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

(57) **Abstract**: Disclosed herein are compositions including optimized nucleic acid sequences that encode antibodies and functional fragments thereof, for expressing synthetic antibodies in vivo, and methods of generating synthetic antibodies in a subject. The disclosure also provides methods of preventing and/or treating disease in a subject using said compositions and methods.

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-1Env-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 ± 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 10μg 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 (μg/mL) ± 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 50μl 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 (μg/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 hyrophobicity 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 metalothionein.

[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 cttggattct 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. 60/939792, 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 papillomoa 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, Caldiserica, 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, Diphyllobothriasis, 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 (Aβ) or a fragment or a variant thereof. The Aβ antigen can comprise an Aβ(X-Y) peptide, wherein the amino acid sequence from amino acid position X to amino acid Y of the human sequence Aβ 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 DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVI (corresponding to amino acid positions 1 to 47; the human query sequence) or variants thereof. The Aβ antigen can comprise an Aβ polypeptide of Aβ(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 Aβ 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). PMSA 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 VCR01 is a neutralizing CD4-binding site-antibody for HIV. HIV-1 VCR01 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), Freunds 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), Freunds 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 μs, 20 μs, 10 μs or 1 μs, 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 underling 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-Bl 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 (SED 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 BamH1 and Xho1 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-1Env-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 ($1\text{-}3x10^5$ cells/2 mL per well in 35 mm culture dish) 24 hours before subsequent transfection with 5 μg of the pVax1 control or pHIV-1Env-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 (2μg /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 concensus 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-Bl 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, 10^4 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-(mean RLU of experimental samples-controls)/mean RLU from controls-no addition control wells)) x 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-1Env-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 tranfected to 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-Bl target cells was performed. The TZM-Bl 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 2-3μg/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 ± SD serum concentrations of 0.31±0.33μg/mL and 1.8±1.1μg/mL, respectively. Furthermore, the TNF inhibitor Adalimumab (Humira) has a mean rough serum concentration of around 6 μg/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±0.13μg/mL) occurring one-week post-administration (FIG. 6D). This level of Ig was relatively similar to the 8.3μg/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-1Env-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 - QVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRG GAVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEH 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 HTCPPCP (SEQ ID NO:11).

[00323] Constant Heavy Region 2 (CH2) of VRC01 IgG - APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK (SEQ ID NO:12).

[00324] Constant Heavy Region 3 (CH3) of VRC01 IgG - GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (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 - EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDR FSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKR (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 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: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 Xho1, 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 lamba). 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 – EFGLSWVFLVAFLRGVQCQRLVESGGGVVQPGSSLRLSCAASGFDFSRQGMHWVR QAPGQGLEWVAFIKYDGSEKYHADSVWGRLSISRDNSKDTLYLQMNSLRVEDTATY FCVREAGGPDYRNGYNYYDFYDGYYNYHYMDVWGKGTTVTVSS (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 – APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK (SEQ ID NO:22).

[00343] Constant Heavy Region 3 (CH3) of HIV-1 Env-PG9 Ig – GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (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 Lamba Light Chain Signal Peptide of HIV-1 Env-PG9 Ig – MAWTPLFLFLLTCCPGGSNS (SEQ ID NO:26).

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

SGVSNRFSGSKSGNTASLTISGLQAEDEGDYYCKSLTSTRRRVFGTGTKLTVL (SEQ ID NO:27).

[00348] Constant Light Region (CL, lamba) of HIV-1 Env-PG9 Ig – GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTT PSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS (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 Xho1, 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 ITNYAPRFQGRITITADRSTSTAYLELNSLRPEDTAVYYCAREGTTGWGWLGKPIGAF AHWGQGTLVTVSS (SEQ ID NO:30).

[00356] Constant Heavy region 1 (CH1) of HIV-1 Env-4E10 Ig – ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV (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 – GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (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 VADRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGQSLSTFGQGTKVE (SEQ ID NO:38).

[00364] Constant Light Region (CL, kappa) of HIV-1 Env-4E10 Ig – KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE (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 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-1Env 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, lamba 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 CHIV-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 μg CHIKV-H and 12.5 μg CHIKV-L). Additionally, a second, third, and fourth group of mice were administered 25 μg 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 underling 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 Xho (CTC GAG) restriction enzyme sites, respectively, used to cloned the nucleic acid sequence into the pVAX1vector 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 BamH1 (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.

CLAIMS

What is claimed is:

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 of claim 1, wherein the recombinant nucleic acid sequence further comprises a Kozak sequence.

17. The method of claim 1, 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 of claim 21, wherein the heavy chain polypeptide comprises a variable heavy region and a constant heavy region 1.

26. The method of claim 21, 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 of claim 21, wherein the light chain polypeptide comprises a variable light region and a constant light region.

28. The method of claim 21, wherein the first recombinant nucleic acid sequence and the second recombinant nucleic acid sequence further comprise a Kozak sequence.

29. The method of claim 21, 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 of claim 1 or 21.

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

GGATCCGCCACC**ATG**GAAACCGACACTCTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCCGGCTCAACAGGCGACGGC
GCTCAGGTCCAGCTGGTCCAGTCTGGAGCTGTGATCAAGACCCCTGGCAGCTCCGTCAAAATTTCTTGCAGAGCAAGTG
GCTACAACTTCCGGGACTATAGCATCCACTGGGTGCGGCTGATTCCTGATAAGGGATTTGAGTGGATCGGCTGGATCAA
GCCACTGTGGGGCGCTGTGTCCTACGCAAGGCAGCTGCAGGGGCGCGTCTCCATGACACGACAGCTGTCTCAGGACCC
AGACGATCCCGATTGGGGGGTGGCCTACATGGAGTTCAGTGGACTGACTCCCGCAGACACCGCCGAATATTTTTGCGTG
CGGAGAGGCTCCTGCGACTACTGTGGGGATTTCCCATGGCAGTATTGGTGTCAGGGAACTGTGGTCGTGGTCTCTAGTG
CATCAACCAAGGGCCCCAGCGTGTTTCCTCTGGCCCCATCAAGCAAAAGTACATCAGGAGGAACTGCAGCTCTGGGAT
GTCTGGTGAAGGATTACTTCCCCGAGCCTGTGACCGTCAGCTGGAACTCCGGAGCACTGACCTCCGGAGTGCACACATT
TCCCGCTGTCCTGCAGTCCTCTGGGCTGTACTCTCTGAGTTCAGTGGTCACAGTGCCTAGCTCCTCTCTGGGCACCCAGA
CATATATCTGCAACGTCAATCATAAGCCAAGTAATACTAAAGTGGACAAGAAAGTCGAACCCAAATCATGTTACCCCT
ATGACGTGCCTGATTATGCT**TGATAA**CTCGAG (SEQ ID NO:6)

FIG. 1

Optimized Nucleic Acid Sequence Encoding IgG Light Chain

GGATCCGCCACCATGGAGACTGATACACTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCTGGCTCAACCGGCGACGGG
GCTCAGGTCCAGATTGTGCTGACCCAGAGCCCTGGCATCCTGTCACTGAGCCCAGGAGAGACCGCAACACTGTTCTGCA
AGGCCTCCCAGGGCGGGAACGCTATGACATGGTACCAGAAACGGAGAGGACAGGTGCCCCGACTGCTGATCTATGACA
CTTCAAGGCGAGCAAGCGGAGTGCCTGATCGATTTGTCGGCAGCGGCTCTGGGACAGACTTCTTTCTGACTATTAATAA
GCTGGACAGAGAGGATTTCGCTGTGTACTATTGCCAGCAGTTTGAATTCTTTGGACTGGGCAGCGAGCTGGAAGTGCAC
AGGACCGTCGCCGCTCCAAGTGTGTTCATTTTTCCCCCTAGCGATGAGCAGCTGAAATCCGGGACAGCCTCTGTGGTCT
GTCTGCTGAACAATTTCTACCCCCGCGAAGCAAAGGTGCAGTGGAAAGTCGACAACGCCCTGCAGAGTGGCAATTCAC
AGGAGAGCGTGACCGAACAGGACTCCAAGGATTCTACATATAGTCTGAGCTCCACTCTGACCCTGTCTAAAGCTGATTA
CGAGAAGCACAAAGTGTATGCATGCGAAGTCACTCATCAGGGCCTGTCTAGTCCTGTGACCAAGAGCTTTAACCGAGG
GGAGTGTTACCCATATGACGTCCCCGATTACGCCTGATAACTCGAG (SEQ ID NO:7)

FIG. 2

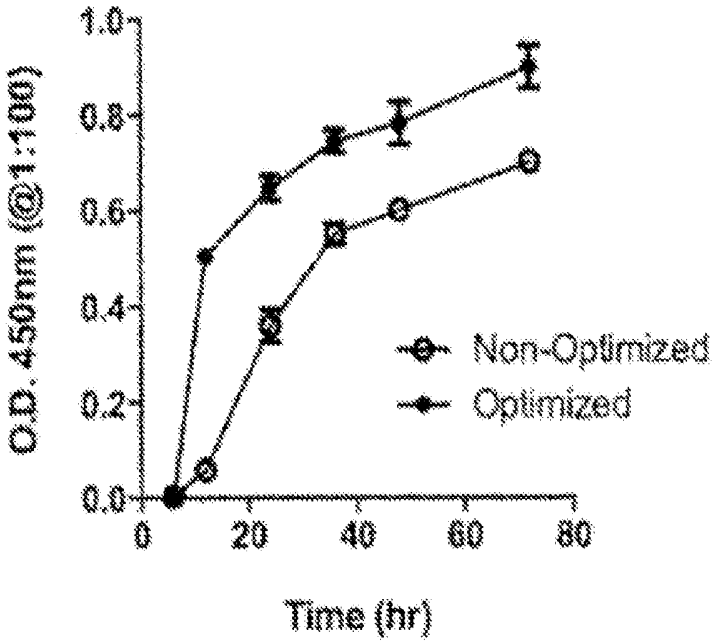
O.D. 450nm (@1:100)
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Time (hr)
Non-Optimized
Optimized

FIG. 3

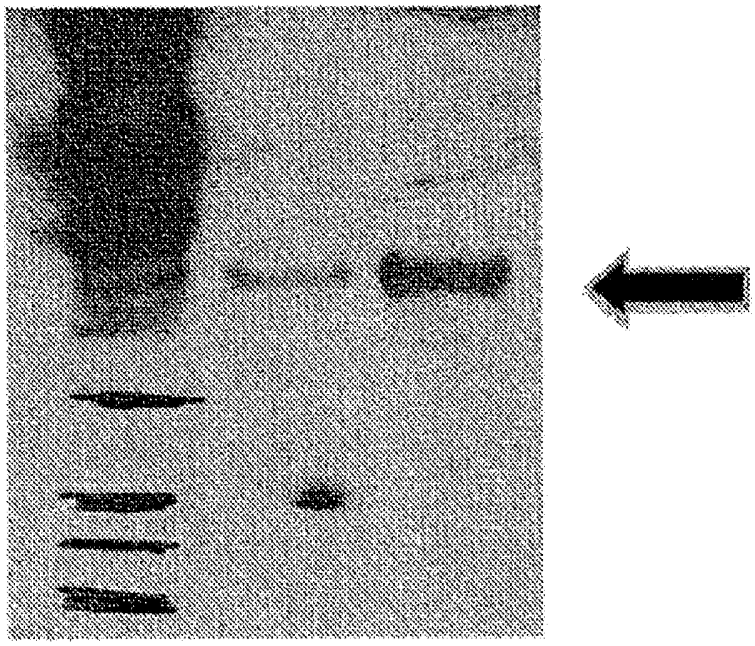

FIG. 4

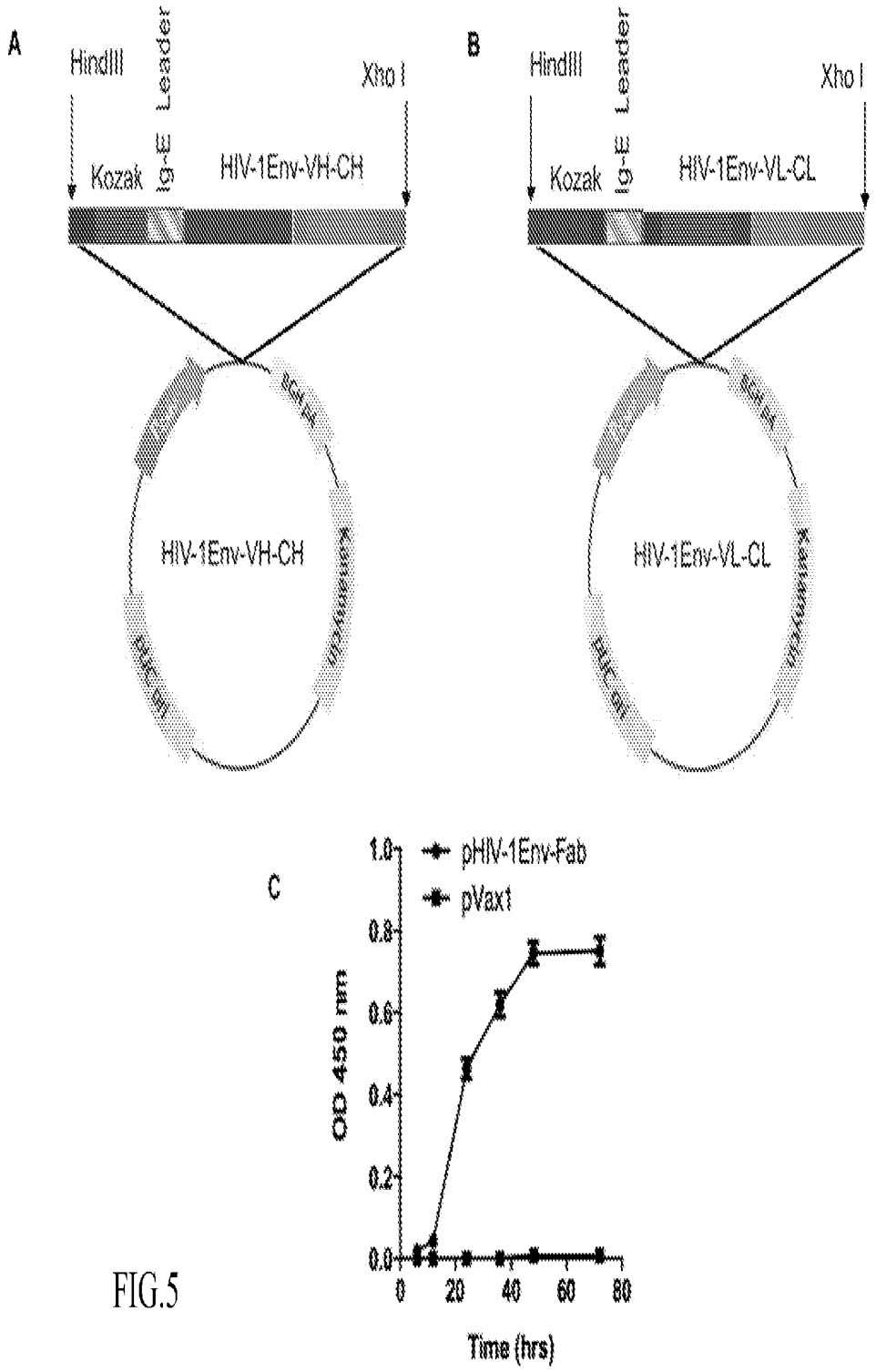
A
HindIII
Leader
Ig-E
Kozak
HIV-1Env-VH-CH
Xho I
B
HindIII
Leader
Ig-E
Kozak
HIV-1Env-VL-CL
Xho I
HIV-1Env-VH-CH
HIV-1Env-VL-CL
C
pHIV-1Env-Fab
pVax1
OD 450 nm
Time (hrs)
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80

FIG.5

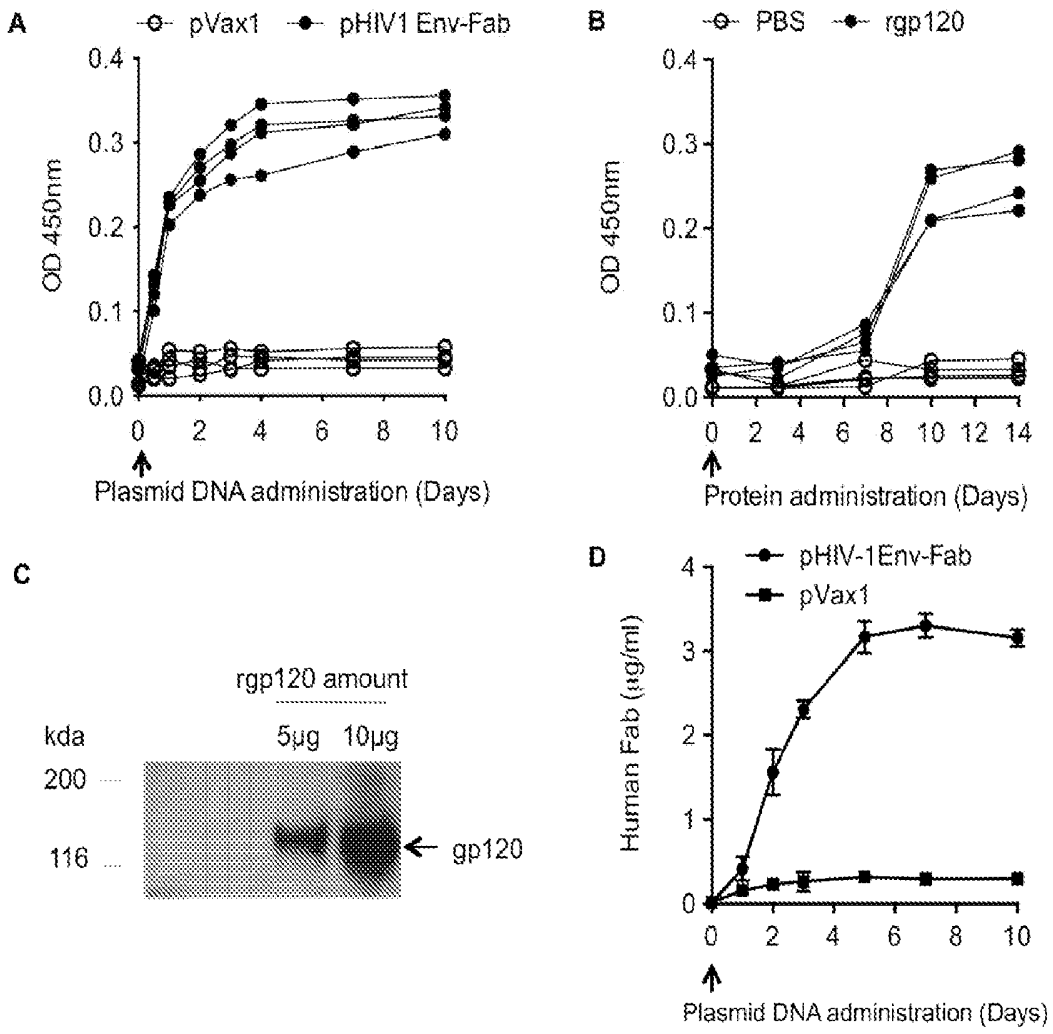
A
pVax1
pHIV1 Env-Fab
OD 450nm
0.4
0.3
0.2
0.1
0.0
0 2 4 6 8 10
Plasmid DNA administration (Days)
B
PBS
rgp120
OD 450nm
0.4
0.3
0.2
0.1
0.0
0 2 4 6 8 10 12 14
Protein administration (Days)
C
rgp120 amount
kda
5μg 10μg
200
116
gp120
D
pHIV-1Env-Fab
pVax1
Human Fab (μg/ml)
4
3
2
1
0
0 2 4 6 8 10
Plasmid DNA administration (Days)

FIG. 6

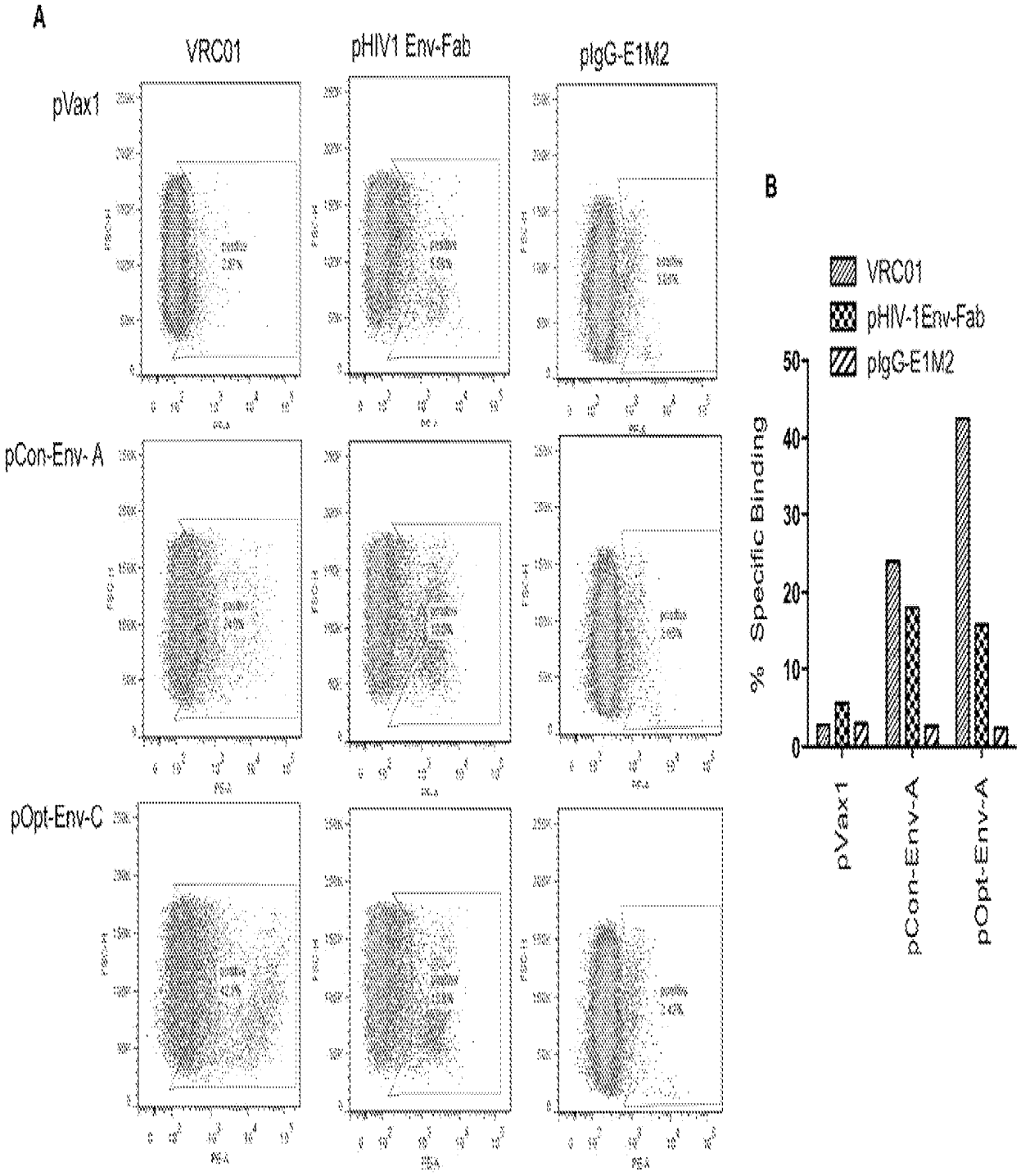
A
VRC01
pHIV1 Env-Fab
pIgG-E1M2
pVax1
pCon-Env-A
pOpt-Env-C
B
VRC01
pHIV-1Env-Fab
pIgG-E1M2
% Specific Binding
0
10
20
30
40
50
pVax1
pCon-Env-A
pOpt-Env-A

