding the HIV-1 VRC01 IgG Light Chain (VL/CL)

GGATCCGCCACCATGGATTGGACTTGGATTCTGTTCTGGTGGCAGCCGCTACCAGAGTCCATTCCGAAATTGTGCTGA
CCCAGTCTCCCGGAACACTGTCTCTGAGTCCTGGCGAGACAGCCATCATTTCCTGTAGGACTTCTCAGTACGGGAGTCT
GGCATGGTATCAGCAGCGACCAGGACAGGCTCCTCGACTGGTCATCTACTCAGGAAGCACTCGGGCAGCCGGCATTCC
CGACCGATTCTCCGGGTCTCGGTGGGGACCTGATTACAACCTGACCATCTCAAATCTGGAAAGCGGAGACTTTGGCGTG
TACTATTGCCAGCAGTATGAGTTCTTTGGGCAGGGAACCAAGGTCCAGGTGGACATCAAACGCACAGTCGCTGCACCA
AGCGTGTTTCATCTTTCCACCCTCAGATGAACAGCTGAAGTCCGGCACC GCCTCTGTGGTGTGCCTGCTGAACAATTTCTA
CCCCCGGGAGGCAAAGGTCCAGTGGAAAGTGGACAACGCCCTGCAGTCTGGCAATAGTCAGGAGTCAGTGACTGAAC
AGGACAGCAAGGATTCCACCTATTCTCTGTCTCTACTCTGACCCTGAGCAAAGCTGATTACGAGAAGCACAAAGTGTA
TGCATGTGAGGTCACCCACCAGGACTGCGGTCACCCGTCACCAAGAGCTTCAATCGCGGAGAGTGTTGATAACTCGA
G (SEQ ID NO:56)

FIG. 54**Amino Acid Sequence of the HIV-1 VRC01 IgG Light Chain (VL/CL)**

MDWTWILFLVAAATRVHSEIVLTQSPGTLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRW
GPDYNLTISNLESGDFGVYYCQYEFFGQGTKVQVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
NALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGEC (SEQ ID NO:57)

FIG. 55

Nucleic Acid Sequence Encoding the Heavy Chain (VH-CH1) of the CHIKV-Env-Fab

GGATCCGCCACCATGGATTGGACATGGAGGATTCTGTTTCTGGTCGCCGCCGCTACTGGAACTCAGGCTCAGGTGCAGC
TGGTGCAGTCAGGGTCCGAACTGAAGAAACCAGGGGCATCTGTGAAGGTCAGTTGCAAAGCCTCAGGCTACACCCTGA
CACGGTATGCCATGACTTGGGTGCGCCAGGCTCCTGGACAGGGACTGGAGTGGATGGGCTGGATCAACACTTACACCG
GAAATCCAACCTTATGTGCAGGGGTTACCGGCCGATTCTGTGTTTTCTCTGGACACTTCCGTCTCTACCGCCTTTCTGCAC
ATTACAAGTCTGAAGGCAGAGGACACTGCCGTGTACTTCTGCGCTAGGGAAGGCGGAGCAAGAGGCTTTGATTATTGG
GGCCAGGGAACCCTGGTGACAGTCAGCTCCGCCAGCACAAAGGGACCCTCCGTGTTCCCACTGGCTCCCTCTAGTAAA
AGTACATCAGGGGGCACTGCCGCTCTGGGATGTCTGGTCAAAGATTACTTCCCCGAACCTGTGACCGTCAGCTGGA
ACTCCGGAGCTCTGACCAGCGGGGTGCATACATTTCCCGCAGTCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTGGT
CACAGTGCCTAGTTCAAGCCTGGGGACACAGACTTATATCTGTAATGTGAACCATAAGCCAAGCAACACCAAAGTGA
CAAAAAAGTGGAACCTAAGAGCTGCTGATAACTCGAG (SEQ ID NO:58)

FIG. 56**Amino Acid Sequence of the Heavy Chain (VH-CH1) of the CHIKV-Env-Fab**

MDWTWRILFLVAAATGTHAQVQLVQSGSELKKPGASVKVSCKASGYTLTRYAMTWVRQAPGQGLEWMGWINTYTGNPT
YVQGFTGRFVFSLDTSVSTAFSLHITSLKAEDTAVYFCAREGGARGFDYWQGGLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
(SEQ ID NO:59)