FIG.7

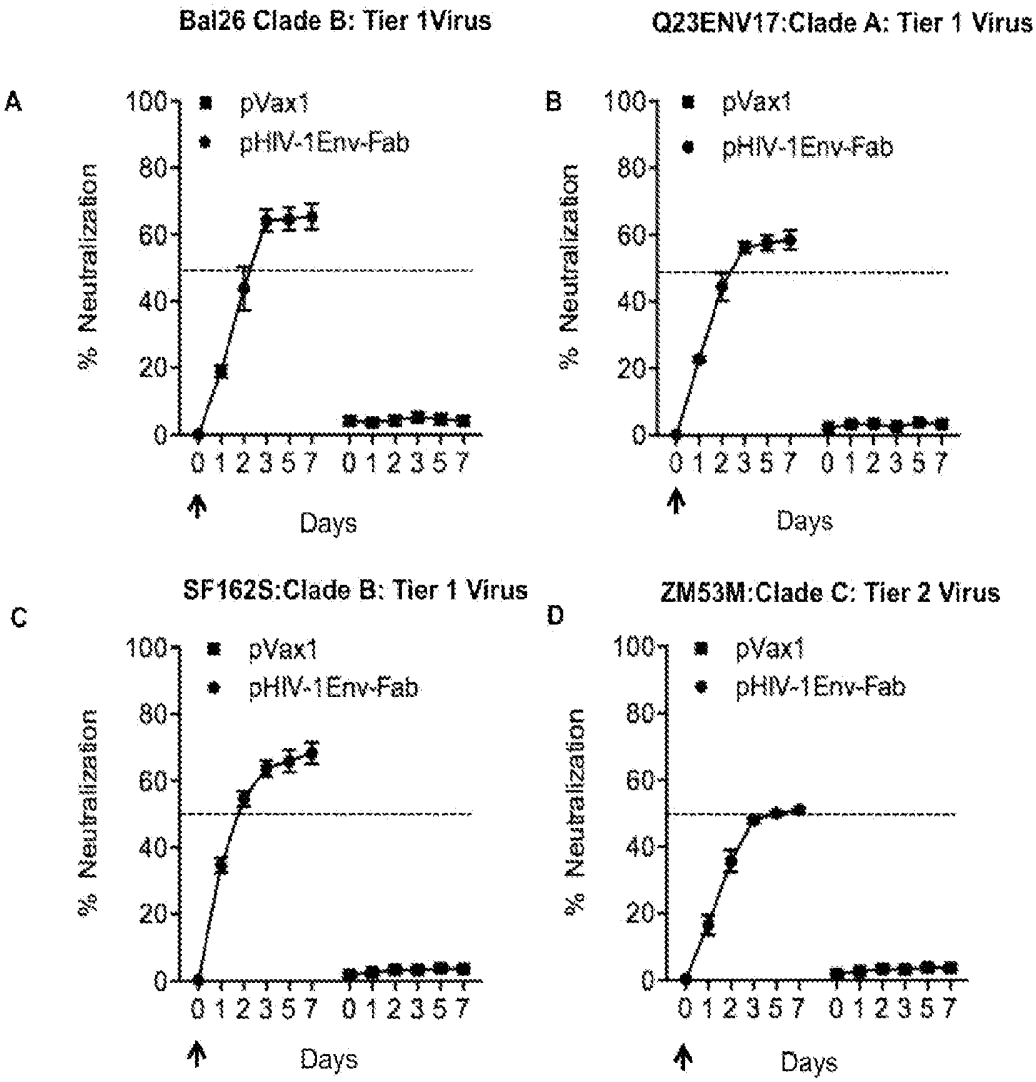
Bal26 Clade B: Tier 1Virus
Q23ENV17:Clade A: Tier 1 Virus
SF162S:Clade B: Tier 1 Virus
ZM53M:Clade C: Tier 2 Virus
A
B
C
D
pVax1
pHIV-1Env-Fab
% Neutralization
Days
0 1 2 3 5 7 0 1 2 3 5 7
0
20
40
60
80
100

FIG. 8

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

AAGCTTGCCGCCACC**ATG**GAGACTGATACACTGCTGCTGTGGGTGCTGCTGCTGTGG
GTGCCAGGGTCAACCGGAGATGGGGCTCAGGTCCAGCTGGTCCAGAGCGGCGGACA
GATGAAGAAACCCGGCGAGAGCATGAGGATCTCCTGCAGAGCATCTGGATACGAGT
TCATCGACTGTACCCTGAACTGGATTAGGCTGGCTCCTGGAAAGAGACCAGAGTGG
ATGGGGTGGCTGAAACCACGAGGGGGAGCAGTGAATTACGCCCGGCCCCTGCAGGG
ACGAGTGACCATGACCAGGGACGTGTACAGCGATACCGCCTTCCTGGAGCTGCGGT
CCCTGACAGTGGACGATACTGCTGTCTACTTCTGCACACGCGGAAAGAACTGTGACT
ATAATTGGGATTTTGAACACTGGGGCCGGGGAACACCCGTGATCGTCAGCTCCCCCA
GTACTAAGGGACCTTCAGTGTTTCCACTGGCCCCCTCTAGTAAATCCACCTCTGGAG
GGACAGCCGCTCTGGGATGCCTGGTGAAAGATTATTTCCCCGAACCTGTGACCGTCA
GTTGGAACTCAGGGGCTCTGACTTCTGGCGTGCACACCTTTCCTGCAGTCCTGCAGT
CAAGCGGGCTGTACAGTCTGTCCTCTGTGGTCACTGTGCCTAGTTCAAGCCTGGGCA
CTCAGACCTATATTTGTAACGTGAATCATAAGCCATCCAATACAAAAGTGGACAAA
AAAGCCGAACCCAAATCCTGTTACCCTTATGATGTGCCCGACTACGCC**TGA**CTCGAG
(SEQ ID NO:3)

FIG. 9

Light Chain (VL-CL) of HIV-1 Env Fab

AAGCTTGCCGCCACC**ATG**GAAACCGATACACTGCTGCTGTGGGTGCTGCTGCTGTGG
GTGCCAGGAAGTACCGGGGATGGGGCTCAGGTCCAGATTGTGCTGACTCAGTCCCCT
GGGACCCTGTCTCTGAGTCCAGGCGAGACAGCTATCATTTCATGCCGAACTAGCCAG
TACGGCAGCCTGGCTTGGTATCAGCAGCGACCAGGACAGGCACCACGACTGGTCAT
CTACTCAGGCAGCACAAGGGCCGCTGGCATCCCCGACAGGTTCTCCGGCAGCAGGT
GGGGGCCTGATTACAACCTGACTATCTCTAATCTGGAGAGTGGGGACTTTGGCGTGT
ACTATTGCCAGCAGTATGAGTTCTTCGGCCAGGGAACTAAGGTGCAGGTGGACATC
AAAAGAACCGTGGCAGCCCCATCCGTCTTCATTTTTCCCCCTTCTGATGAGCAGCTG
AAGTCAGGCACCGCCAGCGTGGTCTGTCTGCTGAACAATTTCTACCCCCGGGAAGCC
AAGGTGCAGTGGAAAGTGGACAACGCTCTGCAGAGTGGAAATTCACAGGAGAGCGT
GACCGAACAGGACTCCAAGGATTCTACATATAGTCTGAGCAGCACCCTGACCCTGA
GTAAAGCAGATTACGAGAAGCACAAAGTGTATGCCTGTGAAGTCACACATCAGGGC
CTGAGGAGCCCCGTGACTAAAAGTTTCAACCGAGGAGAGTGCTACCCTTATGATGTG
CCCGACTACGCC**TAA**CTCGAG (SEQ ID NO:4)

FIG. 10

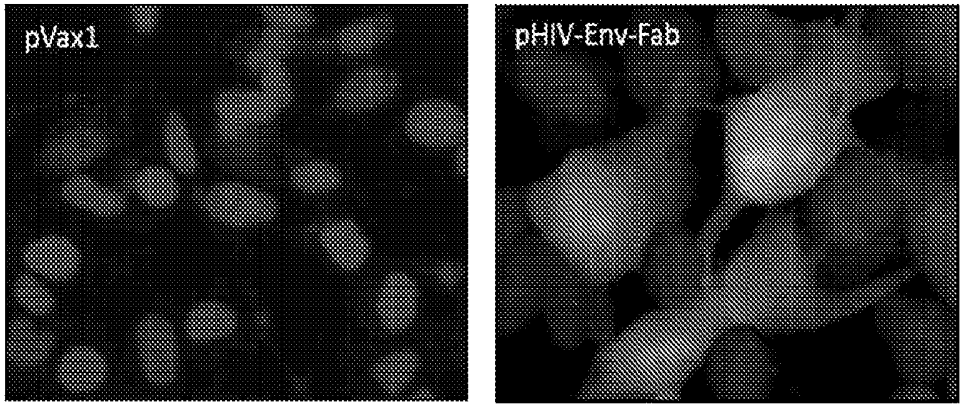
pVax1
pHIV-Env-Fab

FIG. 11

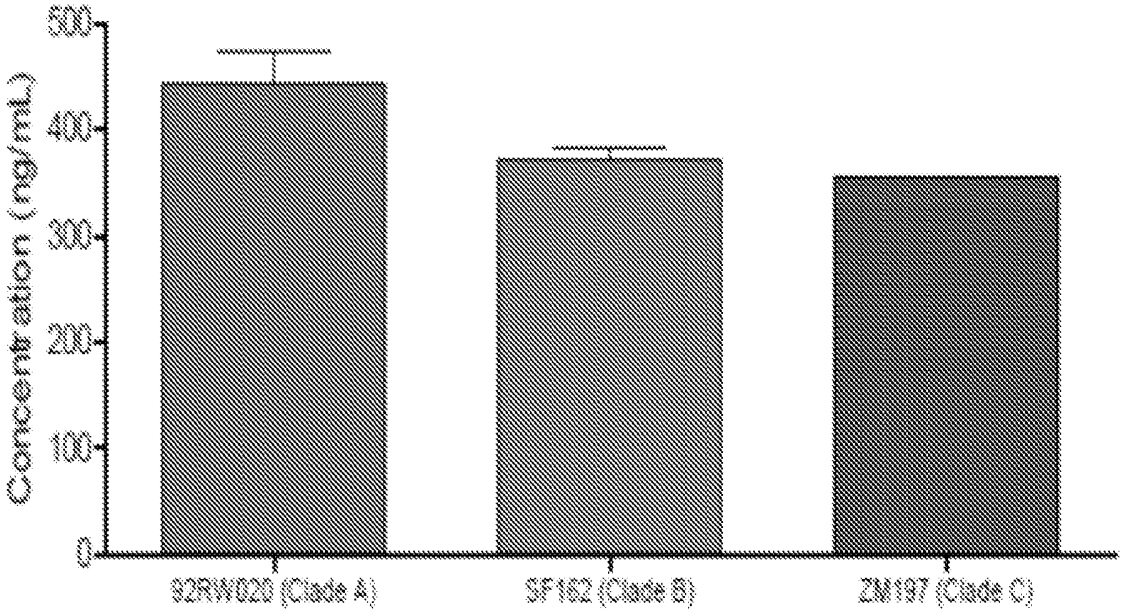
Concentration (ng/mL)
500
400
300
200
100
0
92RW020 (Clade A)
SF162 (Clade B)
ZM197 (Clade C)

FIG. 12

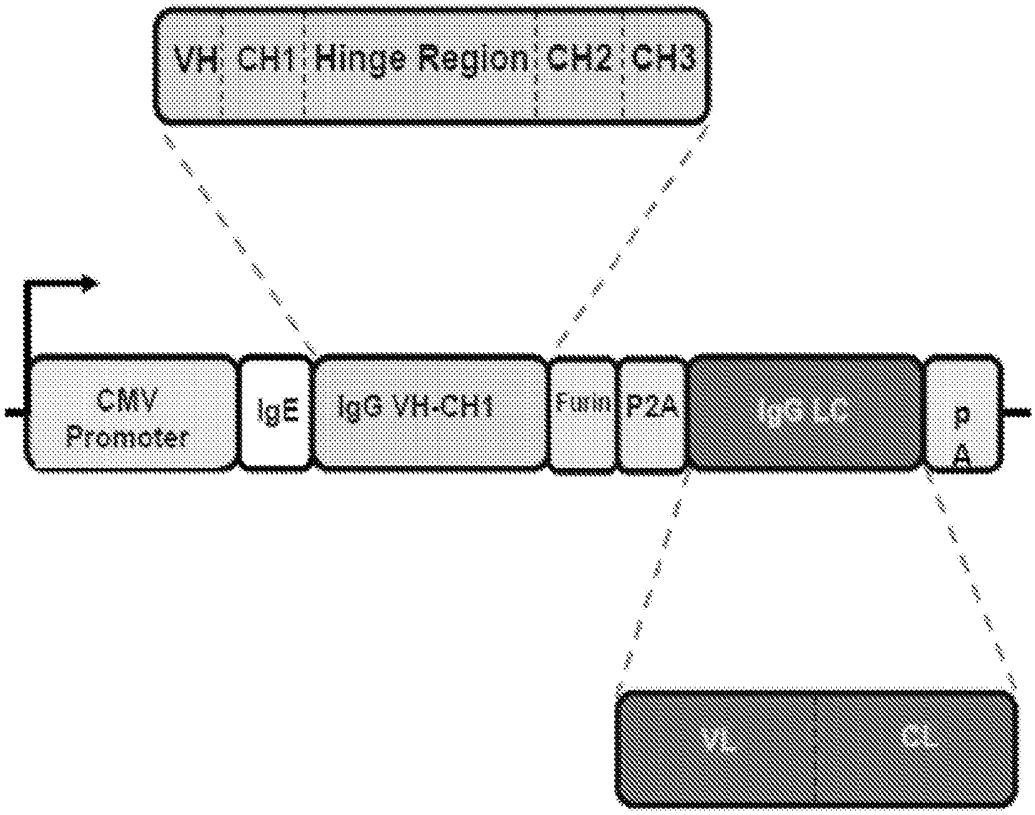
VH
CH1
Hinge Region
CH2
CH3
CMV Promoter
IgE
IgG VH-CH1
Furin
P2A
IgG LC
p A
VL
CL

FIG. 13

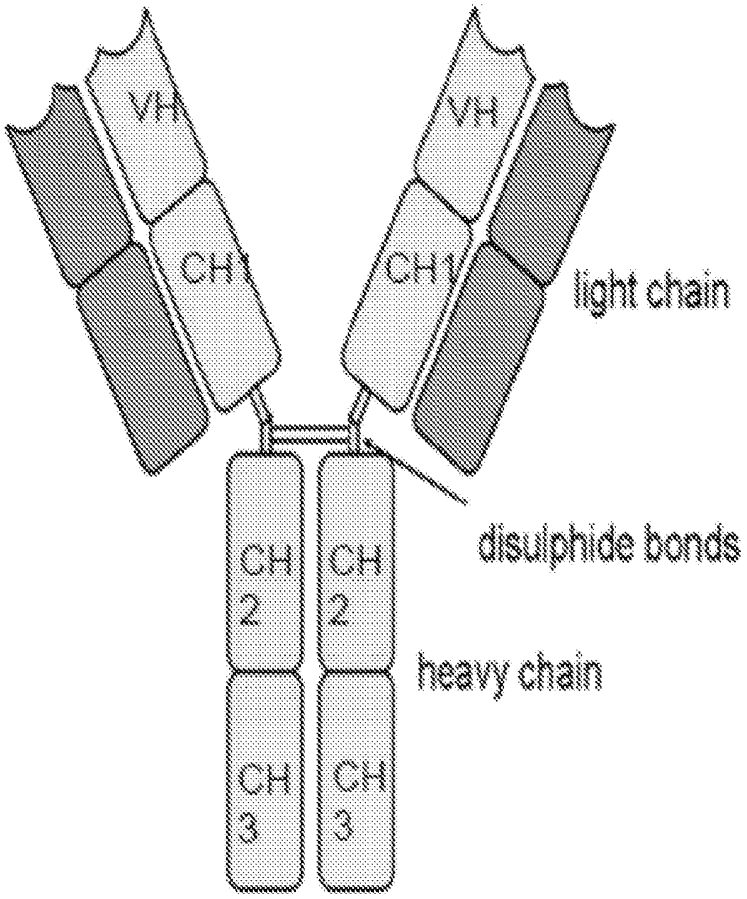
VH
VH
CH1
CH1
light chain
disulphide bonds
CH 2
CH 2
heavy chain
CH 3
CH 3

FIG. 14

VRC01 IgG

MDWTWILFLVAAATRVHSQVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLA
PGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCT
RGKNCDYNWDFEHWGRGTPVIVSSPSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKAEPKSCEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKRGRKRRSGSGATNFSLLKQAGDVEENPGPMDWTWILFLVAAATRVHSEIVLTQ
SPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGP
DYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH
KVYACEVTHQGLRSPVTKSFNRGEC (SEQ ID NO:5)

FIG. 15

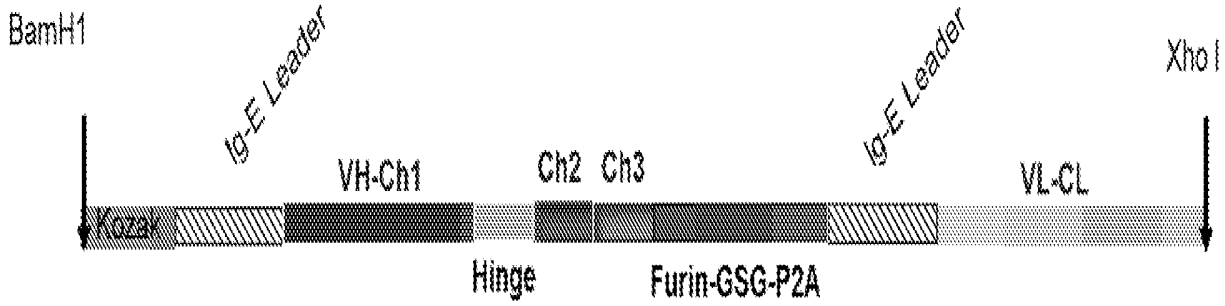

FIG. 16A

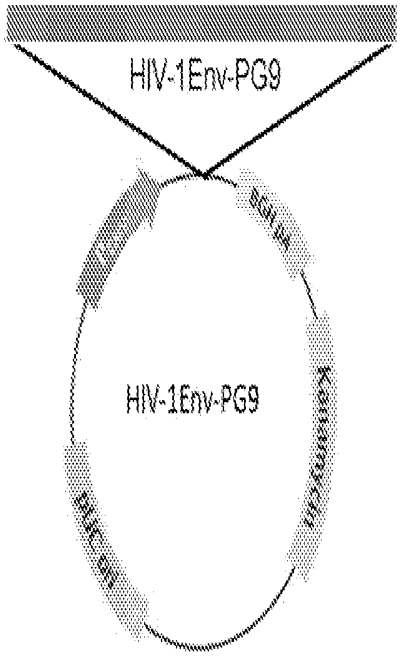

FIG. 16B

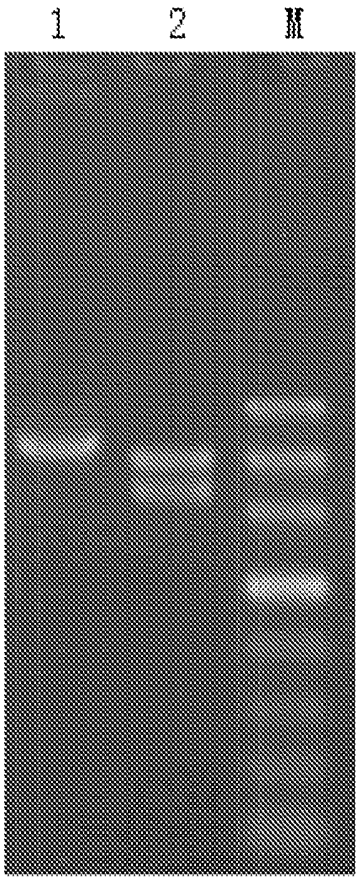

FIG. 16C

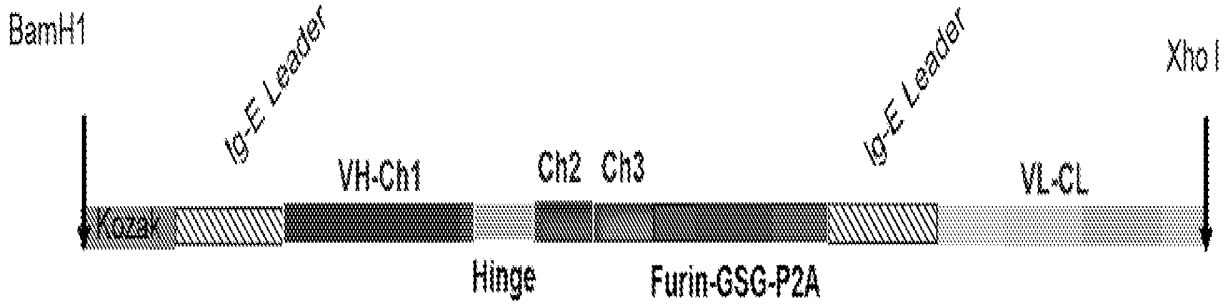

FIG. 17A

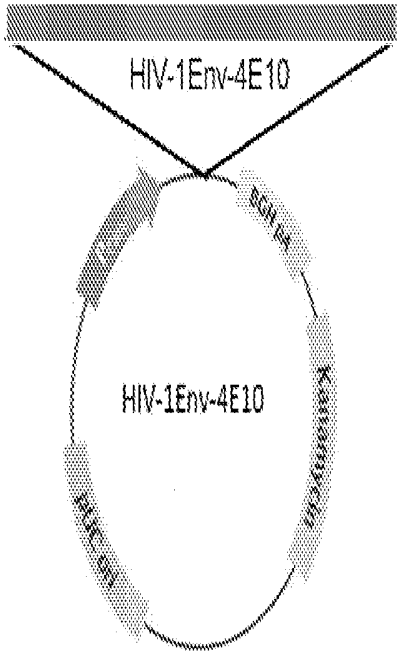

FIG. 17B

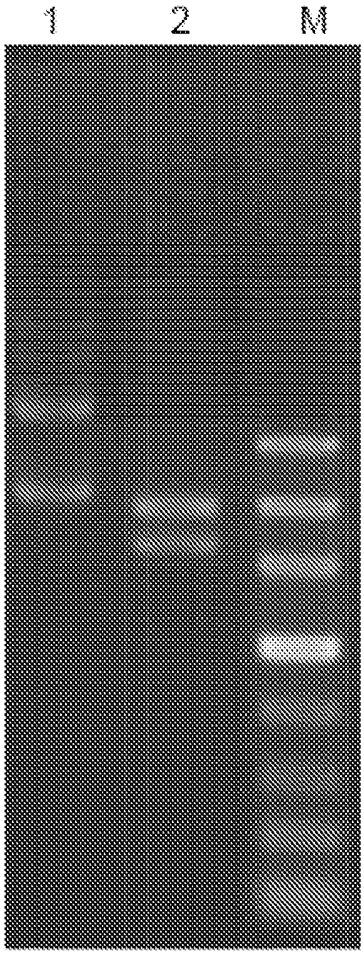

FIG. 17C

Amino Acid Sequence of HIV-1 Env-PG9 Ig (before protease cleavage)
MDWTWRILFLVAAATGTHAEFGLSWVFLVAFLRGVQCQRLVESGGGVVQPGSSLRLSC
AASGFDFSRQGMHWVRQAPGQGLEWVAFIKYDGSEKYHADSVWGRLSISRDNSKDTL
YLQMNSLRVEDTATYFCVREAGGPDYRNGYNYYDFYDGYYNYHYMDVWGKGTTVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKRGRKRRSGSGATNFSLLKQAGD
VEENPGPMAWTPLFLFLLTCCPGGSNSQSALTQPASVSGSPGQSITISCNGTSNDVGGYE
SVSWYQQHPGKAPKVVIYDVSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEGDYYCKS
LTSTRRRVFGTGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAW
KADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTV
APTECS (SEQ ID NO:2)

FIG. 18

Amino Acid Sequence of HIV-1 Env-4E10 Ig (before protease cleavage)
MDWTWRILFLVAAATGTHAQVQLVQSGAEVKRPGSSVTVSCKASGGSFSTYALSWVR
QAPGRGLEWMGGVIPLLTITNYAPRFQGRITITADRSTSTAYLELNSLRPEDTAVYYCAR
EGTTGWGWLGKPIGAFAHWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
LSLSPGKRGRKRRSGSGATNFSLLKQAGDVEENPGPMVLQTQVFISLLLWISGAYGEIVL
TQSPGTQSLSPGERATLSCRASQSVGNNKLAWYQQRPGQAPRLLIYGASSRPSGVADRF
SGSGSGTDFTLTISRLEPEDFAVYYCQQYGQSLSTFGQGTKVEKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK
ADYEKHKVYACEVTHQGLSSPVTKSFNRGE (SEQ ID NO:1)

FIG. 19

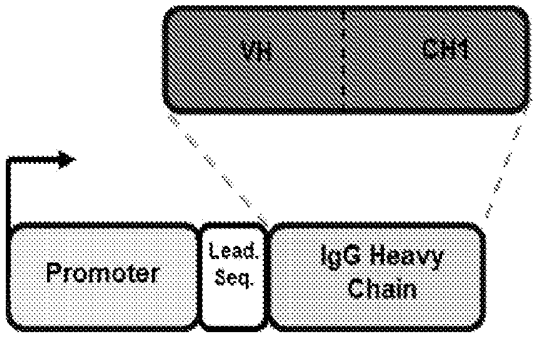

FIG. 20A

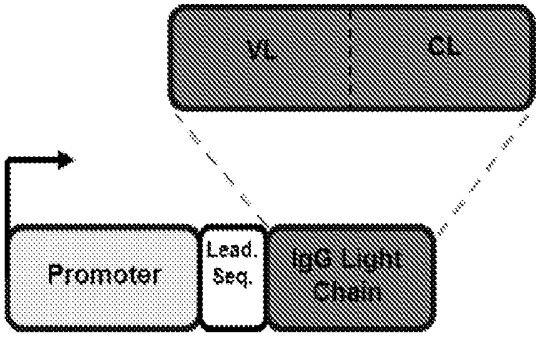

FIG. 20B

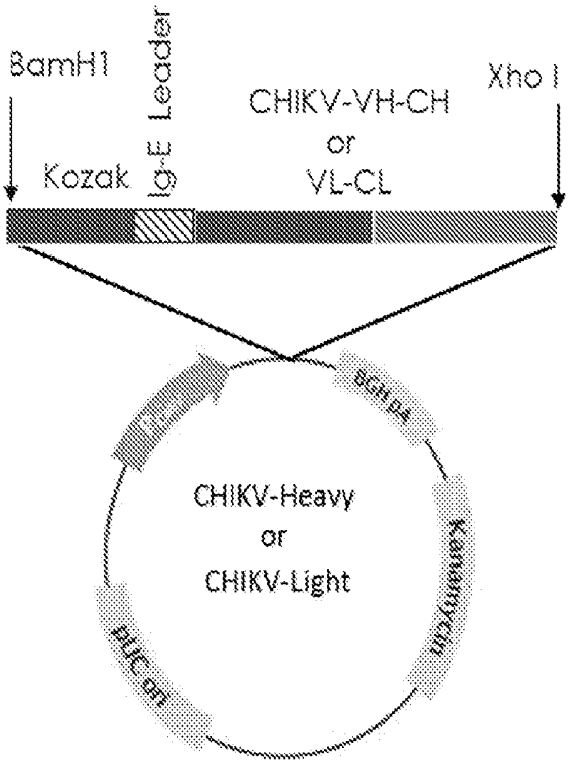
BamH1
Ig-E Leader
Xho I
Kozak
CHIKV-VH-CH
or
VL-CL
CHIKV-Heavy
or
CHIKV-Light
pUC ori
Kanamycin

FIG. 21

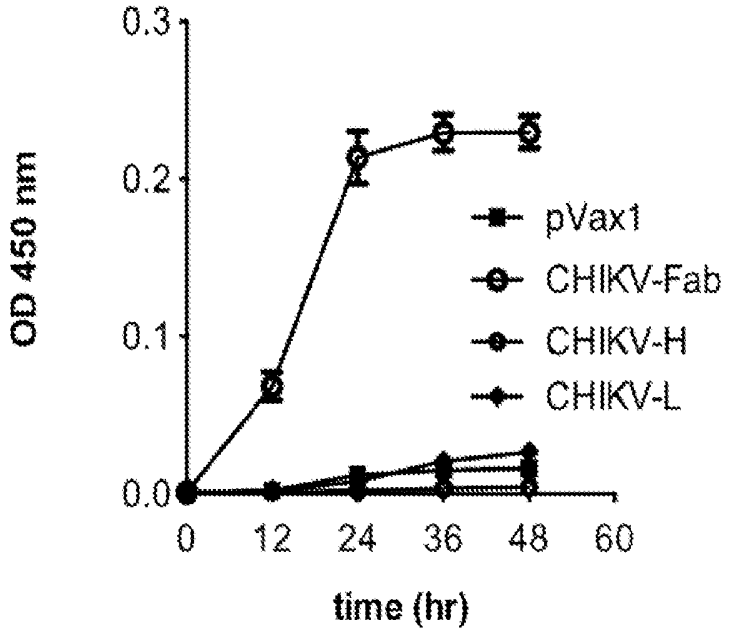
OD 450 nm
0.3
0.2
0.1
0.0
0
12
24
36
48
60
time (hr)
pVax1
CHIKV-Fab
CHIKV-H
CHIKV-L

FIG. 22

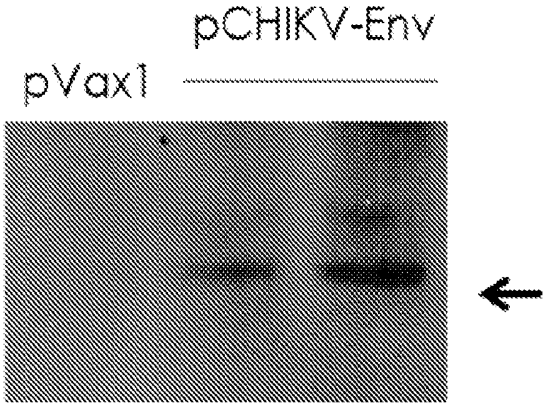

FIG. 23

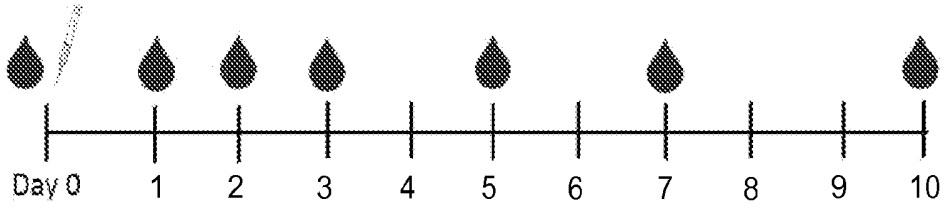

FIG. 24

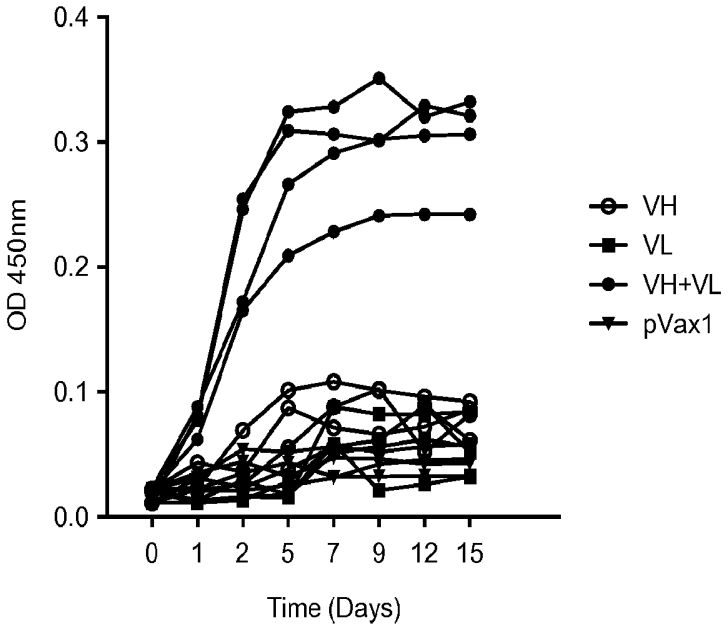

FIG. 25

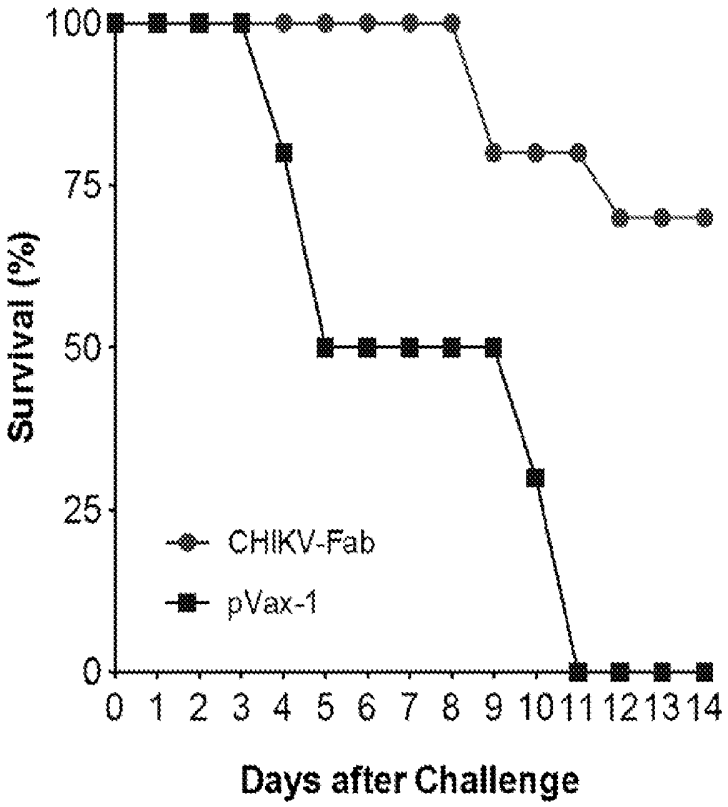
100
75
50
25
0
Survival (%)
CHIKV-Fab
pVax-1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days after Challenge

FIG. 26

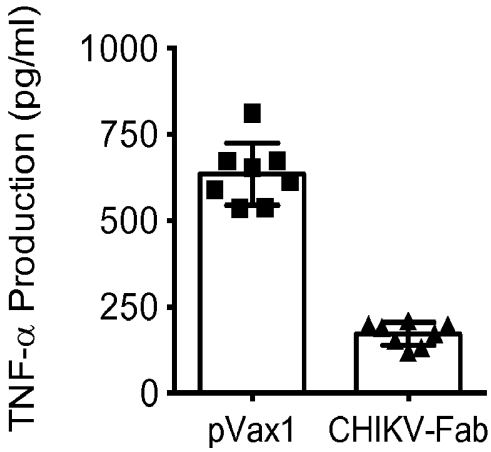
TNF-α Production (pg/ml)
1000
750
500
250
0
pVax1
CHIKV-Fab

FIG. 27

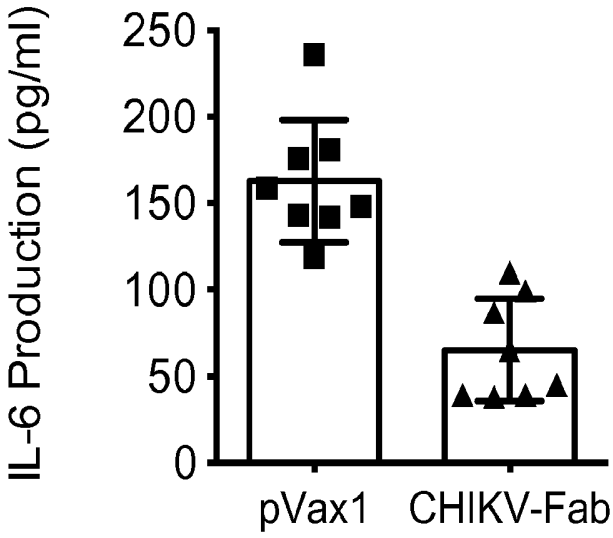

FIG. 28

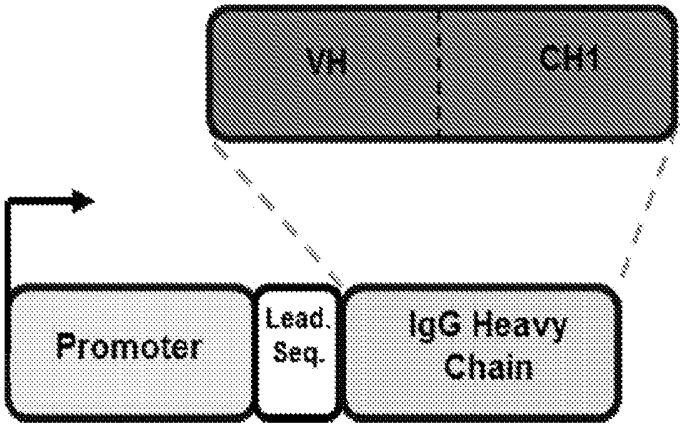
VH
CH1
Promoter
Lead. Seq.
IgG Heavy Chain

FIG. 29

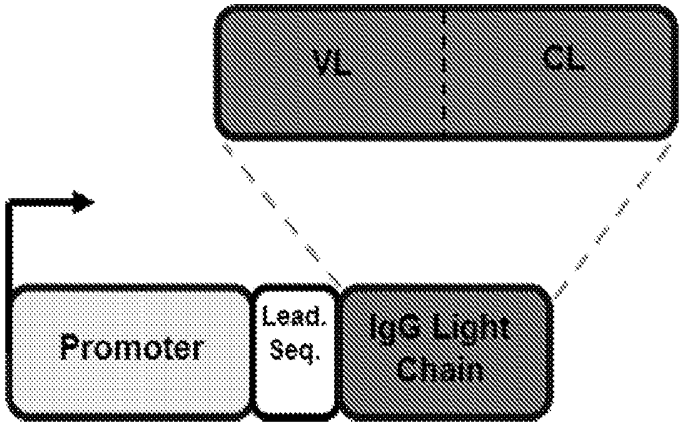
VL
CL
Promoter
Lead. Seq.
IgG Light Chain

FIG. 30

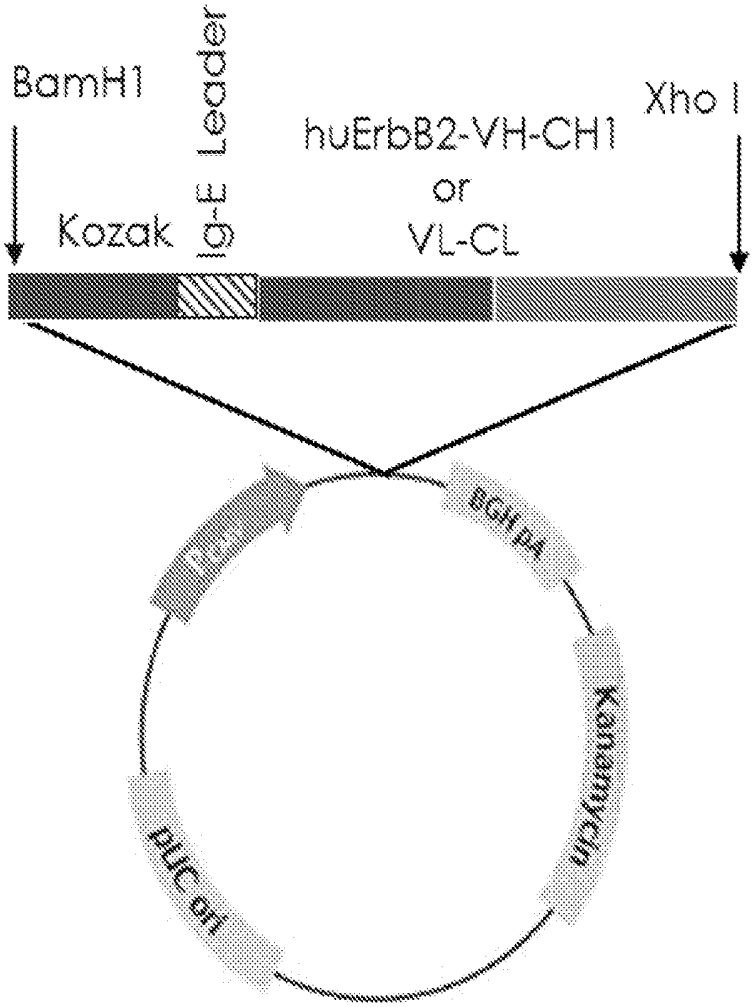
BamH1
Leader
Xho I
huErbB2-VH-CH1
or
VL-CL
Kozak
g-E
hCH pA
Kanamycin
pUC ori

FIG. 31

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

GGATCCGCCACC**ATG**GACTGGACATGGATTCTGTTTCTGGTCGCCGCCGCTACAAGAGTGCATTCCGAAGTGCAGCTGG
TCGAGAGTGGAGGGGGACTGGTGCAGCCCGGCGGATCTCTGCGACTGAGTTGCGCCGCTTCAGGCTTCACCTTTACAGA
CTACACCATGGATTGGGTGAGACAGGCACCTGGCAAGGGACTGGAGTGGGTGGCTGATGTCAACCCAAATAGTGGGGG
CTCAATCTACAACCAGAGGTTCAAGGGCAGGTTCACCCTGAGCGTGGACAGGTCCAAAAACACTCTGTATCTGCAGAT
GAATTCTCTGCGGGCTGAAGATACCGCAGTCTACTATTGCGCCCGCAATCTGGGCCCAAGCTTCTACTTTGACTATTGG
GGGCAGGGCACACTGGTGACTGTCAGCTCCGCTTCTACAAAGGGACCAAGCGTGTTCCCACTGGCACCCTCTAGTAAAT
CCACCTCTGGAGGGACAGCAGCCCTGGGCTGTCTGGTGAAAGACTATTTCCCCGAGCCTGTGACTGTCAGCTGGAACTC
CGGAGCACTGACTAGCGGAGTGCACACCTTTCCAGCCGTCCTGCAGTCAAGCGGCCTGTACTCCCTGTCCTCTGTGGTC
ACAGTGCCTAGTTCAAGCCTGGGAACTCAGACCTATATTTGTAATGTGAACCATAAACCAAGCAATACAAAGGTGGAC
AAGAAGGTGGAACCAAAATCCTGC**TGATAA**CTCGAG (SEQ ID NO:40)

FIG. 32

Amino Acid Sequence of the VH-CH1 of anti-Her-2 Fab

MDWTWILFLVAAATRVHSEVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYN
QRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
(SEQ ID NO:41)

FIG. 33

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

GGATCCGCCACC**ATG**GATTGGACTTGGATTCTGTTCCTGGTCGCCGCCGCTACCCGCGTGCATTCCGATATTCAGATGA
CTCAGAGCCCCTCCTCACTGTCAGCCAGCGTGGGCGACCGAGTCACCATCACATGCAAAGCTTCTCAGGATGTGAGTAT
TGGGGTCGCATGGTACCAGCAGAAGCCAGGCAAAGCACCCAAGCTGCTGATCTATTCCGCCTCTTACAGGTATACAGG
AGTGCCCAGCAGATTCAGTGGCTCAGGAAGCGGGACTGACTTTACTCTGACCATCAGCTCCCTGCAGCCTGAGGATTTC
GCTACCTACTATTGCCAGCAGTACTATATCTACCCATATACCTTTGGCCAGGGAACAAAAGTGGAGATCAAGCGGACCG
TGGCCGCTCCCTCCGTCTTCATTTTTCCCCCTTCTGACGAACAGCTGAAGAGCGGAACAGCAAGCGTGGTCTGTCTGCT
GAACAATTTCTACCCTCGCGAGGCCAAAGTGCAGTGGAAGGTCGATAACGCTCTGCAGTCCGGGAATTCTCAGGAGAG
TGTGACTGAACAGGACTCAAAAGATAGCACCTATTCCCTGTCTAGTACACTGACTCTGAGCAAGGCAGACTACGAAAA
GCACAAAGTGTATGCCTGTGAGGTCACCCACCAGGGGCTGTCAAGTCCCGTCACCAAGTCCTTCAATAGAGGCGAATG
C**TGATAA**CTCGAG (SEQ ID NO:42)

FIG. 34

Amino Acid Sequence of the VL-CL of anti-Her-2 Fab

MDWTWILFLVAAATRVHSDIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW
KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:43)

FIG. 35

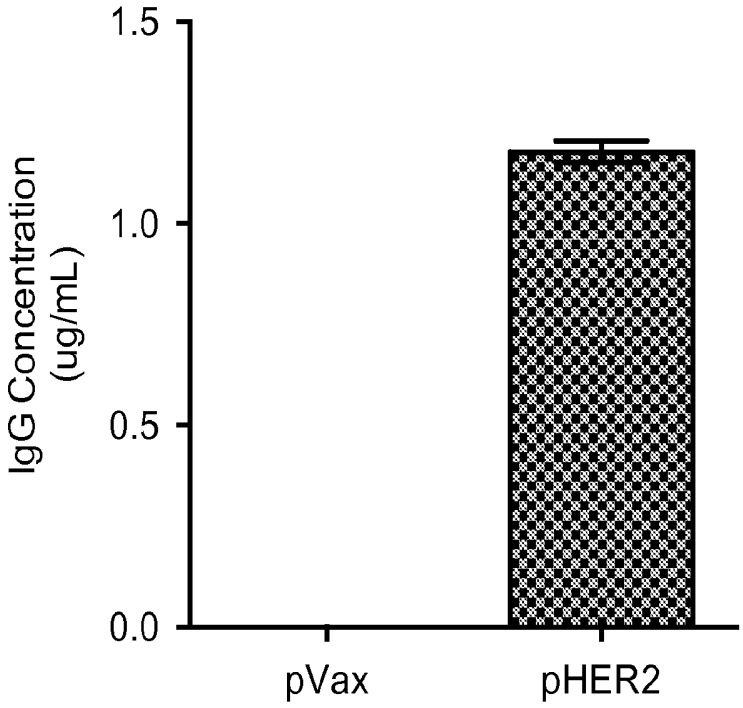

FIG. 36

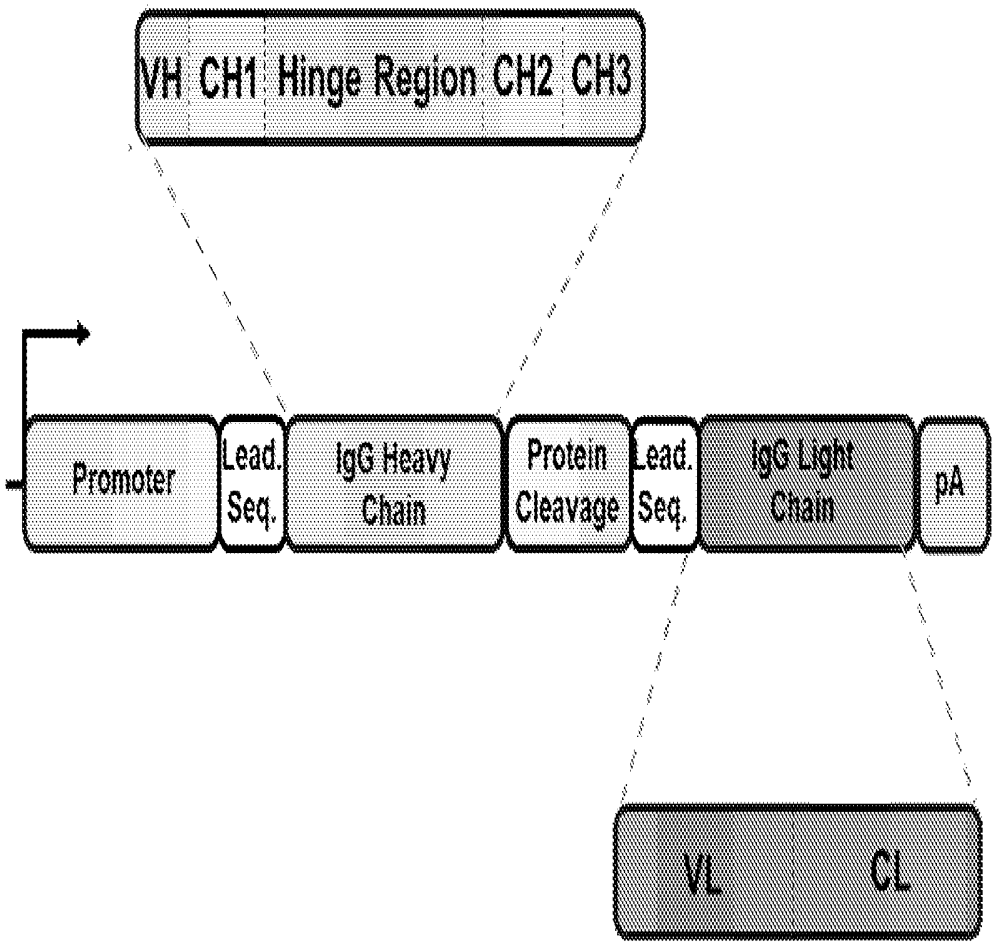
VH CH1 Hinge Region CH2 CH3
Promoter
Lead. Seq.
IgG Heavy Chain
Protein Cleavage
Lead. Seq.
IgG Light Chain
pA
VL
CL