FIG. 57

Nucleic Acid Sequence Encoding the Light Chain (VL-CL) of the CHIKV-Env-Fab

GGATCCGCCACCATGGCATGGACCCCACTGTTCTGTTCTGCTGACTTGTTGTCCTGGCGGGAGCAATTCACAGAGCG
TCCTGACCCAGCCCCCTTCTGTGTCCGGAGCACCAGGACAGCGAGTCACAATCTCTTGCACTGGAAGCTCCTCTAACAT
TGGGGCCAGCCACGACGTGCATTGGTACCAGCAGCTGCCAGGGACCGCTCCCACACTGCTGATCTATGTGAACTCTAAT
AGGCCTAGTGGCGTCCCAGATAGATTTTCAGGGAGCAAGTCCGGCACCTCTGCTAGTCTGGCAATTACAGGACTGCAG
GCTGAGGACGAAGCAGATTACTATTGCCAGAGTTACGACTCAAACCTGTCAGGCAGCGCAGTGTTCCGAGGAGGAACT
AAGCTGACCGTCCTGGGACAGCCCCAAAGCCGCTCCTTCTGTGACCCTGTTTCCCCCTAGTTCAGAGGAACTGCAGGCCA
ACAAGGCTACTCTGGTGTGTCTGATCTCCGACTTCTACCCTGGAGCAGTGACCGTCGCATGGAAGGCCGATAGCTCCCC
AGTGAAAGCTGGGGTCGAGACCACAACTCCCAGCAAGCAGTCCAACAACAAGTACGCAGCCTCTAGTTATCTGTCACT
GACACCTGAACAGTGGAAGAGCCACAAATCCTATTCTTGCCAAGTGACTCATGAGGGCAGTACCGTGGAAGAGACAGT
CGCCCCAACTGAGTGTTCTGATAACTCGAG (SEQ ID NO:60)

FIG. 58**Amino Acid Sequence of the Light Chain (VL-CL) of the CHIKV-Env-Fab**

MAWTPLEFLLLTCCPGGSNSQSVLTQPPSVSGAPGQRVTISCTGSSSNIGASHDVHWHYQQLPGTAPTLLIYVNSNRPSGVPDR
FSGSKSGTSASLAITGLQAEDEADYYCQSYDSNLSGSAVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFY
PGAVTVAWKADSSPVKAGVETTTPSKQSNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS (SEQ ID
NO:61)