FIG. 37

Nucleic Acid Sequence Encoding anti-DENV Human IgG

GGATCCGCCACC**ATG**GACTGGACTTGGAGGATTCTGTTTCTGGTCGCCGCCGCTACTGGGACTCACGCTCAGGCACATC
TGGTCGAATCTGGAGGAGGAGTGGTCCAGCCTGGCCGATCCCTGCGACTGTCTTGCGCAGCTAGCGCCTTCAACTTCAG
CACAAACGCAATGCACTGGGTGCGACAGGCACCAGGCAAGGGACTGGAGTGGGTCGCTGTGATCTCATACGACGGAA
GCCATAAGTACTATGCAGATTCTGTGAAAGGCCGGTTCACCATTTCCAGGGACAATTCTAAGAACACCCTGTATCTGCA
GATGAATAGCCTGCGCGCAGCCGATACCGCAGTGTACTATTGCGCAACTGTCGGCGTGCTGACCTGGCCAGTGAACGC
CGAATACTTTCACCATTGGGGACAGGGCAGTCTGGTCTCAGTGAGCTCCGCAAGTACTAAGGGACCATCAGTGTTCCCA
CTGGCACCCTCTAGTAAATCTACTAGTGGCGGGACCGCTGCACTGGGATGTCTGGTGAAGGACTATTTCCCCGAGCCTG
TCACCGTGAGCTGGAATTCCGGAGCCCTGACAAGCGGCGTCCACACTTTTCCCGCTGTGCTGCAGTCAAGCGGACTGTA
CTCCCTGTCCTCTGTGGTCACTGTGCCTAGTTCAAGCCTGGGCACTCAGACCTATATCTGCAATGTGAACCACAAGCCCT
CTAACACCAAAGTCGACAAGAAAGTGGAACCTAAGAGCTGTGATAAAACACATACTTGCCCACCTTGTCCAGCACCAG
AGCTGCTGGGAGGACCAAGCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACACTGATGATTAGCCGGACACCTGAAG
TCACTTGCGTGGTCGTGGACGTGTCCCACGAGGACCCCGAAGTCAAGTTTAATTGGTACGTGGATGGCGTCGAGGTGCA
TAACGCCAAGACCAAACCCCGGGAGGAACAGTACAATAGCACATATAGAGTCGTGTCCGTCCTGACTGTGCTGCATCA
GGATTGGCTGAATGGGAAGGAGTATAAGTGCAAAGTGTCTAACAAGGCTCTGCCTGCACCAATCGAGAAAACCATTAG
CAAGGCTAAAGGCCAGCCTAGGGAACCACAGGTGTACACACTGCCTCCAAGTCGCGACGAGCTGACCAAGAATCAGGT
CTCCCTGACATGTCTGGTGAAAGGCTTCTATCCATCAGATATCGCCGTGGAGTGGGAAAGCAACGGGCAGCCCGAAAA
CAATTACAAGACCACACCCCCTGTGCTGGACTCTGATGGCAGTTTCTTTCTGTATTCTAAGCTGACCGTGGACAAAAGT
AGATGGCAGCAGGGGAATGTCTTTTCATGTAGCGTGATGCACGAGGCCCTGCACAACCATTACACACAGAAGTCCCTG
TCTCTGAGTCCCGGAAAGAGGGGCCGCAAACGGAGATCAGGGAGCGGAGCTACTAATTTCAGCCTGCTGAAACAGGCA
GGGGATGTGGAGGAAAACCCCGGACCTATGGCTTGGACCCCACTGTTCCTGTTTCTGCTGACATGCTGTCCCGGGGGCA
GCAATTCTCAGAGTGTCCTGACACAGCCACCATCAGTGAGCGGAGCACCAGGACAGAGGGTGACCATCTCCTGCACAG
GCAGCAGCAGCAACATTGGCGCCGGGTACGACGTGCATTGGTATCAGCAGCTGCCCGGCACCGCTCCTAAGCTGCTGA
TCTGTGGCAACAATAACCGCCCATCTGGGGTGCCCGATCGATTCTCCGGCTCTAAAAGTGGGACTTCAGCCAGCCTGGC
TATTACCGGCCTGCAGGCCGAGGACGAAGCTGATTACTATTGCCAGAGCTACGACTCAAGCCTGACCGGAGTCGTGTTC
GGAGGAGGAACCAAGCTGACAGTCCTGGGACAGCCTAAAGCCGCTCCAAGCGTGACACTGTTTCCTCCATCCTCTGAG
GAACTGCAGGCAAACAAGGCCACCCTGGTGTGCCTGATTTCCGACTTCTACCCCGGGGCAGTCACTGTGGCTTGGAAG
GCAGATAGTTCACCTGTCAAAGCCGGAGTGGAGACTACCACACCATCAAAGCAGAGCAATAACAAATACGCAGCCAG
CTCCTATCTGTCCCTGACCCCTGAGCAGTGGAAGTCTCACAAATCCTATTCTTGCCAGGTCACTCACGAAGGAAGCACT
GTGGAGAAAACTGTCGCACCAACCGAATGTAGT**TGATAA**CTCGAG (SEQ ID NO:44)

FIG. 38

Amino Acid Sequence of anti-DENV Human IgG (before protease cleavage to separate heavy and light chain polypeptides)
MDWTWRILFLVAAATGTHAQAHLVESGGGVVQPGRSLRLSCAASAFNFSTNAMHWVRQAPGKGLEWVAVISYDGSHKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAADTAVYYCATVGVLTWPVNAEYFHHWGQGSLVSVSSASTKGPSVFPLAPSSKS
TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE
PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKRGRKRRSGSGATNF
SLLKQAGDVEENPGPMAWTPLFLFLLTCCPGGSNSQSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPK
LLICGNNNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLTGVVFGGGTKLTVLGQPKAAPSVTLFPPSSEEL
QANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTV
APTECS (SEQ ID NO:45)

FIG. 39

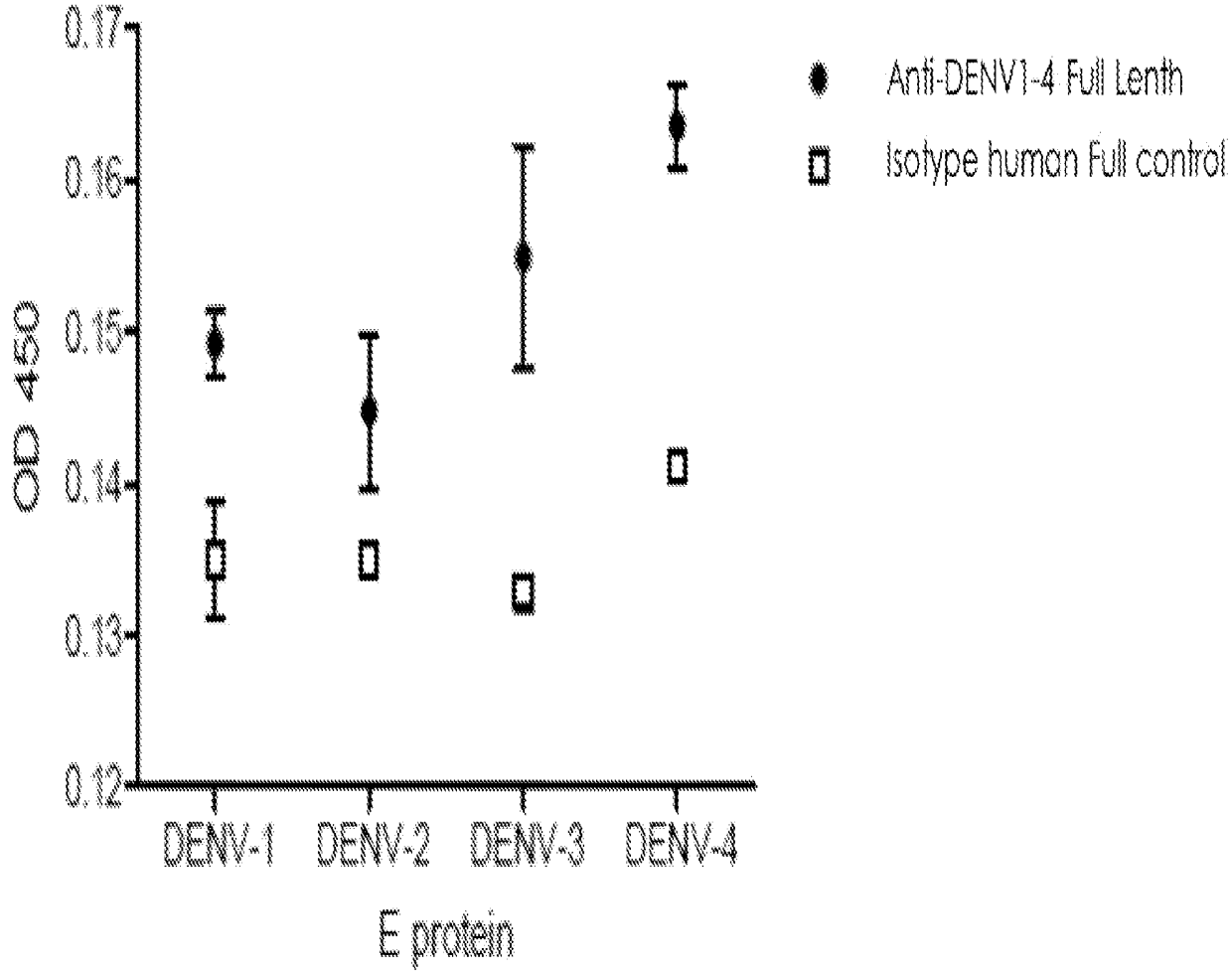
0.17
0.16
0.15
0.14
0.13
0.12
OD 450
DENV-1
DENV-2
DENV-3
DENV-4
E protein
Anti-DENV1-4 Full Lenth
Isotype human Full control

FIG. 40

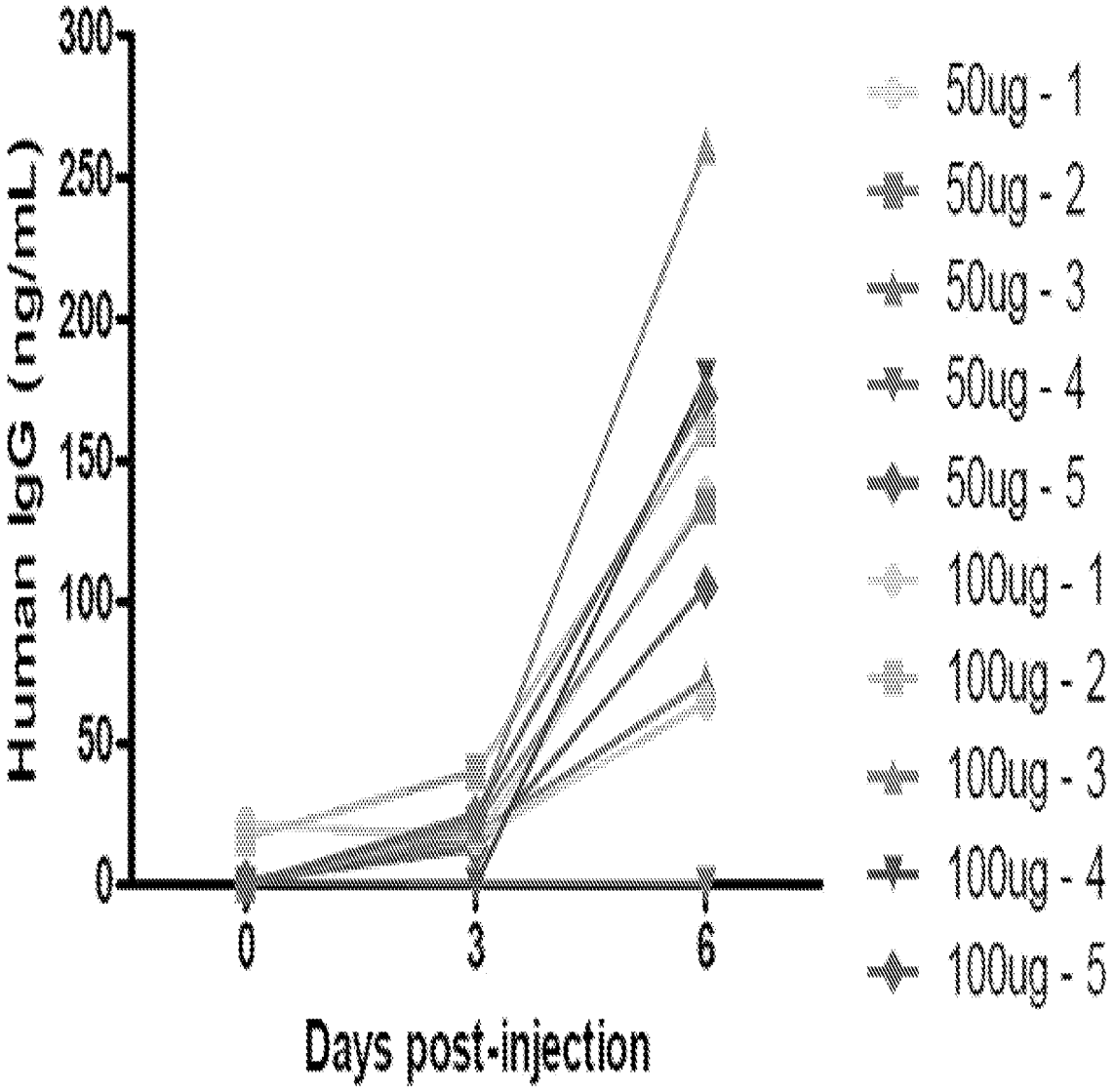
Human IgG (ng/mL)
300
250
200
150
100
50
0
0
3
6
Days post-injection
50ug - 1
50ug - 2
50ug - 3
50ug - 4
50ug - 5
100ug - 1
100ug - 2
100ug - 3
100ug - 4
100ug - 5

FIG. 41

IgG Heavy Chain

METDTLLLWVLLLWVPGSTGDGAQVQLVQSGAVIKTPGSSVKISCRASGYNFRDYSIHWVRLIPDKGFEWIGWIKPLWGAV
SYARQLQGRVSMTRQLSQDPDDPDWGVAYMEFSGLTPADTAEYFCVRRGSCDYCGDFPWQYWCQGTVVVVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKKVEPKSCYPYDVPDYA (SEQ ID NO:46)

FIG. 42

IgG Light Chain

METDTLLLWVLLLWVPGSTGDGAQVQIVLTQSPGILSLSPGETATLFCKASQGGNAMTWYQKRRGQVPRLLIYDTSRRASG
VPDRFVGSGSGTDFFLTINKLDREDFAVYYCQQFEFFGLGSELEVHRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECYPYDVPDYA
(SEQ ID NO:47)

FIG. 43

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

METDTLLLWVLLLWVPGSTGDGAQVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGG
AVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSPSTKGPSVFPLAPSSK
STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKA
EPKSCYPYDVPDYA (SEQ ID NO:48)

FIG. 44

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

METDTLLLWVLLLWVPGSTGDGAQVQIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPD
RFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGECYPYDVPDYA
(SEQ ID NO:49)

FIG. 45

Nucleic Acid Sequence Encoding HIV-1 PG9 Fab

GGATCCGCCACC**ATG**GCAAGACCCCTGTGCACCCTGCTGCTGCTGATGGCAACCCTGGCCGGAGCCCTGGCACAGAGC
GCCCTGACCCAGCCCGCAAGCGTCTCCGGCTCACCAGGCCAGAGCATCACTATTAGTTGCAACGGGACTAGCAACGAC
GTGGGAGGCTATGAGAGTGTCAGCTGGTACCAGCAGCATCCCGGAAAAGCACCAAAAGTGGTCATCTACGATGTCAGT
AAAAGGCCAAGTGGGGTCTCAAATAGGTTCTCAGGGAGTAAATCTGGGAATACAGCATCTCTGACCATCTCCGGACTG
GGCGCAGAAGATGAAGGCGACTACTATTGCAAAAGCCTGACCTCAACCAGACGGCGAGTCTTTGGGACAGGCACCAA
GCTGACAGTCCTGACAGTCGCTGCCCCCTCCGTCTTCATTTTTCCACCTTCAGATGAGCAGCTGAAATCTGGCACTGCAT
CTGTGGTCTGCCTGCTGAACAACTTCTATCCACGAGAGGCCAAGGTGCAGTGGAAAGTGGATAACGCACTGCAGTCCG
GCAATAGTCAGGAAAGCGTGACTGAGCAGGATTCCAAGGACAGTACCTATAGCCTGTCCAGTACACTGACCCTGTCCA
AGGCTGACTACGAAAAACATAAGGTGTATGCATGTGAAGTGACTCACCAGGGACTGAGGTCACCAGTCACTAAGTCTT
TTAACAGGGGAGAGTGCGGCGGGGGAGGATCTGGAGGCGGCGGCTCTGGAGGGGGAGGCTCAGGGGGCGGAGGAAG
CGGCGGAGGAGGGTCCGGAGGAGGAGGCAGTCAGAGACTGGTCGAAAGCGGGGGAGGAGTGGTGCAGCCTGGGTCCT
CACTGAGACTGTCATGCGCTGCCAGTGGCTTTGATTTTTCACGACAGGGAATGCATTGGGTCAGGCAGGCACCCGGACA
GGGCCTGGAATGGGTCGCCTTCATTAAGTACGACGGAAGCGAGAAGTACCATGCCGACTCAGTGTGGGGAAGGCTGAG
CATCTCAAGGGACAACTCAAAGGACACCCTGTACCTGCAGATGAATAGCCTGAGAGTGGAAGATACCGCTACTTATTT
CTGCGTGCGAGAGGCCGGAGGGCCAGATTACCGGAACGGGTACAATTACTATGATTTCTACGACGGCTACTACAATTA
CCATTATATGGATGTCTGGGGCAAAGGAACTACAGTCACCGTGAGCTCCGCAAGTACTAAGGGACCTTCCGTGTTTCCT
CTGGCTCCCAGTTCCAAAAGTACATCCGGAGGAACAGCCGCTCTGGGATGTCTGGTCAAGGACTATTTTCCCGAGCCCG
TGACTGTCTCCTGGAACAGCGGGGCTCTGACAAGCGGGGTGCACACCTTTCCTGCCGTGCTGCAGTCCAGTGGGCTGTA
CAGTCTGTCTAGTGTCGTCACTGTGCCAAGCTCAAGTCTGGGGACCCAGACATACATTTGTAATGTGAACCATAAACCC
TCAAACACCAAAGTGGACAAGAAAGTGGAACCTAAAAGC**TGATAA**CTCGAG (SEQ ID NO:50)

FIG. 46

Amino Acid Sequence of HIV-1 PG9 Fab

MARPLCTLLLLMATLAGALAQSALTQPASVSGSPGQSITISCNGTSNDVGGYESVSWYQQHPGKAPKVVIYDVSKRPSGVSN
RFSGSKSGNTASLTISGLGAEDEGDYYCKSLTSTRRRVFGTGTKLTVLTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGECGGGGSGGGGS
GGGGSGGGGSGGGGSGGGGSQRLVESGGGVVQPGSSLRLSCAASGFDFSRQGMHWVRQAPGQGLEWVAFIKYDGSEKYH
ADSVWGRLSISRDNSKDTLYLQMNSLRVEDTATYFCVREAGGPDYRNGYNYYDFYDGYYNYHYMDVWGKGTTVTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV
NHKPSNTKVDKKVEPKS (SEQ ID NO:51)

FIG. 47

Nucleic Acid Sequence Encoding HIV-1 4E10 Fab

GGATCCGCCACC**ATG**GCAAGACCTCTGTGCACTCTGCTGCTGCTGATGGCTACTCTGGCCGGGGCTCTGGCTGAGATTG
TCCTGACCCAGTCCCCTGGCACTCAGTCACTGTCCCCCGGCGAGCGCGCAACTCTGTCCTGCAGAGCAAGCCAGTCCGT
CGGGAACAACAAGCTGGCATGGTACCAGCAGCGCCCAGGACAGGCACCCAGGCTGCTGATCTACGGAGCAAGCTCCC
GGCCTAGCGGAGTCGCTGATAGATTCTCCGGAAGCGGCTCCGGGACCGATTTCACTCTGACCATCTCCAGGCTGGAACC
TGAGGATTTTGCCGTGTATTACTGTCAGCAGTACGGGCAGAGCCTGTCAACTTTCGGCCAGGGAACTAAAGTCGAAAA
GAGAACCGTGGCCGCACCAAGCGTCTTTATTTTTCCCCCTAGCGATGAACAGCTGAAATCCGGGACTGCTTCCGTGGTC
TGCCTGCTGAATAACTTCTATCCAAGAGAGGCAAAGGTGCAGTGGAAAGTGGACAACGCCCTGCAGAGCGGAAACTCA
CAGGAATCTGTGACAGAGCAGGACTCCAAGGATAGCACATACAGTCTGTCCTCAACTCTGACCCTGTCCAAAGCTGAC
TATGAGAAGCATAAAGTCTACGCATGTGAGGTGACCCACCAGGGACTGAGGTCCCCCGTCACTAAGTCCTTCAATAGA
GGCGAGTGCGGGGGCGGGGGCAGTGGCGGAGGGGGAAGTGGGGGCGGAGGGAGTGGCGGCGGCGGGAGTGGCGGCG
GCGGCTCAGGGGGCGGCGGCTCCCAGGTCCAGCTGGTCCAGAGCGGAGCCGAGGTCAAGAGACCAGGCTCTTCAGTCA
CCGTGAGCTGCAAAGCCAGCGGAGGCTCCTTTAGCACTTACGCCCTGTCATGGGTGCGGCAGGCCCCAGGCCGAGGCC
TGGAGTGGATGGGCGGCGTGATCCCCCTGCTGACCATTACTAACTATGCCCCTAGATTTGGAGGCCGGATCACCATCAC
AGCTGACAGATCCACATCCACAGCTTACCTGGAGCTGAACAGTCTGAGGCCCGAGGACACTGCAGTCTACTACTGTGC
ACGAGAAGGCACCACTGGATGGGGGTGGCTGGGGAAGCCCATCGGGGCTTTTGCACATTGGGGCGGAGGGACACTGGT
GACTGTGAGCTCTGCCAGCACTAAAGGGCCCAGTGTCTTCCCTCTGGCCCCAAGTTCCAAGAGTACATCAGGGGGCACC
GCCGCACTGGGGTGTCTGGTGAAGGATTACTTCCCAGAGCCCGTGACAGTCAGTTGGAACAGCGGCGCTCTGACCAGT
GGGGTGCACACTTTCCCAGCCGTGCTGCAGAGTTCAGGGCTGTACTCCCTGTCCTCAGTGGTGACTGTGCCCTCAAGCA
GTCTGGGGACTCAGACTTACATTTGTAATGTGAACCATAAACCCTCAAATACTAAAGTGGACAAAAAAGTGGAACCAA
AGAGC**TGATAA**CTCGAG (SEQ ID NO:52)

FIG. 48

Amino Acid Sequence of HIV-1 4E10 Fab

MARPLCTLLLLMATLAGALAEIVLTQSPGTQSLSPGERATLSCRASQSVGNNKLAWYQQRPGQAPRLLIYGASSRPSGVADR
FSGSGSGTDFTLTISRLEPEDFAVYYCQQYGQSLSTFGQGTKVEKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGECGGGGSGGGGSGG
GGSGGGGSGGGGSGGGGSQVQLVQSGAEVKRPGSSVTVSCKASGGSFSTYALSWVRQAPGRGLEWMGGVIPLLTITNYAP
RFGGRITITADRSTSTAYLELNSLRPEDTAVYYCAREGTTGWGWLGKPIGAFAHWGGGTLVTVSSASTKGPSVFPLAPSSKST
SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KS (SEQ ID NO:53)

FIG. 49

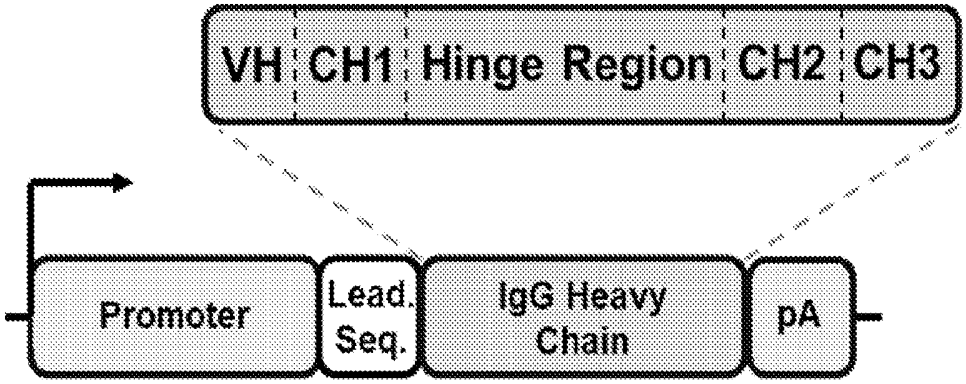
VH
CH1
Hinge Region
CH2
CH3
Promoter
Lead. Seq.
IgG Heavy Chain
pA

FIG. 50

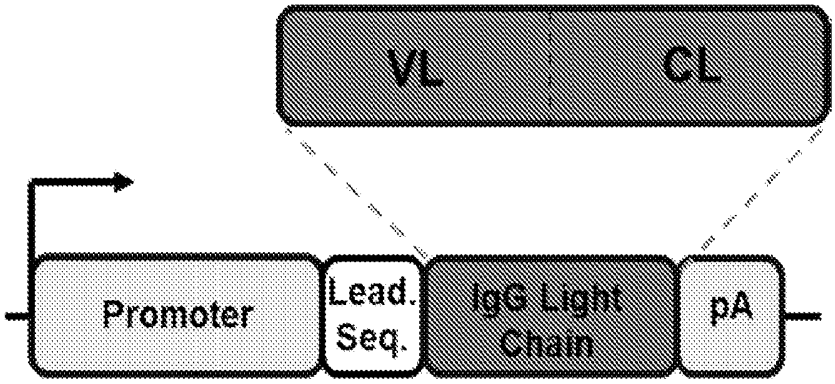
VL
CL
Promoter
Lead. Seq.
IgG Light Chain
pA

FIG. 51

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

GGATCCGCCACC**ATG**GATTGGACATGGATTCTGTTCCTGGTCGCCGCCGCAACTAGAGTGCATTCACAGGTGCAGCTGG
TGCAGTCAGGCGGGCAGATGAAGAAACCCGGCGAGAGTATGCGAATCTCATGCCGGGCTAGCGGGTACGAATTCATCG
ACTGTACCCTGAACTGGATTAGACTGGCACCTGGGAAGAGGCCAGAGTGGATGGGATGGCTGAAACCTAGAGGCGGG
GCAGTGAATTACGCCAGACCACTGCAGGGCAGGGTCACTATGACCCGCGACGTGTATTCTGATACCGCATTCCTGGAG
CTGCGAAGTCTGACAGTCGACGATACTGCCGTGTACTTCTGCACACGGGGCAAGAACTGTGACTATAATTGGGATTTTG
AACACTGGGGCAGGGGGACACCTGTCATTGTGAGCTCCCCAAGTACTAAGGGACCCTCAGTGTTTCCCCTGGCCCCTTC
TAGTAAAAGTACCTCAGGAGGCACAGCCGCTCTGGGATGCCTGGTGAAGGATTACTTCCCTGAGCCAGTCACCGTGAG
TTGGAACTCAGGCGCCCTGACAAGCGGGGTCCATACTTTTCCAGCTGTGCTGCAGTCAAGCGGGCTGTACTCCCTGTCC
TCTGTGGTCACAGTGCCCAGTTCAAGCCTGGGAACACAGACTTATATCTGTAACGTCAATCACAAGCCTAGCAATACTA
AAGTGGACAAGAAAGCCGAGCCTAAGAGCTGCGAACCAAAGTCCTGTGATAAAACCCATACATGCCCTCCCTGTCCAG
CTCCTGAACTGCTGGGCGGCCCATCCGTGTTCCTGTTTCCACCCAAGCCCAAAGACACCCTGATGATTAGCAGGACTCC
TGAGGTCACCTGCGTGGTCGTGGACGTGTCCCACGAGGACCCCGAAGTCAAGTTTAACTGGTACGTGGATGGCGTCGA
AGTGCATAATGCCAAGACAAAACCCCGGGAGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTCCTGACAGTGCT
GCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTGTCTAATAAGGCCCTGCCAGCTCCCATCGAGAAAAC
AATTTCCAAGGCAAAAGGCCAGCCAAGGGAACCCCAGGTGTACACTCTGCCTCCATCCCGCGACGAGCTGACTAAGAA
CCAGGTCTCTCTGACCTGTCTGGTGAAAGGATTCTATCCAAGCGATATCGCCGTGGAGTGGGAATCCAATGGCCAGCCC
GAGAACAATTACAAGACCACACCCCCTGTGCTGGACAGCGATGGCTCCTTCTTTCTGTATTCAAAGCTGACCGTGGATA
AAAGCCGCTGGCAGCAGGGGAACGTCTTTAGCTGCTCCGTGATGCACGAAGCTCTGCACAATCATTACACCCAGAAGT
CTCTGAGTCTGTCACCTGGCAAG**TGATAA**CTCGAG (SEQ ID NO:54)

FIG. 52

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

MDWTWILFLVAAATRVHSQVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYA
RPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSPSTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCE
PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:55)

FIG. 53

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

GGATCCGCCACC**ATG**GATTGGACTTGGATTCTGTTCCTGGTGGCAGCCGCTACCAGAGTCCATTCCGAAATTGTGCTGA
CCCAGTCTCCCGGAACACTGTCTCTGAGTCCTGGCGAGACAGCCATCATTTCCTGTAGGACTTCTCAGTACGGGAGTCT
GGCATGGTATCAGCAGCGACCAGGACAGGCTCCTCGACTGGTCATCTACTCAGGAAGCACTCGGGCAGCCGGCATTCC
CGACCGATTCTCCGGGTCTCGGTGGGGACCTGATTACAACCTGACCATCTCAAATCTGGAAAGCGGAGACTTTGGCGTG
TACTATTGCCAGCAGTATGAGTTCTTTGGGCAGGGAACCAAGGTCCAGGTGGACATCAAACGCACAGTCGCTGCACCA
AGCGTGTTCATCTTTCCACCCTCAGATGAACAGCTGAAGTCCGGCACCGCCTCTGTGGTGTGCCTGCTGAACAATTTCTA
CCCCCGGGAGGCAAAGGTCCAGTGGAAAGTGGACAACGCCCTGCAGTCTGGCAATAGTCAGGAGTCAGTGACTGAAC
AGGACAGCAAGGATTCCACCTATTCTCTGTCCTCTACTCTGACCCTGAGCAAAGCTGATTACGAGAAGCACAAAGTGTA
TGCATGTGAGGTCACCCACCAGGGACTGCGGTCACCCGTCACCAAGAGCTTCAATCGCGGAGAGTGT**TGATAA**CTCGA
G (SEQ ID NO:56)

FIG. 54

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

MDWTWILFLVAAATRVHSEIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRW
GPDYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGEC (SEQ ID NO:57)

FIG. 55

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

GGATCCGCCACC**ATG**GATTGGACATGGAGGATTCTGTTTCTGGTCGCCGCCGCTACTGGAACTCACGCTCAGGTGCAGC
TGGTGCAGTCAGGGTCCGAACTGAAGAAACCAGGGGCATCTGTGAAGGTCAGTTGCAAAGCCTCAGGCTACACCCTGA
CACGGTATGCCATGACTTGGGTGCGCCAGGCTCCTGGACAGGGACTGGAGTGGATGGGCTGGATCAACACTTACACCG
GAAATCCAACTTATGTGCAGGGGTTCACCGGCCGATTCGTGTTTTCTCTGGACACTTCCGTCTCTACCGCCTTTCTGCAC
ATTACAAGTCTGAAGGCAGAGGACACTGCCGTGTACTTCTGCGCTAGGGAAGGCGGAGCAAGAGGCTTTGATTATTGG
GGCCAGGGAACCCTGGTGACAGTCAGCTCCGCCAGCACAAAGGGACCCTCCGTGTTCCCACTGGCTCCCTCTAGTAAA
AGTACATCAGGGGGCACTGCCGCTCTGGGATGTCTGGTCAAAGATTACTTCCCCGAACCTGTGACCGTCAGCTGGAACT
CCGGAGCTCTGACCAGCGGGGTGCATACATTTCCCGCAGTCCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTGGT
CACAGTGCCTAGTTCAAGCCTGGGGACACAGACTTATATCTGTAATGTGAACCATAAGCCAAGCAACACCAAAGTGGA
CAAAAAAGTGGAACCTAAGAGCTGC**TGATAA**CTCGAG (SEQ ID NO:58)

FIG. 56

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

MDWTWRILFLVAAATGTHAQVQLVQSGSELKKPGASVKVSCKASGYTLTRYAMTWVRQAPGQGLEWMGWINTYTGNPT
YVQGFTGRFVFSLDTSVSTAFLHITSLKAEDTAVYFCAREGGARGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
(SEQ ID NO:59)

FIG. 57

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

GGATCCGCCACC**ATG**GCATGGACCCCACTGTTCCTGTTCCTGCTGACTTGTTGTCCTGGCGGGAGCAATTCACAGAGCG
TCCTGACCCAGCCCCCTTCTGTGTCCGGAGCACCAGGACAGCGAGTCACAATCTCTTGCACTGGAAGCTCCTCTAACAT
TGGGGCCAGCCACGACGTGCATTGGTACCAGCAGCTGCCAGGGACCGCTCCCACACTGCTGATCTATGTGAACTCTAAT
AGGCCTAGTGGCGTCCCAGATAGATTTTCAGGGAGCAAGTCCGGCACCTCTGCTAGTCTGGCAATTACAGGACTGCAG
GCTGAGGACGAAGCAGATTACTATTGCCAGAGTTACGACTCAAACCTGTCAGGCAGCGCAGTGTTCGGAGGAGGAACT
AAGCTGACCGTCCTGGGACAGCCCAAAGCCGCTCCTTCTGTGACCCTGTTTCCCCCTAGTTCAGAGGAACTGCAGGCCA
ACAAGGCTACTCTGGTGTGTCTGATCTCCGACTTCTACCCTGGAGCAGTGACCGTCGCATGGAAGGCCGATAGCTCCCC
AGTGAAAGCTGGGGTCGAGACCACAACTCCCAGCAAGCAGTCCAACAACAAGTACGCAGCCTCTAGTTATCTGTCACT
GACACCTGAACAGTGGAAGAGCCACAAATCCTATTCTTGCCAAGTGACTCATGAGGGCAGTACCGTGGAAAAGACAGT
CGCCCCAACTGAGTGTTCC**TGATAA**CTCGAG (SEQ ID NO:60)

FIG. 58

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

MAWTPLFLFLLTCCPGGSNSQSVLTQPPSVSGAPGQRVTISCTGSSSNIGASHDVHWYQQLPGTAPTLLIYVNSNRPSGVPDR
FSGSKSGTSASLAITGLQAEDEADYYCQSYDSNLSGSAVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFY
PGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS (SEQ ID
NO:61)

FIG. 59

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

GGATCCGCCACCATGGATTGGACATGGAGGATTCTGTTTCTGGTCGCCGCCGCTACAGGAACTCACGCCCAGGTGCAG
CTGGTGCAGTCAGGAGCCGAAGTGAAGCGACCAGGCAGCTCCGTCACTGTGTCCTGCAAAGCATCTGGCGGATCATTC
AGCACCTACGCCCTGAGCTGGGTGAGACAGGCTCCTGGACGAGGACTGGAATGGATGGGAGGCGTCATCCCACTGCTG
ACAATTACTAACTACGCCCCCCGATTTCAGGGCAGGATCACCATTACAGCAGACCGCTCCACTTCTACCGCCTATCTGG
AGCTGAATAGCCTGAGACCAGAAGATACCGCAGTGTACTATTGCGCCCGGGAGGGAACCACAGGATGGGGATGGCTG
GGAAAGCCCATCGGGGCTTTCGCACACTGGGGCCAGGGAACCCTGGTCACAGTGTCTAGTGCCAGCACAAAGGGCCCC
TCCGTGTTTCCCCTGGCTCCTTCAAGCAAAAGTACTTCAGGAGGGACCGCCGCTCTGGGATGTCTGGTGAAGGACTACT
TCCCTGAGCCAGTCACCGTGTCCTGGAACTCTGGCGCTCTGACCTCCGGAGTGCATACATTTCCCGCAGTCCTGCAGTC
CTCTGGGCTGTACTCTCTGAGTTCAGTGGTCACTGTGCCTAGCTCCTCTCTGGGCACACAGACTTATATCTGCAACGTGA
ATCACAAGCCCTCCAATACCAAAGTCGACAAGAAAGTGGAACCTAAGTCTTGTGATAAAACCCATACATGCCCACCTT
GTCCAGCACCTGAGCTGCTGGGCGGACCTTCCGTGTTCCTGTTTCCACCCAAGCCAAAAGACACACTGATGATTAGCCG
GACACCTGAAGTGACTTGTGTGGTCGTGGACGTCAGCCACGAGGACCCCGAAGTGAAGTTCAACTGGTACGTGGATGG
CGTCGAGGTGCATAATGCCAAGACCAAACCCAGGGAGGAACAGTACAACTCTACTTATAGGGTCGTGAGTGTCCTGAC
CGTGCTGCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTGTCCAATAAGGCCCTGCCAGCTCCCATCGA
GAAAACAATTTCTAAGGCTAAAGGCCAGCCACGCGAACCCCAGGTGTACACTCTGCCTCCCAGCAGGGACGAGCTGAC
CAAGAACCAGGTGAGTCTGACATGTCTGGTCAAAGGCTTCTATCCAAGCGATATCGCCGTGGAGTGGGAATCCAATGG
ACAGCCCGAAAACAATTACAAGACTACCCCCCCTGTGCTGGACAGTGATGGATCATTCTTTCTGTATTCCAAGCTGACC
GTGGACAAATCTCGCTGGCAGCAGGGGAACGTCTTTAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCATTACACA
CAGAAGTCTCTGAGTCTGTCACCAGGCAAGCGGGGACGCAAAAGGAGAAGCGGGTCCGGCGCTACTAACTTCAGCCTG
CTGAAACAGGCAGGGGATGTGGAGGAAAATCCTGGCCCAATGGTCCTGCAGACCCAGGTGTTTATCTCACTGCTGCTGT
GGATTAGCGGGGCTTATGGCGAAATCGTGCTGACTCAGAGCCCCGGAACCCAGTCTCTGAGTCCTGGGGAGCGCGCTA
CACTGAGCTGTCGAGCATCACAGAGCGTGGGGAACAATAAGCTGGCATGGTACCAGCAGAGGCCTGGCCAGGCTCCAA
GACTGCTGATCTATGGCGCAAGTTCACGGCCTAGCGGAGTGGCAGACCGCTTCTCCGGATCTGGGAGTGGCACCGATTT
TACTCTGACCATTAGCAGGCTGGAGCCAGAAGACTTCGCTGTGTACTATTGCCAGCAGTACGGCCAGTCACTGAGCACA
TTTGGACAGGGGACTAAGGTCGAAAAAAGAACCGTGGCAGCCCCAAGTGTCTTCATTTTTCCACCCTCAGACGAGCAG
CTGAAGAGTGGAACAGCCTCAGTCGTGTGTCTGCTGAACAATTTCTACCCCAGGGAGGCCAAGGTCCAGTGGAAAGTG
GATAACGCTCTGCAGAGCGGCAATTCCCAGGAGTCTGTGACAGAACAGGACAGTAAGGATTCAACTTATAGCCTGAGC
TCCACACTGACTCTGTCCAAAGCAGATTACGAGAAGCACAAAGTGTATGCCTGCGAAGTCACCCATCAGGGACTGTCT
AGTCCTGTGACAAAGTCTTTTAACAGAGGGGAGTGATAACTCGAG (SEQ ID NO:62)

FIG. 60

Nucleic Acid Sequence Encoding HIV-1 Env-PG9 Ig

GGATCCGCCACC**ATG**GACTGGACTTGGAGGATTCTGTTTCTGGTCGCCGCCGCAACTGGAACTCACGCTGAATTTGGAC
TGTCATGGGTCTTTCTGGTGGCCTTTCTGCGAGGGGTCCAGTGCCAGAGGCTGGTGGAGTCCGGAGGAGGAGTGGTCCA
GCCAGGCAGCTCCCTGCGACTGAGTTGTGCCGCTTCAGGGTTCGACTTTTCTAGACAGGGCATGCACTGGGTGCGGCAG
GCACCAGGACAGGGACTGGAGTGGGTGGCTTTCATCAAGTACGACGGAAGTGAAAAATATCATGCCGATTCAGTGTGG
GGGCGGCTGTCAATTAGCCGCGACAACTCCAAGGATACCCTGTACCTGCAGATGAATTCTCTGAGGGTCGAGGACACA
GCTACTTATTTCTGCGTGAGGGAAGCAGGCGGACCTGATTACAGAAACGGGTATAATTACTATGACTTTTACGATGGCT
ACTATAACTACCACTATATGGACGTGTGGGGCAAGGGAACCACAGTCACAGTGTCTAGTGCATCAACTAAAGGCCCAA
GCGTGTTTCCCCTGGCCCCTTCAAGCAAGTCCACTTCTGGAGGAACCGCAGCACTGGGATGTCTGGTGAAGGATTACTT
CCCTGAGCCAGTCACCGTGAGTTGGAACTCAGGCGCCCTGACTAGCGGAGTCCATACCTTTCCTGCTGTGCTGCAGTCC
TCTGGGCTGTACAGCCTGAGTTCAGTGGTCACAGTGCCAAGCTCCTCTCTGGGCACCCAGACATATATCTGCAACGTGA
ATCACAAGCCTAGCAATACTAAGGTCGACAAAAGAGTGGAACCAAAGAGCTGTGATAAAACTCATACCTGCCCACCTT
GTCCAGCACCTGAGCTGCTGGGAGGGCCTTCCGTGTTCCTGTTTCCACCCAAGCCAAAAGACACCCTGATGATTAGCCG
GACACCAGAAGTCACTTGCGTGGTCGTGGACGTGAGCCACGAGGACCCCGAAGTCAAGTTTAACTGGTACGTGGATGG
CGTCGAGGTGCATAATGCTAAGACAAAACCACGGGAGGAACAGTACAACTCCACATATCGCGTCGTGTCTGTCCTGAC
TGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTATAAGTGCAAAGTGTCCAATAAGGCACTGCCAGCCCCCATCGA
GAAAACCATTTCTAAGGCCAAAGGCCAGCCACGAGAACCCCAGGTGTACACACTGCCTCCAAGTAGGGACGAGCTGAC
TAAGAACCAGGTCTCTCTGACCTGTCTGGTGAAAGGCTTCTATCCCTCTGATATCGCTGTGGAGTGGGAAAGTAATGGA
CAGCCTGAAAACAATTACAAGACTACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTATAGCAAGCTGACCG
TGGACAAATCCAGATGGCAGCAGGGGAACGTCTTTAGTTGCTCAGTGATGCACGAGGCACTGCACAATCATTACACCC
AGAAAAGCCTGTCCCTGTCTCCTGGCAAGAGGGGAAGAAAAAGGAGAAGTGGGTCAGGCGCAACAAACTTCAGCCTG
CTGAAGCAGGCCGGAGATGTGGAGGAAAATCCTGGGCCAATGGCTTGGACCCCCCTGTTCCTGTTTCTGCTGACATGCT
GTCCTGGCGGAAGCAACTCCCAGTCTGCACTGACACAGCCAGCAAGTGTGTCAGGGAGCCCAGGACAGAGCATCACCA
TTTCCTGTAACGGCACAAGCAATGACGTCGGGGGCTACGAGTCCGTGTCTTGGTATCAGCAGCATCCTGGAAAGGCCCC
AAAAGTCGTGATCTACGATGTCAGCAAACGCCCCTCTGGGGTGAGTAACCGATTCAGTGGATCAAAGAGCGGGAATAC
CGCTTCTCTGACAATTAGTGGCCTGCAGGCAGAGGACGAAGGAGATTACTATTGCAAATCACTGACAAGCACTCGGCG
CCGAGTCTTCGGAACCGGGACAAAGCTGACTGTGCTGGGCCAGCCCAAAGCTGCACCTAGCGTGACCCTGTTTCCACCC
AGTTCAGAGGAACTGCAGGCTAATAAGGCAACACTGGTGTGTCTGATCTCCGACTTCTACCCTGGCGCTGTCACTGTGG
CCTGGAAGGCTGATAGCTCCCCAGTCAAAGCAGGAGTGGAAACAACTACCCCCTCCAAGCAGTCTAACAACAAGTACG
CCGCTTCTAGTTATCTGTCACTGACTCCCGAGCAGTGGAAGAGCCACAAATCCTATTCTTGCCAGGTGACCCATGAGGG
CTCCACTGTCGAAAAGACCGTGGCCCCTACAGAGTGTTCT**TGATAA**CTCGAG (SEQ ID NO:63)

FIG. 61

Nucleic Acid Sequence Encoding VRC01 IgG

GGATCCGCCACC**ATG**GATTGGACATGGATTCTGTTCCTGGTCGCCGCCGCAACTAGAGTGCATTCACAGGTGCAGCTGG
TGCAGTCAGGCGGGCAGATGAAGAAACCCGGCGAGAGTATGCGAATCTCATGCCGGGCTAGCGGGTACGAATTCATCG
ACTGTACCCTGAACTGGATTAGACTGGCACCTGGGAAGAGGCCAGAGTGGATGGGATGGCTGAAACCTAGAGGCGGG
GCAGTGAATTACGCCAGACCACTGCAGGGCAGGGTCACTATGACCCGCGACGTGTATTCTGATACCGCATTCCTGGAG
CTGCGAAGTCTGACAGTCGACGATACTGCCGTGTACTTCTGCACACGGGGCAAGAACTGTGACTATAATTGGGATTTTG
AACACTGGGGCAGGGGGACACCTGTCATTGTGAGCTCCCCAAGTACTAAGGGACCCTCAGTGTTTCCCCTGGCCCCTTC
TAGTAAAAGTACCTCAGGAGGCACAGCCGCTCTGGGATGCCTGGTGAAGGATTACTTCCCTGAGCCAGTCACCGTGAG
TTGGAACTCAGGCGCCCTGACAAGCGGGGTCCATACTTTTCCAGCTGTGCTGCAGTCAAGCGGGCTGTACTCCCTGTCC
TCTGTGGTCACAGTGCCCAGTTCAAGCCTGGGAACACAGACTTATATCTGTAACGTCAATCACAAGCCTAGCAATACTA
AAGTGGACAAGAAAGCCGAGCCTAAGAGCTGCGAACCAAAGTCCTGTGATAAAACCCATACATGCCCTCCCTGTCCAG
CTCCTGAACTGCTGGGCGGCCCATCCGTGTTCCTGTTTCCACCCAAGCCCAAAGACACCCTGATGATTAGCAGGACTCC
TGAGGTCACCTGCGTGGTCGTGGACGTGTCCCACGAGGACCCCGAAGTCAAGTTTAACTGGTACGTGGATGGCGTCGA
AGTGCATAATGCCAAGACAAAACCCCGGGAGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTCCTGACAGTGCT
GCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTGTCTAATAAGGCCCTGCCAGCTCCCATCGAGAAAAC
AATTTCCAAGGCAAAAGGCCAGCCAAGGGAACCCCAGGTGTACACTCTGCCTCCATCCCGCGACGAGCTGACTAAGAA
CCAGGTCTCTCTGACCTGTCTGGTGAAAGGATTCTATCCAAGCGATATCGCCGTGGAGTGGGAATCCAATGGCCAGCCC
GAGAACAATTACAAGACCACACCCCCTGTGCTGGACAGCGATGGCTCCTTCTTTCTGTATTCAAAGCTGACCGTGGATA
AAAGCCGCTGGCAGCAGGGGAACGTCTTTAGCTGCTCCGTGATGCACGAAGCTCTGCACAATCATTACACCCAGAAGT
CTCTGAGTCTGTCACCTGGCAAGAGGGGACGAAAACGGAGAAGCGGCAGCGGAGCTACAAACTTCAGCCTGCTGAAA
CAGGCAGGCGACGTGGAGGAAAATCCTGGGCCAATGGATTGGACTTGGATTCTGTTCCTGGTGGCAGCCGCTACCAGA
GTCCATTCCGAAATTGTGCTGACCCAGTCTCCCGGAACACTGTCTCTGAGTCCTGGCGAGACAGCCATCATTTCCTGTA
GGACTTCTCAGTACGGGAGTCTGGCATGGTATCAGCAGCGACCAGGACAGGCTCCTCGACTGGTCATCTACTCAGGAA
GCACTCGGGCAGCCGGCATTCCCGACCGATTCTCCGGGTCTCGGTGGGGACCTGATTACAACCTGACCATCTCAAATCT
GGAAAGCGGAGACTTTGGCGTGTACTATTGCCAGCAGTATGAGTTCTTTGGGCAGGGAACCAAGGTCCAGGTGGACAT
CAAACGCACAGTCGCTGCACCAAGCGTGTTCATCTTTCCACCCTCAGATGAACAGCTGAAGTCCGGCACCGCCTCTGTG
GTGTGCCTGCTGAACAATTTCTACCCCCGGGAGGCAAAGGTCCAGTGGAAAGTGGACAACGCCCTGCAGTCTGGCAAT
AGTCAGGAGTCAGTGACTGAACAGGACAGCAAGGATTCCACCTATTCTCTGTCCTCTACTCTGACCCTGAGCAAAGCTG
ATTACGAGAAGCACAAAGTGTATGCATGTGAGGTCACCCACCAGGGACTGCGGTCACCCGTCACCAAGAGCTTCAATC
GCGGAGAGTGT**TGATAA**CTCGAG (SEQ ID NO:64)

FIG. 62

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) **Date of publication of the international search report**:
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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.