FIG. 59

Nucleic Acid Sequence Encoding HIV-1 Env-4E10 Ig

GGATCCGCCACCATGGATTGGACATGGAGGATTCTGTTTCTGGTCGCCGCCGCTACAGGAACTCACGCCCAGGTGCAG
CTGGTGCAGTCAGGAGCCGAAGTGAAGCGACCAGGCAGCTCCGTCAGTGTCTGCAAAGCATCTGGCGGATCATTC
AGCACCTACGCCCTGAGCTGGGTGAGACAGGCTCCTGGACGAGGACTGGAATGGATGGGAGGCGTCATCCCACTGCTG
ACAATTACTAACTACGCCCCCGATTTACAGGGCAGGATCACCATTACAGCAGACCGCTCCACTTCTACCGCCTATCTGG
AGCTGAATAGCCTGAGACCAGAAGATACCGCAGTGTACTATTGCGCCCGGGAGGGAACACAGGATGGGGATGGCTG
GGAAAGCCCATCGGGGCTTTTCGCACACTGGGGCCAGGGAACCTGGTTCACAGTGTCTAGTGCCAGCACAAAGGGCCCC
TCCGTGTTTCCCCTGGCTCCTTCAAGCAAAAGTACTTCAGGAGGGACCGCCGCTCTGGGATGTCTGGTGAAGGACTACT
TCCCTGAGCCAGTCACCGTGTCTGGAACCTCTGGCGCTCTGACCTCCGGAGTGCATACATTTCCCGCAGTCCTGCAGTC
CTCTGGGCTGTACTCTCTGAGTTCAGTGGTCACTGTGCCTAGCTCCTCTCTGGGCACACAGACTTATATCTGCAACGTGA
ATCACAAGCCCTCCAATACCAAAGTCGACAAGAAAGTGAACCTAAGTCTTGTGATAAAACCCATACATGCCCACCTT
GTCCAGCACCTGAGCTGCTGGGCGGACCTTCCGTGTTCTGTTCACCCAAGCCAAAAGACACACTGATGATTAGCCG
GACACCTGAAGTGACTTGTGTGGTCGTGGACGTCAGCCACGAGGACCCCGAAGTGAAGTTCAACTGGTACGTGGATGG
CGTCGAGGTGCATAATGCCAAGACCAAACCCAGGGAGGAACAGTACAACCTCTACTTATAGGGTCGTGAGTGTCTGAC
CGTGCTGCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTGTCCAATAAGGCCCTGCCAGCTCCCATCGA
GAAAACAATTTCTAAGGCTAAAGGCCAGCCACGCGAACCCCAAGGTGTACACTCTGCCTCCCAGCAGGGACGAGCTGAC
CAAGAACCAGGTGAGTCTGACATGTCTGGTCAAAGGCTTCTATCCAAGCGATATCGCCGTGGAGTGGGAATCCAATGG
ACAGCCCGAAAACAATTACAAGACTACCCCCCTGTGCTGGACAGTGATGGATCATTCTTTCTGTATTCCAAGCTGACC
GTGGACAAATCTCGCTGGCAGCAGGGGAACGTCTTTAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCATTACACA
CAGAAGTCTCTGAGTCTGTCACCAGGCAAGCGGGGACGCAAAAGGAGAAGCGGGTCCGGCGCTACTAACTTCAGCCTG
CTGAAACAGGCAGGGGATGTGGAGGAAAATCCTGGCCCAATGGTCTGCAGACCCAGGTGTTTATCTCACTGCTGCTGT
GGATTAGCGGGGCTTATGGCGAAATCGTGCTGACTCAGAGCCCCGGAACCCAGTCTCTGAGTCCTGGGGAGCGCGCTA
CACTGAGCTGTCGAGCATCACAGAGCGTGGGGAACAATAAGCTGGCATGGTACCAGCAGAGGCCTGGCCAGGCTCCAA
GACTGCTGATCTATGGCGCAAGTTCACGGCCTAGCGGAGTGGCAGACCGCTTCTCCGGATCTGGGAGTGGCACCGATTT
TACTCTGACCATTAGCAGGCTGGAGCCAGAAGACTTCGCTGTGTACTATTGCCAGCAGTACGGCCAGTCACTGAGCACA
TTTGGACAGGGGACTAAGGTCGAAAAAAGAACCGTGGCAGCCCCAAGTGTCTTCATTTTCCACCCTCAGACGAGCAG
CTGAAGAGTGGAACAGCCTCAGTCGTGTGTCTGCTGAACAATTTCTACCCCAGGGAGGCCAAGGTCCAGTGGAAAGTG
GATAACGCTCTGCAGAGCGGCAATTCAGGAGTCTGTGACAGAACAGGACAGTAAGGATTCACTTATAGCCTGAGC
TCCACACTGACTCTGTCCAAAGCAGATTACGAGAAGCACAAAGTGTATGCCTGCGAAGTCACCCATCAGGGACTGTCT
AGTCCTGTGACAAAGTCTTTTAACAGAGGGGAGTGATAACTCGAG (SEQ ID NO:62)