WO 2014/093894 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/75137

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/75137

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 48-50
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, claims 1-47, 51-52, drawn to 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; or 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; or using the method for preventing or treating a disease in a subject; as well as the product produced by the method. The first invention is restricted to the recombinant nucleic acid further comprises a promoter that is CMV, and the method is directed to treating HIV and SEQ ID NOs: 1 and 3-4 (Specification: para [00292] - SEQ ID NO: 3, VH-CH1; and SEQ ID NO: 4, VL-CL). Group I+ will be searched to the extent that it reads on the recombinant nucleic acid further comprises a promoter that is CMV and SEQ ID NOs: 1 and 3-4, without fee. It is believed that claims 1-6, 10-15, 19-27, 31-37, 51 read on this first named invention. **********Continued in the extra sheet**********

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6, 10-15, 19-27, 31-37, and 51, limited to SEQ ID NOs: 1 and 3-4.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/75137

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 48/00; A61K 39/395; A61K 39/00; C07H 21/04 (2014.01)
USPC - 514/44R; 424/130.1; 424/133.1; 536/23.53
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 48/00; A61K 39/395; A61K 39/00; C07H 21/04; A61K 39/42 (2014.01)
USPC - 514/44R; 424/130.1; 424/133.1; 536/23.53; 424/159.1, 424/160.1; 424/174.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - A61K 48/00; A61K 39/395; A61K 39/00; C07H 21/04; A61K 39/42 (2014.01) - see keyword below
USPC - 514/44R; 424/130.1; 424/133.1; 536/23.53; 424/159.1, 424/160.1; 424/174.1 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); PatBase; Medline, Google: vector, cassette, construct, expressing, encoding, antibody, HIV, synthesize, heavy chain, light chain, promoter, CMV, antigen, cytomegalovirus, constant, CH1, CH2, CH3, CL, VRC02, VRC01, VRC03, scFV, C.gamma.1, polynucleotide, nucleic acid, pharmaceutical, composition, pathogen, treating,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	US 2012/0282264 A1 (MASCOLA et al.) 08 November 2012 (08.11.2012), para [0003], [0041-[0043], [0101], [0102], [0127]-[0130], [0133], [0134], [0162], [0164], [0166], [0170], [0188], [0191], [0221], [0238], [0249], [0253], [0271], [0274]-[0276], [0300], [0305], [0312], [0350], [0366], [0371], [0434], and [0484]	1-6, 10-15, 21-27, 31-35, and 51 --- 19-20, 36-37
A	US 2012/0269723 A1 (BRINKMANN et al.) 25 October 2012 (25.10.2012), Abstract, para [0288], and SEQ ID NO: 44 (943 a.a.), amino acid residues betweeen 1-678	19, 36
A	US 2012/0232133 A1 (BALAZS et al.) 13 September 2012 (13.09.2012), para [0135], and SEQ ID NO: 24 (6418 nt), nucleotides between 1237-1969 and 2805-3450	20, 37
A	KIM et al. Two-promoter vector is highly efficient for overproduction of protein complexes. Protein Sci. 2004, Vol. 13(6), p..1698-1703. Abstract; pg 1700, Fig 1; and pg 1701, Fig 3	10-12, 23-24,
A	ELLISON et al.. The nucleotide sequence of a human immunoglobulin C.gamma.1 gene. Nucleic Acids Res. 1982, Vol. 10(13), p. 4071-4079. Abstract	13-14, 25-26
A	NCHU_Administrator, Microsoft PowerPoint - week 3 Antibody structure Ag-Ab interactions, NCHU, Power Point, 2007 [online]. [Retrieved on 2014.02.12]. Retrieved from the Internet: <URL: http://www.as.nchu.edu.tw/lab/5c/course/antibody/week%203%20Antibody%20structure%20Ag-Ab%20interactions.pdf> pg 1-44, pg 6, Antibody structure by modern techniques	13-14, 25-26

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 February 2014 (14.02.2014)	20 MAY 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/75137

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FANG et al. An antibody delivery system for regulated expression of therapeutic levels of monoclonal antibodies in vivo. Mol Ther. 2007, Vol. 15(6), p. 1153-9. Entire documentation, especially Abstract.	1-6, 10-15, 19-27, 31-37, 51

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/75137

Continuation of:
Box No III (unity of invention is lacking)

Applicants must indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: 1) wherein the recombinant nucleic acid sequence further comprises a third nucleic acid sequence encoding a protease cleavage sites (1-15, 91-27, 31-37, 51); or 2) treating CHIKV, directed to SEQ ID NOs: 58-61 (claims 1-6, 10-15, 21-27, 31-34, 38-40, 51).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Feature
Among Groups I+, each additional nucleic acid encoding a third element is structurally and functionally different from all other nucleic acids encoding a third element; and the method for treating each disorder requires a functionally different antibody, and each SEQ ID NO represents a structurally different nucleotide or protein sequence; and each antibody is structurally different from all others.

Common Technical Features
The inventions of Groups I+ share the technical feature of generating a synthetic antibody in a subject, 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; or
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; and
a method of preventing or treating a disease in a subject, comprising generating a synthetic antibody in a subject.

The inventions of claims 31-47 of Groups I+ further share the technical feature of preventing or treating a disease in a subject, comprising generating a synthetic antibody in a subject.

The inventions of Claims 19-20 and 36-37 of Groups I+ further share the technical feature of a nucleic acid encoding an antibody for treating or preventing HIV.

However, these shared technical features do not represent a contribution over prior art as being anticipated by US 2012/0282264 A1 to MASCOLA et al. (hereinafter 'Mascola') as follows:

Mascola discloses a method of generating a synthetic antibody in a subject (para [0350] - 'administration of the antibody results in a reduction in the establishment of HIV infection and/or reducing subsequent HIV disease progression in a subject...administering to the subject a therapeutically effective amount of ... a nucleic acid encoding the antibody', wherein 'administering to the subject a therapeutically effective amount of ... a nucleic acid encoding the antibody' is for '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 (para [0350] - 'administering to the subject a therapeutically effective amount of ... a nucleic acid encoding the antibody, thereby preventing or treating the HIV-1 infection'; para [0305] - 'Any of the nucleic acids encoding any of the antibodies, V.sub.H and/or V.sub.L, ...or fragment... can be expressed'; para [0166]; para [0366] - 'Plasmids including nucleic acid sequences encoding the VRC03 heavy chain and VRC03 light chain'; para [0345] - 'Compositions ... antibodies that specifically bind gp120 ... functional fragments'; para [0484] - 'treat HIV in a human subject by administration ...gp120...human neutralizing mAbs' para [0253] - 'a novel class of gp120 antibodies, ... VRC03').
Mascola further discloses using a cytomegalovirus (CMV) promoter for antibody expression (para [0312] - 'The expression of nucleic acids encoding the isolated proteins...The promoter can be any promoter of interest, including a cytomegalovirus promoter').

Mascola discloses a method of generating a synthetic antibody in a subject (para [0350] - 'administration of the antibody results in a reduction in the establishment of HIV infection and/or reducing subsequent HIV disease progression in a subject...administering to the subject a therapeutically effective amount of ... a nucleic acid encoding the antibody', wherein 'administering to the subject a therapeutically effective amount of ... a nucleic acid encoding the antibody' is for '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 (para [0350] - 'administering to the subject a therapeutically effective amount of ... a nucleic acid encoding the antibody, thereby preventing or treating the HIV-1 infection'; para [0305] - 'Any of the nucleic acids encoding any of the antibodies, V.sub.H and/or V.sub.L, ...or fragment... can be expressed'; para [0166]; para [0042] - 'VRC01 precisely targets the CD4-defined site of vulnerability on HIV-1 gp120'),
---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 (para [0305] - 'Any of the nucleic acids encoding any of the antibodies, V.sub.H and/or V.sub.L, ...or fragment... can be expressed'; para [0366] - 'Plasmids including the nucleic acids encoding VRC01 heavy chain, VRC01 light chain, ...were deposited ...VRC01 heavy chain... PTA-10412, VRC01 light chain ...PTA-10411'; para [0042] - 'VRC01 precisely targets the CD4 -defined site of vulnerability on HIV-1 gp120', wherein ' VRC01' indicating 'wherein the synthetic antibody is generated by the first and second polypeptides').

**********Continued in the next extra sheet**********

Continuation of:
The previous extra sheet - Box No III (unity of invention is lacking)

Mascola further discloses a method of preventing or treating a disease in a subject, the method comprising generating a synthetic antibody in a subject (para [0350] - 'administering to the subject a therapeutically effective amount of ... a nucleic acid encoding the antibody, thereby preventing or treating the HIV-1 infection'; para [0305] - 'Any of the nucleic acids encoding any of the antibodies, V.sub.H and/or V.sub.L, ...or fragment... can be expressed'; para [0166]; para [0366] - "Plasmids including the nucleic acids encoding VRC01 heavy chain, VRC01 light chain...Plasmids including nucleic acid sequences encoding the VRC03 heavy chain and VRC03 light chain'; para [0253] - 'a novel class of gp120 antibodies, as exemplified by VRC01, ...VRC03'; para [0170] - 'an antigen is derived from HIV, such as a gp120 polypeptide').

Furthermore, Mascola further discloses a nucleic acid encoding an antibody for treating or preventing HIV (para [0253] - 'a novel class of gp120 antibodies, as exemplified by VRC01, ...VRC03'; para [0170] - 'an antigen is derived from HIV, such as a gp120 polypeptide'; Para [0129]-[0130] - ' the nucleic acid sequence of the heavy chain of gp120-specific antibody VRC01... the nucleic acid sequence of the light chain of gp120-specific antibody VRC01'; para [0101] -[0102] - 'heavy chain of gp120-specific antibody VRC01... the light chain of gp120-specific antibody VRC01'; para [0133]-[0134] - the nucleic acid sequence of the heavy chain of gp120-specific antibody VRC03....the nucleic acid sequence of the light chain of gp120-specific antibody VRC03'; para [0127]-[0128] - 'the amino acid sequence of the heavy chain of gp120-specific antibody VRC03....the amino acid sequence of the light chain of gp120-specific antibody VRC03'; para [0336])

Without a shared special technical feature, the inventions lack unity with one another.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note re item 4: Claims 48-50 are not drafted in accordance with the second and third sentences of Rule 6.4 (a). These claims are improper multiple dependent claims.

(19)中华人民共和国国家知识产权局

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权利要求书3页 说明书45页 附图35页

(54)发明名称

DNA抗体构建体及其使用方法

(57)摘要

公开了包含编码抗体的重组核酸序列的组合物。还公开了通过给予受试者所述组合物在受试者中产生合成抗体的方法。本公开还提供了使用所述组合物和产生方法预防和/或治疗受试者疾病的方法。

CN 104853782 A

1. 一种在受试者中产生合成抗体的方法，所述方法包括向所述受试者施用包含编码抗体或其片段的重组核酸序列的组合物，其中所述重组核酸序列在所述受试者中表达以产生所述合成抗体。

2. 根据权利要求 1 所述的方法，其中所述抗体包含重链多肽或其片段和轻链多肽或其片段。

3. 根据权利要求 2 所述的方法，其中所述重链多肽或其片段由第一核酸序列编码并且所述轻链多肽或其片段由第二核酸序列编码。

4. 根据权利要求 3 所述的方法，其中所述重组核酸序列包含所述第一核酸序列和所述第二核酸序列。

5. 根据权利要求 4 所述的方法，其中所述重组核酸序列还包含用于在所述受试者中将所述第一核酸序列和所述第二核酸序列表达为单个转录物的启动子。

6. 根据权利要求 5 所述的方法，其中所述启动子是巨细胞病毒 (CMV) 启动子。

7. 根据权利要求 5 所述的方法，其中所述重组核酸序列还包含编码蛋白酶切割位点的第三核酸序列，其中所述第三核酸序列位于所述第一核酸序列与第二核酸序列之间。

8. 根据权利要求 7 所述的方法，其中所述受试者的蛋白酶识别并且切割所述蛋白酶切割位点。

9. 根据权利要求 8 所述的方法，其中所述重组核酸序列在所述受试者中表达以产生抗体多肽序列，其中所述抗体多肽序列包含所述重链多肽或其片段、所述蛋白酶切割位点和所述轻链多肽或其片段，其中由所述受试者产生的所述蛋白酶识别并且切割所述抗体多肽序列的蛋白酶切割位点，从而产生切割的重链多肽和切割的轻链多肽，其中所述合成抗体由所述切割的重链多肽和所述切割的轻链多肽产生。

10. 根据权利要求 4 所述的方法，其中所述重组核酸序列包含用于将所述第一核酸序列表达为第一转录物的第一启动子和用于将所述第二核酸序列表达为第二转录物的第二启动子，其中所述第一转录物被翻译成第一多肽，并且所述第二转录物被翻译成第二多肽，其中所述合成抗体由所述第一和第二多肽产生。

11. 根据权利要求 10 所述的方法，其中所述第一启动子和所述第二启动子相同。

12. 根据权利要求 11 所述的方法，其中所述启动子是巨细胞病毒 (CMV) 启动子。

13. 根据权利要求 2 所述的方法，其中所述重链多肽包含可变重区和恒定重区 1。

14. 根据权利要求 2 所述的方法，其中所述重链多肽包含可变重区、恒定重区 1、铰链区、恒定重区 2 和恒定重区 3。

15. 根据权利要求 2 所述的方法，其中所述轻链多肽包含可变轻区和恒定轻区。

16. 根据权利要求 1 所述的方法，其中所述重组核酸序列还包含 Kozak 序列。

17. 根据权利要求 1 所述的方法，其中所述重组核酸序列还包含免疫球蛋白 (Ig) 信号肽。

18. 根据权利要求 17 所述的方法，其中所述 Ig 信号肽包括 IgE 或 IgG 信号肽。

19. 根据权利要求 1 所述的方法，其中所述重组核酸序列包含编码 SEQ ID NO:1、2、5、41、43、45、46、47、48、49、51、53、55、57、59 和 61 的至少一个氨基酸序列的核酸序列。

20. 根据权利要求 1 所述的方法，其中所述重组核酸序列包含 SEQ ID NO:3、4、6、7、40、42、44、50、52、54、56、58、60、62 和 63 的至少一个核酸序列。

21. 一种在受试者中产生合成抗体的方法，所述方法包括向所述受试者施用包含编码重链多肽或其片段的第一重组核酸序列和编码轻链多肽或其片段的第二重组核酸序列的组合物，其中所述第一重组核酸序列在所述受试者中表达以产生第一多肽，并且所述第二重组核酸在所述受试者中表达以产生第二多肽，其中所述合成抗体由所述第一和第二多肽产生。

22. 根据权利要求 21 所述的方法，其中所述第一重组核酸序列还包含用于在所述受试者中表达所述第一多肽的第一启动子以及其中所述第二重组核酸序列还包含用于在所述受试者中表达所述第二多肽的第二启动子。

23. 根据权利要求 22 所述的方法，其中所述第一启动子和第二启动子相同。

24. 根据权利要求 23 所述的方法，其中所述启动子是巨细胞病毒 (CMV) 启动子。

25. 根据权利要求 21 所述的方法，其中所述重链多肽包含可变重区和恒定重区 1。

26. 根据权利要求 21 所述的方法，其中所述重链多肽包含可变重区、恒定重区 1、铰链区、恒定重区 2 和恒定重区 3。

27. 根据权利要求 21 所述的方法，其中所述轻链多肽包含可变轻区和恒定轻区。

28. 根据权利要求 21 所述的方法，其中所述第一重组核酸序列和所述第二重组核酸序列还包含 Kozak 序列。

29. 根据权利要求 21 所述的方法，其中所述第一重组核酸序列和所述第二重组核酸序列还包含免疫球蛋白 (Ig) 信号肽。

30. 根据权利要求 29 所述的方法，其中所述 Ig 信号肽包括 IgE 或 IgG 信号肽。

31. 一种预防或治疗受试者的疾病的方法，所述方法包括根据权利要求 1 或 21 所述的方法在受试者中产生合成抗体。

32. 根据权利要求 31 所述的方法，其中所述合成抗体对于外来抗原是特异性的。

33. 根据权利要求 32 所述的方法，其中所述外来抗原来源于病毒。

34. 根据权利要求 33 所述的方法，其中所述病毒是人免疫缺陷病毒 (HIV)、基孔肯雅热病毒 (CHIKV) 或登革热病毒。

35. 根据权利要求 34 所述的方法，其中所述病毒是 HIV。

36. 根据权利要求 35 所述的方法，其中所述重组核酸序列包含编码 SEQ ID NO:1、2、5、46、47、48、49、51、53、55 和 57 的至少一个氨基酸序列的核酸序列。

37. 根据权利要求 35 所述的方法，其中所述重组核酸序列包含 SEQ ID NO:3、4、6、7、50、52、55、56、62、63 和 64 的至少一个核酸序列。

38. 根据权利要求 34 所述的方法，其中所述病毒是 CHIKV。

39. 根据权利要求 38 所述的方法，其中所述重组核酸序列包含编码 SEQ ID NO:59 和 61 的至少一个氨基酸序列的核酸序列。

40. 根据权利要求 38 所述的方法，其中所述重组核酸序列包含 SEQ ID NO:58 和 60 的至少一个核酸序列。

41. 根据权利要求 34 所述的方法，其中所述病毒是登革热病毒。

42. 根据权利要求 41 所述的方法，其中所述重组核酸序列包含编码 SEQ ID NO:45 的至少一个氨基酸序列的核酸序列。

43. 根据权利要求 41 所述的方法，其中所述重组核酸序列包含 SEQ ID NO:44 的至少一

个核酸序列。

44. 根据权利要求 31 所述的方法，其中所述合成抗体对于自体抗原是特异性的。

45. 根据权利要求 44 所述的方法，其中所述自体抗原是 Her2。

46. 根据权利要求 45 所述的方法，其中所述重组核酸序列包含编码 SEQ ID NO:41 和 43 的至少一个氨基酸序列的核酸序列。

47. 根据权利要求 45 所述的方法，其中所述重组核酸序列包含 SEQ ID NO:40 和 42 的至少一个核酸序列。

48. 一种通过根据权利要求 1-47 所述的方法的任一方法产生的产物。

49. 根据权利要求 48 所述的产物，其中所述产物是能够表达功能性抗体的单个 DNA 质粒。

50. 根据权利要求 48 所述的产物，其中所述产物由两种不同的能够表达功能性抗体组分的 DNA 质粒组成，所述功能性抗体组分在体内组合以形成功能性抗体。

51. 一种治疗受试者的病原体感染的方法，其包括 ：

施用编码对于所述病原体是特异性的合成抗体的核苷酸序列。

52. 根据权利要求 51 所述的方法，其还包括 ：

施用所述病原体的抗原以在所述受试者中产生免疫应答。

DNA 抗体构建体及其使用方法

[0001] 相关申请的交叉参考

[0002] 本申请要求 2012 年 12 月 13 日提交的美国临时申请 No. 61/737,094、2013 年 9 月 23 日提交的美国临时申请 No. 61/881,376 以及 2013 年 10 月 28 日提交的美国临时申请 No. 61/896,646 的优先权，所述美国临时申请全部在此通过引用并入。

[0003] 政府利益的声明

[0004] 本发明是在由美国国立卫生研究院授予的合同号 HHSN272200800063C 和 5-P30-AI-045008-13 下由政府资助进行的。政府具有本发明的某些权利。

技术领域

[0005] 本发明涉及包含用于在体内产生合成抗体或其片段的重组核酸序列的组合物，以及通过施用所述组合物预防和 / 或治疗受试者疾病的方法。

背景技术

[0006] 免疫球蛋白分子包含轻链 (L) 和重链 (H) 的每一种类型的 2 条链，所述轻链和重链通过半胱氨酸残基之间的二硫键 (显示为 S-S) 共价连接。重链 (VH) 和轻链 (VL) 的可变结构域促成抗体分子的结合位点。重链恒定区由 3 个恒定结构域 (CH1、CH2 和 CH3) 和 (柔性) 铰链区组成。轻链也具有恒定结构域 (CL)。重链和轻链的可变区包含 4 个框架区 (FR ;FR1、FR2、FR3 和 FR4) 和 3 个互补决定区 (CDR ;CDR1、CDR2 和 CDR3)。因此，这些是在体内难以装配的非常复杂的遗传系统。

[0007] 靶向单克隆抗体 (mAb) 代表过去 25 年中最重要的医学治疗进展之一。该类型的基于免疫的疗法现在被常规地用于抗宿主的自身免疫性疾病，治疗癌症以及感染性疾病。对于恶性肿瘤，将许多目前使用的基于免疫球蛋白 (Ig) 的疗法与针对肿瘤的细胞毒性化疗方案组合。该组合方法已显著提高总生存率。多种 mAb 制剂被批准用于针对特定癌症，包括瑞图宣 (利妥昔单抗)，一种靶向 CD20 以用于治疗非霍奇金氏淋巴瘤 (Non-Hodgkins lymphoma) 的嵌合 mAb，和伊匹单抗 (Yervoy)，一种阻断 CTLA-4 并且已被用于治疗黑色素瘤和其它恶性肿瘤的人 mAb。此外，贝伐单抗 (阿瓦斯丁) 是另一个突出的人源化 mAb，其靶向 VEGF 和肿瘤新血管形成，并已用于治疗结直肠癌。也许最为人所知的用于治疗恶性肿瘤的 mAb 是曲妥珠单抗 (赫赛汀)，一种已被证明在一个亚组的患者中具有相当的抗乳腺癌功效的靶向 HER2/neu 的人源化制剂。此外，许多 mAb 用于治疗自身免疫性病症和特定的血液病症。

[0008] 除了癌症治疗以外，多克隆 Ig 的被动转移介导抗许多感染性疾病包括白喉、甲型和乙型肝炎、狂犬病、破伤风、水痘和呼吸道合胞病毒 (RSV) 的保护性功效。事实上，在当来不及通过主动接种来产生保护性 Ig 的情况下，几种多克隆 Ig 制剂提供为旅行至疾病流行地区的个体提供抗特定传染性病原体的临时保护。此外，在具有免疫缺陷的儿童中，帕利珠单抗 (Synagis)，一种靶向 RSV 感染的 mAb，已被证明在临床上保护免受 RSV 感染。

[0009] mAb 疗法的临床影响是令人印象深刻的。然而，仍然存在限制该治疗方法的使用和

传播的问题。这些问题中的一些包括这些复杂生物制品的高生产成本，这可限制它们在更广泛群体中，特别在其中它们可具有巨大影响的发展中国家使用。此外，对重复施用 mAb 以获得和维持功效的频繁需要在物流和患者依从性方面可成为障碍。此外，这些抗体制剂的长期稳定性通常很短并且在最佳状态以下。因此，在本领域仍然需要可以以安全且成本有的方式递送至受试者的合成抗体分子。此外，已论述了合成抗体的鉴定和表达方法；然而，所述蛋白的生产仍然是有问题的并且昂贵。

[0010] 免疫疗法和免疫调节提供了治疗模式，所述模式通过与受试者的免疫系统一起运行或调节或刺激受试者的免疫系统以击退病原体或杀死患病细胞来治疗疾病。疫苗提供了一类可刺激细胞和体液免疫系统以用于预防，并且在某些情况下治疗疾病的药物。例如，流感疫苗可帮助受试者产生针对流感病毒的记忆应答并且帮助预防将来的感染。然而，现有的担忧是关于触发快速发病机制（其中快速中和抗体应答可以是有益的）的病原体，诸如，例如热带病毒如基孔肯雅热或登革热或埃博拉病毒。在这样的情况下，如果受试者不具有已建立且有效的记忆应答，那么宿主体液应答的延迟可证明是致命的。此外，立即产生中和抗体对于帮助在病毒完全感染并且适应宿主之前免受有问题的病毒诸如 HIV 感染可能是有益的。需要可提供立即记忆应答，或更优选地中和抗体应答；必要时，可与刺激宿主免疫应答的疫苗配对以用于联合治疗的疫苗。

发明内容

[0011] 简述

[0012] 本发明涉及在受试者中产生合成抗体的方法。所述方法可包括向受试者施用包含编码抗体或其片段的重组核酸序列的组合物。可在受试者中表达所述重组核酸序列以产生合成抗体。