FIG. 60

Nucleic Acid Sequence Encoding HIV-1 Env-PG9 Ig

GGATCCGCCACCATGGACTGGACTTGGAGGATTCTGTTTCTGGTCGCCGCCGCAACTGGAACTCACGCTGAATTTGGAC
TGTCATGGGTCTTTCTGGTGGCCTTTCTGCGAGGGGTCCAGTGCCAGAGGCTGGTGGAGTCCGGAGGAGGAGTGGTCCA
GCCAGGCAGCTCCCTGCGACTGAGTTGTCCGCTTCAGGGTTCGACTTTTCTAGACAGGGCATGCACTGGGTGCGGCAG
GCACCAGGACAGGGACTGGAGTGGGTGGCTTTCATCAAGTACGACGGAAGTAAAAATATCATGCCGATTCAGTGTGG
GGCGGGCTGTCAATTAGCCGCGACAACCTCCAAGGATACCCTGTACCTGCAGATGAATTCTCTGAGGGTCGAGGACACA
GCTACTTATTTCTGCGTGAGGGAAGCAGGCGGACCTGATTACAGAAACGGGTATAATTACTATGACTTTTACGATGGCT
ACTATAACTACCACTATATGGACGTGTGGGGCAAGGGAACACAGTCACAGTGTCTAGTGCATCAACTAAAGGCCCAA
GCGTGTTCCTCCCTGGCCCCCTCAAGCAAGTCCACTTCTGGAGGAACCGCAGCACTGGGATGTCTGGTGAAGGATTACTT
CCCTGAGCCAGTCACCGTGAGTTGGAAGTCAAGGCGCCCTGACTAGCGGAGTCCATACCTTTCCTGCTGTGCTGCAGTCC
TCTGGGCTGTACAGCCTGAGTTCAGTGGTCACAGTGCCAAGCTCCTCTCTGGGCACCCAGACATATATCTGCAACGTGA
ATCACAAGCCTAGCAATACTAAGGTGACAAAAAGAGTGGAACCAAAGAGCTGTGATAAAACTCATACCTGCCACCTT
GTCCAGCACCTGAGCTGCTGGGAGGGCCTTCCGTGTTCTGTTTCCACCCAAGCCAAAAGACACCCTGATGATTAGCCG
GACACCAGAAGTCACTTGCCTGGTCTGTGGACGTGAGCCACGAGGACCCCGAAGTCAAGTTTAACTGGTACGTGGATGG
CGTCGAGGTGCATAATGCTAAGACAAAACACGGGAGGAACAGTACAACCTCCACATATCGCGTGTGTCTGTCTGCTGAC
TGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTATAAGTGCAAAGTGTCCAATAAGGCACTGCCAGCCCCCATCGA
GAAAACCATTTCTAAGGCCAAAGGCCAGCCACGAGAACCCCAAGGTGTACACACTGCCTCCAAGTAGGGACGAGCTGAC
TAAGAACCAGGTCTCTCTGACCTGTCTGGTGAAGGCTTCTATCCCTCTGATATCGCTGTGGAGTGGGAAAGTAATGGA
CAGCCTGAAAACAATTACAAGACTACCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCTGTATAGCAAGCTGACCG
TGGACAAATCCAGATGGCAGCAGGGGAACGTCTTTAGTTGCTCAGTGATGCACGAGGCACTGCACAATCATTACACCC
AGAAAAGCCTGTCCCTGTCTCCTGGCAAGAGGGGAAGAAAAAGGAGAAGTGGGTGAGGCGCAACAACTTCAGCCTG
CTGAAGCAGGCCGGAGATGTGGAGGAAAATCCTGGGCCAATGGCTTGGACCCCCCTGTTCTGTTTCTGCTGACATGCT
GTCCTGGCGGAAGCAACTCCAGTCTGCACTGACACAGCCAGCAAGTGTGTGAGGAGCCAGGACAGAGCATCACCA
TTTCTGTAAACGGCACAAGCAATGACGTGGGGGCTACGAGTCCGTGTCTTGGTATCAGCAGCATCCTGGAAAGGCCCC
AAAAGTCGTGATCTACGATGTCAGCAAACGCCCCCTCTGGGGTGAGTAACCGATTCACTGGATCAAAGAGCGGGAATAC
CGCTTCTCTGACAATTAGTGGCTGCAGGCAGAGGACGAAGGAGATTACTATTGCAAATCACTGACAAGCACTCGGCG
CCGAGTCTTCGGAACCGGGACAAAGCTGACTGTGCTGGGCCAGCCAAAGCTGCACCTAGCGTGACCCTGTTTCCACCC
AGTTCAGAGGAAGTGCAGGCTAATAAGGCAACACTGGTGTGTCTGATCTCCGACTTCTACCCTGGCGCTGTCACTGTGG
CCTGGAAGGCTGATAGCTCCCAAGTCAAAGCAGGAGTGGAACAACCTACCCCTCCAAGCAGTCTAACAACAAGTACG
CCGCTTCTAGTTATCTGTCACTGACTCCCGAGCAGTGGAAGAGCCACAAATCCTATTCTTGCCAGGTGACCCATGAGGG
CTCCACTGTGAAAAAGACCGTGGCCCCCTACAGAGTGTCTTGATAACTCGAG (SEQ ID NO:63)

FIG. 61

Nucleic Acid Sequence Encoding VRC01 IgG

GGATCCGCCACCATGGATTGGACATGGATTCTGTTCTGGTCGCCGCCGCAACTAGAGTGCATTCACAGGTGCAGCTGG
TGCAGTCAGGCGGGCAGATGAAGAAACCCGGCGAGAGTATGCCAATCTCATGCCGGGCTAGCGGGTACGAATTCATCG
ACTGTACCCTGAACTGGATTAGACTGGCACCTGGGAAGAGGCCAGAGTGGATGGGATGGCTGAAACCTAGAGGCGGG
GCAGTGAATTACGCCAGACCACTGCAGGGCAGGGTCACTATGACCCGCGACGTGTATTCTGATACCGCATTCCTGGAG
CTGCCAAGTCTGACAGTCGACGATACTGCCGTGTACTTCTGCACACGGGGCAAGAACTGTGACTATAATTGGGATTTTG
AACTGGGGCAGGGGGACACCTGTCTATTGTGAGCTCCCCAAGTACTAAGGGACCCCTCAGTGTTCCTGGCCCCCTTC
TAGTAAAAGTACCTCAGGAGGCACAGCCGCTCTGGGATGCCTGGTGAAGGATTACTTCCTGAGCCAGTCACCGTGAG
TTGGAATCAGGCGCCCTGACAAGCGGGTCCATACTTTTCCAGCTGTGCTGCAGTCAAGCGGGCTGTACTCCCTGTCC
TCTGTGGTCACAGTGCCAGTTCAAGCCTGGGAACACAGACTTATCTGTAACGTCAATCACAAGCCTAGCAATACTA
AAGTGGACAAGAAAGCCGAGCCTAAGAGCTGCGAACCAAAGTCCTGTGATAAAACCCATACATGCCCTCCCTGTCCAG
CTCCTGAACTGCTGGGCGGCCCATCCGTGTTCTGTTTCCACCCAAGCCCAAAGACACCCTGATGATTAGCAGGACTCC
TGAGGTCACCTGCGTGGTCTGGACGTGTCCACGAGGACCCCGAAGTCAAGTTTAACTGGTACGTGGATGGCGTCGA
AGTGCATAATGCCAAGACAAAACCCCGGGAGGAACAGTACAACCTACCTATAGAGTCGTGAGTGTCTGACAGTGCT
GCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTGTCTAATAAGGCCCTGCCAGCTCCCATCGAGAAAAC
AATTTCCAAGGCAAAAGGCCAGCCAAGGGAACCCAGGTGTACACTCTGCCTCCATCCCGCGACGAGCTGACTAAGAA
CCAGGTCTCTCTGACCTGTCTGGTGAAAGGATTCTATCCAAGCGATATCGCCGTGGAGTGGGAATCCAATGGCCAGCCC
GAGAACAAATTACAAGACCACACCCCTGTGCTGGACAGCGATGGCTCCTTCTTTCTGTATTCAAAGCTGACCGTGGATA
AAAGCCGCTGGCAGCAGGGGAACGTCTTTAGCTGCTCCGTGATGCACGAAGCTCTGCACAATCATTACACCCAGAAGT
CTCTGAGTCTGTACCTGGCAAGAGGGGACGAAAACGGAGAAGCGGCAGCGGAGCTACAACTTCAGCCTGCTGAAA
CAGGCAGGCGACGTGGAGGAAAATCCTGGGCCAATGGATTGGACTTGGATTCTGTTCTCTGGTGGCAGCCGCTACCAGA
GTCCATTCCGAAATTGTGCTGACCCAGTCTCCCGGAACACTGTCTCTGAGTCCTGGCGAGACAGCCATCATTTCTGTGTA
GGACTTCTCAGTACGGGAGTCTGGCATGGTATCAGCAGCGACCAGGACAGGCTCCTCGACTGGTCATCTACTCAGGAA
GCACTCGGGCAGCCGGCATTCCCGACCGATTCTCCGGGTCTCGGTGGGGACCTGATTACAACCTGACCATCTCAAATCT
GGAAAGCGGAGACTTTGGCGTGTACTATTGCCAGCAGTATGAGTTCCTTGGGCAGGGAACCAAGGTCCAGGTGGACAT
CAAACGCACAGTCGCTGCACCAAGCGTGTTCATCTTTCCACCCCTCAGATGAACAGCTGAAGTCCGGCACCGCCTCTGTG
GTGTGCCTGCTGAACAATTTCTACCCCCGGGAGGCAAAGGTCCAGTGGAAAGTGGACAACGCCCTGCAGTCTGGCAAT
AGTCAGGAGTCAGTGAACAGGACAGCAAGGATTCCACCTATTCTGTCTCTACTCTGACCCTGAGCAAAGCTG
ATTACGAGAAGCACAAAGTGTATGCATGTGAGGTCACCCACCAGGGACTGCGGTCACCCGTCACCAAGAGCTTCAATC
GCGGAGAGTGTGATAACTCGAG (SEQ ID NO:64)

FIG. 62