[0013] 所述抗体可包含重链多肽或其片段和轻链多肽或其片段。重链多肽或其片段可由第一核酸序列编码并且轻链多肽或其片段可由第二核酸序列编码。重组核酸序列可包含第一核酸序列和第二核酸序列。重组核酸序列还可包含用于将第一核酸序列和第二核酸序列作为单个转录物在受试者中表达的启动子。所述启动子可以是巨细胞病毒 (CMV) 启动子。

[0014] 所述重组核酸序列还可包含编码蛋白酶切割位点的第三核酸序列。所述第三核酸序列可位于第一核酸序列与第二核酸序列之间。受试者的蛋白酶可识别并切割所述蛋白酶切割位点。

[0015] 可在受试者中表达重组核酸序列以产生抗体多肽序列。抗体多肽序列可包含重链多肽或其片段、蛋白酶切割位点和轻链多肽或其片段。由受试者产生的蛋白质酶可识别并切割抗体多肽序列的蛋白酶切割位点，从而产生切割的重链多肽和切割的轻链多肽。合成抗体可由切割的重链多肽和切割的轻链多肽产生。

[0016] 所述重组核酸序列可包含用于将第一核酸序列表达为第一转录物的第一启动子和用于将第二核酸序列表达为第二转录物的第二启动子。所述第一转录物可被翻译成第一多肽，并且所述第二转录物可被翻译成第二多肽。合成抗体可由第一和第二多肽产生。第一启动子和第二启动子可以相同。所述启动子可以是巨细胞病毒 (CMV) 启动子。

[0017] 所述重链多肽可包含可变重区和恒定重区 1。所述重链多肽可包含可变重区、恒定重区 1、铰链区、恒定重区 2 和恒定重区 3。所述轻链多肽可包含可变轻区和恒定轻区。

[0018] 所述重组核酸序列还可包含 Kozak 序列。所述重组核酸序列还可包含免疫球蛋白 (Ig) 信号肽。所述 Ig 信号肽可包括 IgE 或 IgG 信号肽。

[0019] 所述重组核酸序列可包含编码 SEQ ID NO:1、2、5、41、43、45、46、47、48、49、51、53、55、57、59 和 61 的至少一个氨基酸序列的核酸序列。所述重组核酸序列可包含 SEQ ID NO:3、4、6、7、40、42、44、50、52、54、56、58、60、62 和 63 的至少一个核酸序列。

[0020] 本发明还涉及在受试者中产生合成抗体的方法。所述方法可包括向受试者施用包含编码重链多肽或其片段的第一重组核酸序列和编码轻链多肽或其片段的第二重组核酸序列的组合物。可在受试者中表达第一重组核酸序列以产生第一多肽，并且可在受试者中表达第二重组核酸以产生第二多肽。合成抗体可由所述第一和第二多肽产生。

[0021] 所述第一重组核酸序列还可包含用于在受试者中表达第一多肽的第一启动子。所述第二重组核酸序列还可包含用于在受试者中表达第二多肽的第二启动子。所述第一启动子和第二启动子可以相同。所述启动子可以是巨细胞病毒 (CMV) 启动子。

[0022] 所述重链多肽可包含可变重区和恒定重区 1。所述重链多肽可包含可变重区、恒定重区 1、铰链区、恒定重区 2 和恒定重区 3。所述轻链多肽可包含可变轻区和恒定轻区。

[0023] 所述第一重组核酸序列和第二重组核酸序列还可包含 Kozak 序列。所述第一重组核酸序列和第二重组核酸序列还可包含免疫球蛋白 (Ig) 信号肽。所述 Ig 信号肽可包括 IgE 或 IgG 信号肽。

[0024] 本发明还涉及预防或治疗受试者的疾病的方法。所述方法可包括按照上述方法之一在受试者中产生合成抗体。所述合成抗体可对于外来抗原是特异性的。所述外来抗原可来源于病毒。所述病毒可以是人免疫缺陷病毒 (HIV)、基孔肯雅热病毒 (CHIKV) 或登革热病毒。

[0025] 所述病毒可以是HIV。所述重组核酸序列可包含编码SEQ ID NO:1、2、5、46、47、48、49、51、53、55 和 57 的至少一个氨基酸序列的核酸序列。所述重组核酸序列可包含 SEQ ID NO:3、4、6、7、50、52、55、56、62 和 63 的至少一个核酸序列。

[0026] 所述病毒可为 CHIKV。所述重组核酸序列可包含编码 SEQ ID NO:59 和 61 的至少一个氨基酸序列的核酸序列。所述重组核酸序列可包含 SEQ ID NO:58 和 60 的至少一个核酸序列。

[0027] 所述病毒可为登革热病毒。所述重组核酸序列可包含编码 SEQ ID NO:45 的至少一个氨基酸序列的核酸序列。所述重组核酸序列包含 SEQ ID NO:44 的至少一个核酸序列。

[0028] 所述合成抗体可以对于自体抗原是特异性的。所述自体抗原可为 Her2。所述重组核酸序列可包含编码 SEQ ID NO:41 和 43 的至少一个氨基酸序列的核酸序列。所述重组核酸序列可包含 SEQ ID NO:40 和 42 的至少一个核酸序列。

[0029] 本文中描述的本发明的一方面包括本文中描述的核苷酸产物，其在一些情况下由一个核苷酸构建体组成，以及在一些情况下由两个不同的核苷酸构建体组成。

[0030] 本发明的一方面涉及治疗病原体感染的方法，包括施用编码对于病原体是特异性的合成抗体的核苷酸序列，并且在一些情况下还施用病原体的抗原以在受试者中产生免疫答应。

附图说明

[0031] 图 1 显示编码如实施例 1 中描述的 IgG 重链的核酸序列。

[0032] 图 2 显示编码如实施例 1 中描述的 IgG 轻链的核酸序列。

[0033] 图 3 显示标绘时间（小时）对 OD 450nm(1:100 的组织培养上清液的稀释度）的图。

[0034] 图 4 显示蛋白质印迹的图像。

[0035] 图 5 显示 pHIV-1Env-Fab 的产生和表达的确认。(A 和 B) 使用 VRC01 重 (H) 和轻 (L) 可变链 Ig 基因设计 pHIV-1 Env Fab 抗 -gp120 Fab 表达构建体的环状质粒图谱。当构建 Fab 质粒时包括几个修饰以提高表达的水平。将如显示的 Fab VL 和 VH 片段基因单独地克隆在 pVax1 载体的 BamH1 与 Xho1 限制位点之间。(C)pHIV-1 Env Fab 的体外表达。该图显示 293T 细胞转染后 pHIV-1 Env Fab 的表达的时间动力学。表示表达的指定的值是一式三份的孔的平均 OD450nm±SD。作为对照，也用 pVax1 主链转染 293T 细胞。

[0036] 图 6 显示由 pHIV-1 Env Fab 产生的抗 HIV Env 特异性 Fab 的时间产生的测量。(A) 抗 -HIV1 Fab 的产生的时程。在施用 pHIV-1 Env Fab 后，在 10 天的时期中通过 ELISA 测量终稀释度为 1:100 的血清中的特异性 Fab 的产量，并将其表示为 OD450nm。来自施以 pVax1 的小鼠的血清用作阴性对照。(B) 在用重组 gp120(rgp120) 免疫后抗 -gp120 抗体应答的比较测量。如实施例 2 中所述，通过 rgp120 的单次注射免疫小鼠，随后测量抗 -gp120 抗体的产量达 10 天，并将其表示为 OD450nm 值。将 PBS 用作本研究的阴性对照注射。(C) 通过免疫印迹分析确认 HIV1Env-Fab 结合。如实施例中所指示的，将 5 或 10μg 的 gp120 注射至 SDS-PAGE，并进行硝酸纤维素印迹，随后用来自施以 pHIV-1 Env Fab 的小鼠的血清孵育印迹。所述免疫印迹表明，实验性血清识别结合的 rgp120，从而确认产生的 Fab 的特异性。(D) 人 IgG1Fab 的时间定量，测量为 pHIV-1Env-Fab 施用后小鼠血清中的 IgG1。在指定的时间点上利用标准 ELISA 试剂盒测量 IgG1，并表示为 Fab(μg/mL)±SD。来自施以 pVax1 的小鼠的血清用作阴性对照。在 x- 轴上指定的时间点分析血清样品。在 (A)、(B) 和 (D) 中展示的图中显示的箭头指示 DNA 质粒施用的点。

[0037] 图 7 显示 HIV1 Env Fab 与进化枝 A HIV Env 糖蛋白的 FACS 结合分析。(A)FACS 扫描指示抗 -HIV1Env-Fab 与 HIV-1 进化枝 A Env 糖蛋白的结合。将表达共有 (pCon-Env-A) 或“优化的”(pOpt-Env-A)HIV-1 进化枝 A 包膜的 DNA 转染进 293T 细胞。转染后 2 天，用纯化的天然 VRC01 Ig，从 pHIV-1 Env Fab 产生的血清（在单次质粒施用后 48 小时收集的）或从 pIgG-E1M2 施用产生的对照 Ig 对细胞进行染色。将血清和 VRC01 抗体分别在 50μl PBS 中以 1:4 或 1:100 稀释，并在室温下孵育 30 分钟。随后用适当的缀合有第二藻红蛋白 (PE) 的 Ig 对细胞进行染色，随后针对单重态和活细胞对所述细胞进行门控以进行 FACS 分析。在每一个扫描中指示阳性细胞的百分比结合。(B)FACS 结合数据的图解表示法。将每一个 Ig/ 血清测试组中的染色细胞的数目（即指示表达水平）除以本底染色值，并表示为作为经测试的不同 HIV 进化枝 A Env 制剂的函数的 y- 轴上的特异性结合的百分比。

[0038] 图 8 显示来自施以 pHIV-1Env-Fab 的小鼠的血清对 HIV-1 的中和的时程。在图中指定的时间点上收集用于分析血清的中和活性的血清。使用一小组 HIV-1 假型病毒：Bal26（小组 A；进化枝 B，Tier 1）、Q23Env17（小组 B；进化枝 A，Tier 1）、SF162S（小组 C；进化枝 B，Tier 1）和 ZM53M（小组 D；进化枝 C，Tier 2）在 TZM-BL 细胞中进行中和分析。如实施例 2 中所描绘的，以 0.01 的 MOI 感染细胞，并在含有从 pHIV-1 Env Fab 施用产生的

Fab 的血清（1:50 的终稀释度）存在的情况下孵育所述细胞。显示了百分比中和值，其计算描述于实施例 2 中。同样地，在每一个图中提供了水平线，其指示实验性血清介导 50％病毒中和所处的大致时间点。

[0039] 图 9 显示编码实施例 2-7 中描述的 HIV-1 Env Fab 的重链 (VH-CH1) 的核酸序列。

[0040] 图 10 显示编码实施例 2-7 中描述的 HIV-1 Env Fab 的轻链 (VL-CL) 的核酸序列。

[0041] 图 11 显示用编码 HIV Env 的质粒转染的细胞的免疫荧光。用来自 pVAX1（左图框）或 pHIV-Env-Fab（右图框）的制剂对细胞进行染色。

[0042] 图 12 显示绘制抗原的类型对比血清浓度 (ng/mL) 的图。

[0043] 图 13 显示编码合成人 IgG1 抗体的构建体的示意图。

[0044] 图 14 显示由图 13 的构建体编码的装配的抗体（在表达后）的示意图。

[0045] 图 15 显示 VRC01 IgG 的氨基酸序列。

[0046] 图 16 显示 (A) 编码 HIV-1 Env-PG9 Ig 的构建体的示意图；(B) 含有 (A) 的构建体的载体的示意图；和 (C) 染色的凝胶的图像。

[0047] 图 17 显示 (A) 编码 HIV-1 Env-4E10 Ig 的构建体的示意图；(B) 含有 (A) 的构建体的载体的示意图；和 (C) 染色的凝胶的图像。

[0048] 图 18 显示在用费林蛋白酶切割之前的 HIV-1 Env-PG9 Ig 的氨基酸序列。

[0049] 图 19 显示利用费林蛋白酶切割之前的 HIV-1 Env-4E10 Ig 的氨基酸序列。

[0050] 图 20 显示 (A) 编码 CHIKV-Env-Fab 的重 (VH-CH1) 链的构建体的示意图；和 (B) 编码 CHIKV-Env-Fab 的重 (VL-CL) 链的构建体的示意图。

[0051] 图 21 显示含有编码 CHIKV-Env-Fab 的重 (VH-CH1) 或轻 (VL-CL) 链的构建体的表达载体的示意图。

[0052] 图 22 显示绘制以小时 (hr) 计的时间对比 OD450nm 的图。

[0053] 图 23 显示免疫印迹的图像。

[0054] 图 24 显示 DNA 施用的定时以及获得预出血和出血的示意图。

[0055] 图 25 显示绘制以天数计的时间对比 OD450nm 的图。

[0056] 图 26 显示绘制攻击后天数对比百分比存活率的图。

[0057] 图 27 显示绘制小鼠组对比 TNF-α 的 pg/mL 的图。

[0058] 图 28 显示绘制小鼠组对比 IL-6 的 pg/mL 的图。

[0059] 图 29 显示说明编码 VH-CH1 并且在启动子的控制下的构建体的示意图。

[0060] 图 30 显示说明编码 VL-CL 并且在启动子的控制下的构建体的示意图。

[0061] 图 31 显示说明被克隆进表达载体的编码抗 -Her-2 Fab 的 VH-CH1 或 VL-CL 的构建体的示意图。

[0062] 图 32 显示编码抗 -Her-2 Fab 的 VH-CH1 的核酸序列。

[0063] 图 33 显示由图 32 的核酸序列编码的氨基酸序列（即，抗 -Her-2 Fab 的 VH-CH1 的氨基酸序列）。

[0064] 图 34 显示编码抗 -Her-2 Fab 的 VL-CL 的核酸序列。

[0065] 图 35 显示由图 34 的核酸序列编码的氨基酸序列（即，抗 -Her-2 Fab 的 VL-CL 的氨基酸序列）。

[0066] 图 36 显示绘制转染的细胞的类型对比 IgG 浓度（μg/mL）的图。

[0067] 图 37 显示说明编码免疫球蛋白 G(IgG) 重链的可变重区 (VH)、可变重恒定区 1(CH1)、铰链区、可变重恒定区 2(CH2)、可变重恒定区 3(CH3) 以及编码 IgG 轻链的可变轻区 (VL) 和可变轻恒定区 (CL) 的构建体的示意图。IgG 的重链和轻链由蛋白酶切割位点分隔，并且各自位于信号肽（由前导序列编码）之后。

[0068] 图 38 显示编码抗 - 登革热病毒 (DENV) 人 IgG 的核酸序列。

[0069] 图 39 显示由图 39 的核酸序列编码的氨基酸序列（即，抗 -DENV 人 IgG 的氨基酸序列）。在该氨基酸序列中，蛋白酶切割还未发生来将重链和轻链分离成两个单独的多肽。

[0070] 图 40 显示绘制小鼠组对比 OD 450nm 的图。

[0071] 图 41 显示绘制注射后天数对比人 IgG 浓度 (ng/mL) 的图。

[0072] 图 42 显示由图 1 的核酸序列编码的氨基酸序列（即，SEQ ID NO:6）。该氨基酸序列是下文实施例 1 中描述的 IgG 重链的氨基酸序列。

[0073] 图 43 显示由图 2 的核酸序列编码的氨基酸序列（即，SEQ ID NO:7）。该氨基酸序列是下文实施例 1 中描述的 IgG 轻链的氨基酸序列。

[0074] 图 44 显示由图 9 的核酸序列编码的氨基酸序列（即，SEQ ID NO:3）。该氨基酸序列是实施例 2-7 中描述的 HIV-1 Env-Fab 的重链 (VH-CH1) 的氨基酸序列。

[0075] 图 45 显示由图 10 的核酸序列编码的氨基酸序列（即，SEQ ID NO:4）。该氨基酸序列是实施例 2-7 中描述的 HIV-1 Env-Fab 的轻链 (VL-CL) 的氨基酸序列。

[0076] 图 46 显示编码下文实施例 11 中描述的 HIV-1 PG9 单链 Fab(scFab) 的核酸序列。

[0077] 图 47 显示由图 46 的核酸序列编码的氨基酸序列（即，SEQ ID NO:50）。该氨基酸序列是下文实施例 11 中描述的 HIV-1 PG9 scFab 的氨基酸序列。

[0078] 图 48 显示编码下文实施例 13 中描述的 HIV-1 4E10 单链 Fab(scFab) 的核酸序列。

[0079] 图 49 显示由图 48 的核酸序列编码的氨基酸序列（即，SEQ ID NO: 52）。该氨基酸序列是下文实施例 13 中描述的 HIV-1 4E10 scFab 的氨基酸序列。

[0080] 图 50 显示说明编码免疫球蛋白 G(IgG) 重链的可变重区 (VH)、可变重恒定区 1(CH1)、铰链区、可变重恒定区 2(CH2)、可变重恒定区 3(CH3) 的构建体的示意图。编码 IgG 重链的核酸序列位于前导序列之后。

[0081] 图 51 显示说明编码 IgG 轻链的可变轻区 (VL) 和可变轻恒定区 (CL) 的构建体的示意图。编码 IgG 轻链的核酸序列位于前导序列之后。

[0082] 图 52 显示编码下文实施例 9 中描述的 HIV-1 VRC01 IgG1 重链的核酸序列。

[0083] 图 53 显示由图 52 的核酸序列编码的氨基酸序列（即，SEQ ID NO:54）。该氨基酸序列是下文实施例 9 中描述的 HIV-1 VRC01 IgG1 重链的氨基酸序列。

[0084] 图 54 显示编码下文实施例 9 中描述的 HIV-1 VRC01 IgG 轻链的核酸序列。

[0085] 图 55 显示由图 54 的核酸序列编码的氨基酸序列（即，SEQ ID NO:56）。该氨基酸序列是下文实施例 9 中描述的 HIV-1 VRC01 IgG 轻链的氨基酸序列。

[0086] 图 56 显示编码下文实施例 14 中描述的 CHIKV-Env-Fab 的重链 (VH-CH1) 的核酸序列。

[0087] 图 57 显示由图 56 的核酸序列编码的氨基酸序列（即，SEQ ID NO:58）。该氨基酸序列是下文实施例 14 中描述的 CHIKV-Env-Fab 的重链 (VH-CH1) 的氨基酸序列。

[0088] 图 58 显示编码下文实施例 14 中描述的 CHIKV-Env-Fab 的轻链 (VL-CL) 的核酸序

列。

[0089] 图 59 显示由图 58 的核酸序列编码的氨基酸序列（即，SEQ ID NO:60）。该氨基酸序列是下文实施例 14 中描述的 CHIKV-Env-Fab 的轻链 (VL-CL) 的氨基酸序列。

[0090] 图 60 显示编码下文实施例 12 中描述的 HIV-1 Env-4E10 Ig 的核酸序列。

[0091] 图 61 显示编码下文实施例 10 中描述的 HIV-1 Env-PG9 Ig 的核酸序列。

[0092] 图 62 显示编码 VRC01 IgG 的核酸序列 (SEQ ID NO:64)。

具体实施方式

[0093] 本发明涉及包含编码抗体、其片段、其变体或其组合的重组核酸序列的组合物。可向有此需要的受试者施用所述组合物以促进合成抗体的体内表达和形成。

[0094] 具体地，从所述重组核酸序列表达的重链和轻链多肽可装配成合成抗体。所述重链多肽和轻链多肽可彼此相互作用，以便装配导致合成抗体能够结合抗原，相较于未按本文中所描述装配的抗体更具免疫原性，并且能够引发或诱发抗所述抗原的免疫应答。

[0095] 此外，这些合成抗体比响应抗原诱发的免疫应答而产生的抗体更快地在受试者中产生。合成抗体能够有效地结合并中和一系列抗原。合成抗体也能够有效地保护免受疾病侵袭和 / 或促进疾病的存活。

[0096] 1. 定义

[0097] 除非另有定义，否则本文使用的所有技术和科学术语具有与本领域普通技术人员理解的含义相同的含义。在冲突的情况下，以本文件（包括定义）为准。下文中描述了优选的方法和材料，尽管可在本发明的实施或测试中使用与本文中描述的那些方法和材料类似或等同的方法和材料。本文中提及的所有出版物、专利申请、专利和其它参考文献通过引用整体并入。本文中公开的材料、方法以及实施例仅仅是说明性的并非意在限制。

[0098] 如本文中所用，术语“包含 / 包括”、“包括”、“具有”、“有”、“可以”、“含有”及其变型意欲为不排除另外的行为或结构的可能性的开放式过渡短语、术语或词语。除非上下文另有明确说明，否则单数形式“一个 (a)”、“和 (and)”以及“该 (the)”包括复数参考物。本公开还涵盖“包含 / 包括”本文中提出的实施方案或组件、“由所述实施方案或组件组成”和“基本上由所述实施方案或组件组成”的其它实施方案，无论是否明确地显示。

[0099] “抗体”可意指种类 IgG、IgM、IgA、IgD 或 IgE 或其片段、片段或其衍生物的抗体（包括 Fab、F(ab')2、Fd 和单链抗体）及其衍生物。抗体可以是从哺乳动物的血清样品分离的抗体、多克隆抗体、亲和纯化的抗体或其混合物，所述抗体展现足够的对期望的表位或来源于其的序列的结合特异性。

[0100] 如在本文中可互换使用的“抗体片段”或“抗体的片段”是指包含抗原结合位点或可变区的完整抗体的部分。所述部分不包括完整抗体的 Fc 区域的恒定重链结构域（即取决于抗体同种型，CH2、CH3 或 CH4）。抗体片段的实例包括但不限于 Fab 片段、Fab' 片段、Fab'-SH 片段、F(ab')2 片段、Fd 片段、Fv 片段、双链抗体、单链 Fv(scFv) 分子、仅含 1 个轻链可变结构域的单链多肽、含有轻链可变结构域的 3 个 CDR 的单链多肽、仅含 1 个重链可变区的单链多肽和含有重链可变区的 3 个 CDR 的单链多肽。

[0101] “抗原”是指具有在宿主中产生免疫应答的能力的蛋白质。抗原可被抗体识别并结合。抗原可源于身体内部或源自外部环境。

[0102] 如本文中所用，“编码序列”或“编码核酸”可意指包含编码本文中所示的抗体的核苷酸序列的核酸（RNA 或 DNA 分子）。编码序列还可包括有效地连接于能够在向其施用了核酸的个体或哺乳动物的细胞中指导表达的调控元件（包括启动子和多聚腺苷酸化信号）的起始和终止信号。编码序列还可包括编码信号肽的序列。

[0103] 如本文中所用，“互补”或“互补的”可意指可核酸，可意指核酸分子的核苷酸或核苷酸类似物之间的沃森 - 克里克 (Watson-Crick)（例如，A-T/U 和 C-G）或 Hoogsteen 碱基配对。

[0104] 如本文中所用，“恒定电流”定义由组织或限定所述组织的细胞在整个递送至相同组织的电脉冲的持续过程中接收或经历的电流。从本文中描述的电穿孔装置递送电脉冲。该电流在电脉冲的整个过程中在所述组织中维持恒定的安培度，因为本文中提供的电穿孔装置具有反馈元件，优选地具有瞬时反馈。反馈元件可测量整个脉冲持续过程中组织（或细胞）的阻抗，并使电穿孔装置改变其电能输出（例如，提高电压），这样相同组织中的电流在整个电脉冲（大约数微秒）中以及在脉冲间维持恒定。在一些实施方案中，反馈元件包括控制器。

[0105] 如本文中所用，“电流反馈”或“反馈”可互换使用，并且可意指提供的电穿孔装置的主动响应，其包括测量组织中电极之间的电流和相应地改变由 EP 装置递送的能量输出，以将电流维持在恒定水平。该恒定水平由用户在开始脉冲序列或电处理之前预先设定。反馈可由电穿孔组件例如电穿孔装置的控制器来实现，因为其中的电路能够连续监测组织中电极之间的电流并将该监测的电流（或组织内的电流）与预设电流相比较，以及连续进行能量输出调整以将监测的电流维持在预设水平。反馈回路可以是瞬时的，因为其为模拟闭环反馈。

[0106] 如本文中所用的“分散电流”可意指从本文中描述的电穿孔装置的各种针电极阵列递送的电流的模式，其中所述模式使待电穿孔的组织的任何区域上的电穿孔相关热应激的发生降至最低或优选消除。

[0107] 如本文中可互换使用的，“电穿孔”、“电 - 透化”或“电动增强”（“EP”）可指使用跨膜电场脉冲来诱发生物膜中的微观通路（孔）；它们的存在允许生物分子诸如质粒、寡核苷酸、siRNA、药物、离子和水从细胞膜的一侧通过进入另一侧。

[0108] 如本文中所用，“内源性抗体”可指在被施以有效剂量的抗原以诱发体液免疫应答的受试者中产生的抗体。

[0109] 如本文中所用，“反馈机制”可指通过软件或硬件（或固件）进行的过程，该过程接收期望的组织的阻抗（在递送能量脉冲之前，期间和 / 或之后）并将其与现有值（优选地当前）相比较，随后调整被递送的能量的脉冲以达到预设值。可通过模拟闭环电路进行反馈机制。

[0110] “片段”可意指具有功能的，即可结合至期望的靶并具有与全长抗体相同的期望的作用的抗体的多肽片段。除从 N 和 / 或 C 末端失去至少一个氨基酸外，抗体的片段可与全长抗体 100％相同，在每一种情况下具有或不具有信号肽和 / 或位置 1 上的甲硫氨酸。片段可包含 20％或更多、25％或更多、30％或更多、35％或更多、40％或更多、45％或更多、50％或更多、55％或更多、60％或更多、65％或更多、70％或更多、75％或更多、80％或更多、85％或更多、90％或更多、91％或更多、92％或更多、93％或更多、94％或更多、95％或更多、96％或

更多、97％或更多、98％或更多、99％或更多百分比的特定全长抗体的长度，不包括任何添加的异源信号肽。片段可包括多肽的片段，所述多肽的片段与抗体具有95％或更高、96％或更高、97％或更高、98％或更高或99％或更高同一性，并且额外地包含当计算百分比同一性时被包括的N末端甲硫氨酸或异源信号肽。片段还可包含N末端甲硫氨酸和/或信号肽，诸如免疫球蛋白信号肽，例如IgE或IgG信号肽。可将N末端甲硫氨酸和/或信号肽连接于抗体的片段。

[0111] 在具有或不具有编码信号肽和/或位置1上的甲硫氨酸的序列的每一种情况下，编码抗体的核酸序列的片段可与全长100％相同，除从5’和/或3’末端失去至少一个核苷酸外。片段可包含20％或更多、25％或更多、30％或更多、35％或更多、40％或更多、45％或更多、50％或更多、55％或更多、60％或更多、65％或更多、70％或更多、75％或更多、80％或更多、85％或更多、90％或更多、91％或更多、92％或更多、93％或更多、94％或更多、95％或更多、96％或更多、97％或更多、98％或更多、99％或更多百分比的特定全长编码序列的长度，不包括任何添加的异源信号肽。片段可包含编码多肽的片段，所述多肽与抗体具有95％或更高、96％或更高、97％或更高、98％或更高或99％或更高的同一性，并且额外地任选地包含当计算百分比同一性时未包括的编码N末端甲硫氨酸或异源信号肽的序列。片段还可包含N末端甲硫氨酸和/或信号肽，诸如免疫球蛋白信号肽例如IgE或IgG信号肽的编码序列。可将编码N末端甲硫氨酸和/或信号肽的编码序列连接于编码序列的片段。

[0112] 如本文中所用的“遗传构建体”是指包含编码蛋白质诸如抗体的核苷酸序列的DNA或RNA分子。所述编码序列包括有效地连接于能够在向其施用了核酸分子的个体的细胞中指导表达的调控元件（包括启动子和多聚腺苷酸化信号）的起始和终止信号。如本文中所用，术语“可表达的形式”是指基因构建体，所述基因构建体含有有效地连接于编码蛋白质的编码序列的必需调控元件，以便当存在于个体的细胞中时，所述编码序列可被表达。

[0113] 如在本文中在两个或更多个核酸或多肽序列的上下文中所用，“相同的”或“同一性”可意指序列具有指定百分比的在指定区域上相同的残基。可通过如下步骤计算百分比：将两个序列最佳地比对，在指定的区域上比较两条序列，测定在两个序列中在其上存在相同残基的位置的数目以产生匹配位置的数目，将匹配位置的数目除以指定区域中的位置总数，及将结果乘以100来产生序列同一性的百分比。在其中两条序列具有不同长度或比对产生一个或多个交错末端以及指定的比较区域仅包括单个序列的情况下，将单个序列的残基包括在计算的分母而非分子中。当比较DNA和RNA时，可胸腺嘧啶(T)和尿嘧啶(U)可被认为是等同的。人工或通过使用计算机序列算法诸如BLAST或BLAST 2.0来获得同一性。

[0114] 当讨论反馈机制时可使用如本文中所用的“阻抗”，并且可按照欧姆定律将其转换成电流值，从而使得能够与预设电流比较。

[0115] 如本文中所用的“免疫应答”可意指宿主的免疫系统，例如哺乳动物的免疫系统，响应一种或多种核酸和/或肽的引入的激活。免疫应答可以以细胞或体液应答的形式或这两种形式存在。

[0116] 如本文中所用的“核酸”或“寡核苷酸”或“多核苷酸”可意指共价连接在一起的至少两个核苷酸。单链的描述也限定了互补链的序列。因此，核酸还包括描述的单链的互补链。核酸的许多变体可出于相同的目的作为给定的核酸使用。因此，核酸还包括基本上相同的核酸和其补体。单链提供可在严格杂交条件下与靶序列杂交的探针。因此，核酸还包

括在严格杂交条件下杂交的探针。

[0117] 核酸可以是单链或双链的，或可含有双链和单链序列的部分。核酸可以是DNA（基因组DNA和cDNA）、RNA或杂交体，其中核酸可含有脱氧核糖核苷酸和核糖核苷酸的组合，碱基（包括尿嘧啶、腺嘌呤、胸腺嘧啶、胞嘧啶、鸟嘌呤、肌苷、黄嘌呤次黄嘌呤、异胞嘧啶和异鸟嘌呤）的组合。核酸可以通过化学合成法或通过重组法获得。

[0118] 如本文中所用的“有效连接的”可意指基因的表达在与其空间上连接的启动子的控制下。启动子可位于在其控制下的基因的5’（上游）或3’（下游）。启动子与基因之间的距离可与在所述启动子所源自的基因中该启动子与其控制的基因之间的距离大致相同。如在本领域中是已知的，可接受该距离的变化而不丧失启动子功能。

[0119] 如本文中所用的“肽”、“蛋白质”或“多肽”可意指氨基酸的连接的序列，并且可以是天然的、合成的或者经修饰的或天然和合成的组合。

[0120] 如本文中所用的“启动子”可意指能够赋予、激活或增强核酸在细胞中的表达的合成或天然来源的分子。启动子可包含一个或多个特定的转录调控序列以进一步增强所述序列的表达和/或改变其的空间表达和/或时间表达。启动子还可包含与转录起始位点相距多达数千碱基对的远端增强子或阻遏子元件。启动子可源自包括病毒、细菌、真菌、植物、昆虫和动物的来源。启动子可针对其中发生表达的细胞、组织或器官或针对表达发生所处的发育阶段，或响应外部刺激（诸如生理应激、病原体、金属离子或诱导剂）而组成型地，或差异地调控基因组分的表达。启动子的代表性实例包括噬菌体T7启动子、噬菌体T3启动子、SP6启动子、lac操纵子-启动子、tac启动子、SV40晚期启动子、SV40早期启动子、RSV-LTR启动子、CMV IE启动子、SV40早期启动子或SV 40晚期启动子和CMV IE启动子。

[0121] “信号肽”和“前导序列”在本文中可互换使用，并且是指可被连接在本文中所示的蛋白质的氨基末端上的氨基酸序列。信号肽/前导序列通常指导蛋白质的定位。本文中使用的信号肽/前导序列优选促进蛋白质从产生其的细胞分泌。在从细胞分泌后，通常从蛋白质的其余部分（通常称为成熟蛋白）切割信号肽/前导序列。信号肽/前导序列连接在蛋白质的N末端上。

[0122] 如本文中所用的“严格杂交条件”可意指第一核酸序列（例如，探针）将与第二核酸序列（例如，靶）（诸如在核酸的复杂混合物中）杂交的条件。严格条件是序列依赖性的，并且在不同的情况下是不同的。可选择比特定序列在限定的离子强度、pH下的热熔点（T_m）低约5-10℃作为严格条件。T_m可以是50％的与靶互补的探针在平衡（由于靶序列过量存在，因此在T_m下，50％的探针在平衡时被占据）时与靶序列杂交时的温度（在限定的离子强度、pH和核酸浓度下）。严格条件可以是在pH 7.0至8.3下盐浓度低于约1.0 M钠离子，诸如约0.01-1.0 M的钠离子浓度（或其它盐）以及温度为至少约30℃（对于短探针（例如，约10-50个核苷酸））和至少约60℃（对于长探针（例如，大于约50个核苷酸）的那些条件。严格条件还可通过添加去稳定剂诸如甲酰胺来实现。对于选择性或特异性杂交，阳性信号可以为至少2至10倍本底杂交。示例性严格杂交条件包括下列条件：50％甲酰胺、5x SSC和1％ SDS在42℃下孵育，或者5x SSC、1％ SDS在65℃下孵育，在65℃于0.2x SSC和0.1％ SDS中洗涤。

[0123] 如本文中可互换所用的“受试者”和“患者”是指任何脊椎动物，包括但不限于哺乳动物（例如，牛、猪、骆驼、美洲驼、马、山羊、兔、绵羊、仓鼠、豚鼠、猫、狗、大鼠和小鼠、非

人灵长类动物（例如，猴，诸如食蟹猴或恒河猴、黑猩猩等）和人）。在一些实施方案中，受试者可以是人或非人。受试者或患者可以正接受其他形式的治疗。

[0124] 如本文中所用的“基本上互补的”可意指第一序列与第二序列的互补序列在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或更多个核苷酸或氨基酸的区域上具有至少60％、65％、70％、75％、80％、81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％同一性，或两条序列在严格杂交条件下杂交。

[0125] 如本文中所用的“基本上相同的”可意指第一与第二序列在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或更多个核苷酸或氨酸的区域上至少60％、65％、70％、75％、80％、81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％相同，或对于核酸，如果第一序列与第二序列的补体基本上互补的话，则它们是基本上相同的。

[0126] 如本文中所用的“合成抗体”是指由本文中描述的重组核酸序列编码并且在受试者中产生的抗体。

[0127] 如本文中所用的“治疗”或“处理”可意指通过预防、抑制、压制或完全消除疾病来保护受试者免受疾病侵害。预防疾病包括在疾病发作之前向受试者施用本发明的疫苗。抑制疾病包括在疾病引发后但在其临床表现之前向受试者施用本发明的疫苗。压制疾病包括在疾病临床表现后向受试者施用本发明的疫苗。

[0128] 如本文中针对核酸所用的“变体”可意指(i)参照的核苷酸序列的部分或片段；(ii)参照的核苷酸序列或其部分的补体；(iii)与参照核酸或其互补序列基本上同一的核酸；或(iv)在严格条件下与参照核酸、其补体或与其基本上相同的序列杂交的核酸。

[0129] 对于肽或多肽，“变体”是指在氨基酸序列上相异于氨基酸的插入、缺失或保守取代，但保留至少一种生物活性的肽或多肽。变体还可意指具有与参照蛋白质的氨基酸序列基本上相同的氨基酸序列的，保留至少一种生物活性的蛋白质。氨基酸的保守取代，即利用具有相似性质（例如，疏水性、带电区域的程度和分布）的不同氨基酸替代氨基酸，在本领域中被公认为通常牵涉较小变化。如本领域中所理解的，这些较小变化可部分通过考虑氨基酸的亲疏水性指数(hydropathic index)来鉴定。Kyte等人，J. Mol.Biol. 157:105-132(1982)。氨基酸的亲疏水性指数基于对其疏水性和电荷的考虑。在本领域中已知具有相似亲疏水性指数的氨基酸可被取代并且仍然保留蛋白质功能。在一个方面，具有±2的亲疏水性指数的氨基酸被取代。氨基酸的亲水性还可用于显示可导致保留生物功能的蛋白质的取代。在肽的上下文中考虑氨基酸的亲水性允许计算该肽的最大局部平均亲水性，这是一种已被报导与抗原性和免疫原性密切相关的有用的度量。美国专利No. 4,554,101(通过引用完全并入本文)。如本领域中所理解的，具有相似亲水性值的氨基酸的取代可导致保留生物活性例如免疫原性的肽。可利用彼此亲水性值在±2以内的氨基酸进行取代。氨基酸的疏水性指数和亲水性值受该氨基酸的特定侧链影响。与该观察一致，与生物功能相容的氨基酸取代经了解取决于氨基酸，并且具体地那些氨基酸的侧链的相对相似性，如通过疏水性、亲水性、电荷、大小和其它性质显示的。

[0130] 变体可以是在全长的全基因序列或其片段上基本上相同的核酸序列。核酸序列

可在全长的基因序列或其片段上具有 80％、81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或 100％的同一性。变体可以是在全长的氨基酸序列或其片段上基本上相同的氨基酸序列。氨基酸序列可在全长的所述氨基酸序列或其片段上具有 80％、81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或 100％的同一性。

[0131] 如本文中所用的“载体”可意指含有复制起始点的核酸序列。载体可以是质粒、噬菌体、细菌人工染色体或酵母人工染色体。载体可以是 DNA 或 RNA 载体。载体可以是自主复制的染色体外载体或整合进宿主基因组的载体。

[0132] 对于本文中数值范围的引述，明确地以相同的精度包括其间的每一个中介数。例如，对于 6-9 的范围，除了 6 和 9 外还包括数值 7 和 8，而对于范围 6.0-7.0，明确地包括数值 6.0、6.1、6.2、6.3、6.4、6.5、6.6、6.7、6.8、6.9 和 7.0。

[0133] 2. 组合物

[0134] 本发明涉及包含编码抗体、其片段、其变体或其组合的重组核酸序列的组合物。当向有此需要的受试者施用时，所述组合物可导致在受试者中产生合成抗体。所述合成抗体可结合存在于受试者中的靶分子（即，抗原）。这样的结合可中和抗原，阻断另一种分子例如蛋白质或核酸对抗原的识别，以及引发或诱发对抗原的免疫应答。

[0135] 所述合成抗体可治疗、预防被施以所述组合物的受试者的疾病和 / 或保护其免受所述疾病侵害。所述合成抗体通过结合抗原可治疗、预防被施以所述组合物的受试者的疾病和 / 或保护其免受所述疾病侵害。所述合成抗体可提高被施以所述组合物的受试者的疾病存活率。所述合成抗体可提供至少约 50％、55％、60％、65％、70％、75％、80％、85％、90％、95％或 100％的被施以所述组合物的受试者的疾病存活率。在其它实施方案中，所述合成抗体可提供至少约 65％、66％、67％、68％、69％、70％、71％、72％、73％、74％、75％、76％、77％、78％、79％或 80％的被施以所述组合物的受试者的疾病存活率。

[0136] 所述组合物可导致在向受试者施用所述组合物至少约 1 小时、2 小时、3 小时、4 小时、5 小时、6 小时、7 小时、8 小时、9 小时、10 小时、11 小时、12 小时、13 小时、14 小时、15 小时、20 小时、25 小时、30 小时、35 小时、40 小时、45 小时、50 小时或 60 小时内在所述受试者中产生所述合成抗体。所述组合物可导致在向受试者施用所述组合物至少约 1 天、2 天、3 天、4 天、5 天、6 天、7 天、8 天、9 天或 10 天内在所述受试者中产生所述合成抗体。所述组合物可导致在向受试者施用所述组合物约 1 小时至约 6 天、约 1 小时至约 5 天、约 1 小时至约 4 天、约 1 小时至约 3 天、约 1 小时至约 2 天、约 1 小时至约 1 天、约 1 小时至约 72 小时、约 1 小时至约 60 小时、约 1 小时至约 48 小时、约 1 小时至约 36 小时、约 1 小时至约 24 小时、约 1 小时至约 12 小时或约 1 小时至约 6 小时内在所述受试者中产生所述合成抗体。

[0137] 当向有此需要的受试者施用时，所述组合物可在受试者中导致比被施以抗原以诱发体液免疫应答的受试者的内源抗体的产生更快的合成抗体的产生。所述组合物可在被施以抗原以诱发体液免疫应答的的受试者的内源抗体产生之前至少约 1 天、2 天、3 天、4 天、5 天、6 天、7 天、8 天、9 天或 10 天导致所述合成抗体的产生。

[0138] 本发明的组合物可具有有效组合物的期望特性，诸如是安全的，以便所述组合物不引起疾病或死亡；具有抵抗疾病的保护作用；以及提供施用的容易性、极少的副作用、生物稳定性和每剂量的低成本。

[0139] 3. 重组核酸序列

[0140] 如上所述，所述组合物可包含重组核酸序列。所述重组核酸序列可编码抗体、其片段、其变体或其组合。在下文中更详细地描述了抗体。

[0141] 所述重组核酸序列可以是异源核酸序列。所述重组核酸序列可包括至少一个异源核酸序列或一个或多个异源核酸序列。

[0142] 所述重组核酸序列可以是优化的核酸序列。这样的优化可增强或改变抗体的免疫原性。优化还可改进转录和 / 或翻译。优化可包括以下的一个或多个方面 ：低 GC 含量的前导序列，以增加转录 ；mRNA 稳定性和密码子优化 ；添加 kozak 序列 (例如，GCC ACC) 以增加翻译 ；添加编码信号肽的免疫球蛋白 (Ig) 前导序列 ；和尽可能消除顺式作用序列基序 (即，内部 TATA 盒)。

[0143] a. 重组核酸序列构建体

[0144] 所述重组核酸序列可包括一个或多个重组核酸序列构建体。所述重组核酸序列构建体可包括在下文中更详细地描述的一个或多个组件。

[0145] 所述重组核酸序列构建体可包括编码重链多肽、其片段、其变体或其组合的异源核酸序列。所述重组核酸序列构建体可包括编码轻链多肽、其片段、其变体或其组合的异源核酸序列。所述重组核酸序列构建体还可包括编码蛋白酶或蛋白酶切割位点的异源核酸序列。所述重组核酸序列构建体可包括一个或多个前导序列，其中每个前导序列编码信号肽。所述重组核酸序列构建体可包括一个或多个启动子、一个或多个内含子、一个或多个转录终止区、一个或多个起始密码子、一个或多个终止密码子 (termination or stop codon) 和 / 或一个或多个多聚腺苷酸化信号。所述重组核酸序列构建体还可包括一个或多个接头或标签序列。所述标签序列可编码血凝素 (HA) 标签。

[0146] (1) 重链多肽

[0147] 所述重组核酸序列构建体可包括编码重链多肽、其片段、其变体或其组合的异源核酸。所述重链多肽可包括可变重链 (VH) 区和 / 或至少一个恒定重链 (CH) 区。所述至少一个恒定重链区可包括恒定重链区 1(CH1)、恒定重链区 2(CH2) 和恒定重链区 3(CH3) 和 / 或铰链区。

[0148] 在一些实施方案中，所述重链多肽可包括 VH 区和 CH1 区。在其它实施方案中，所述重链多肽可包括 VH 区、CH1 区、铰链区、CH2 区和 CH3 区。

[0149] 所述重链多肽可包括互补决定区 (“CDR”) 组。所述 CDR 组可含有 VH 区的 3 个高变区。从重链多肽的 N 末端开始，这此 CDR 分别被命名为“CDR1”、“CDR2”和“CDR3”。重链多肽的 CDR1、CDR2 和 CDR3 可促成抗原的结合或识别。

[0150] (2) 轻链多肽

[0151] 所述重组核酸序列构建体可包括编码轻链多肽、其片段、其变体或其组合的异源核酸序列。所述轻链多肽可包括可变轻链 (VL) 区和 / 或恒定轻链 (CL) 区。

[0152] 所述轻链多肽可包括互补决定区 (“CDR”) 组。所述 CDR 组可含有 VL 区的 3 个高变区。从轻链多肽的 N 末端开始，这些 CDR 分别被命名为“CDR1”、“CDR2”和“CDR3”。轻链多肽的 CDR1、CDR2 和 CDR3 可促成抗原的结合或识别。

[0153] (3) 蛋白酶切割位点

[0154] 所述重组核酸序列构建体可包括编码蛋白酶切割位点的异源核酸序列。所述蛋白

酶切割位点可被蛋白酶或肽酶识别。所述蛋白酶可以是肽链内切酶或内切蛋白酶，例如但不限于费林蛋白酶、弹性蛋白酶、HtrA、钙蛋白酶、胰蛋白酶、胰凝乳蛋白酶、胰蛋白酶和胃蛋白酶。所述蛋白酶可以是费林蛋白酶。在其它实施方案中，所述蛋白酶可以是丝氨酸蛋白酶、苏氨酸蛋白酶、半胱氨酸蛋白酶、天冬氨酸蛋白酶、金属蛋白酶、谷氨酸蛋白酶或切割内部肽键（即，不切割 N 末端或 C 末端肽键）的任何蛋白酶。

[0155] 所述蛋白酶切割位点可包括一个或多个促进或增强切割效率的氨基酸序列。所述一个或多个氨基酸序列可促进或增强形成或产生分离的多肽的效率。所述一个或多个氨基酸序列可包括 2A 肽序列。

[0156] (4) 接头序列

[0157] 所述重组核酸序列构建体可包括一个或多个接头序列。所述接头序列可在空间上分离或连接一个或多个本文中描述的组件。在其它实施方案中，所述接头序列可编码在空间上分离或连接两个或更多个多肽的氨基酸序列。

[0158] (5) 启动子

[0159] 所述重组核酸序列构建体可包括一个或多个启动子。所述一个或多个启动子可以是能够驱动基因表达和调控基因表达的任何启动子。这样的启动子是经由 DNA 依赖性 RNA 聚合酶的转录所需的顺式作用序列元件。用于指导基因表达的启动子的选择取决于特定应用。可将启动子在重组核酸序列构建体中置于离转录起始位点与其在天然背景中离转录起始位点的距离相同的距离。然而，可接受该距离的变化而不丧失启动子功能。

[0160] 可将所述启动子有效地连接于编码重链多肽和 / 或轻链多肽的异源核酸序列。所述启动子可以是经显示对于在真核细胞中的表达是有效的启动子。有效地连接于编码序列的启动子可以是 CMV 启动子，来自猿猴病毒 40(SV40) 的启动子，诸如 SV40 早期启动子和 SV40 晚期启动子、小鼠乳房肿瘤病毒 (MMTV) 启动子、人免疫缺陷病毒 (HIV) 启动子诸如牛免疫缺陷病毒 (BIV) 长末端重复 (LTR) 启动子、莫洛尼病毒启动子 (Moloney virus)、禽类白血病病毒 (ALV) 启动子、巨细胞病毒 (CMV) 启动子诸如 CMV 立即早期启动子、爱泼斯坦 - 巴尔病毒 (Epstein Barr virus, EBV) 启动子或劳斯肉瘤病毒 (Rous sarcoma virus, RSV) 启动子。所述启动子还可以是来自人基因诸如人肌动蛋白、人肌球蛋白、人血红蛋白、人肌肉肌酸、人多角体蛋白或人金属硫蛋白的启动子。

[0161] 所述启动子可以是组成型启动子或诱导型启动子，所述诱导型启动子仅当宿主细胞被暴露于某些特定外部刺激时起始转录。在多细胞生物体的情况下，所述启动子还可于对特定的组织或器官或发育阶段是特异性的。所述启动子还可以是组织特异性启动子，诸如肌肉或皮肤特异性启动子（天然或合成的）。此类启动子的实例描述于美国专利申请公开 no.US20040175727（其内容以其整体并入本文）中。

[0162] 所述启动子可与增强子相联。增强子可位于编码序列的上游。所述增强子可以是人肌动蛋白、人肌球蛋白、人血红蛋白、人肌肉肌酸或病毒增强子，诸如来自 CMV、FMDV、RSV 或 EBV 的增强子。多核苷酸功能增强子描述于美国专利 No.5,593,972、5,962,428 和 W094/016737（所述每一个专利或专利申请公开的内容通过引用完全并入）中。

[0163] (6) 内含子

[0164] 所述重组核酸序列构建体可包括一个或多个内含子。每一个内含子可包括功能剪接供体和受体位点。所述内含子可包括剪接的增强子。所述内含子可包括一个或多个有效

剪接所需的信号。

[0165] (7) 转录终止区

[0166] 所述重组核酸序列构建体可包括一个或多个转录终止区。所述转录终止区可位于编码序列的下游以提供高效终止。所述转录终止区可获自与上述启动子相同的基因，或可获自一个或多个不同的基因。

[0167] (8) 起始密码子

[0168] 所述重组核酸序列构建体可包括一个或多个起始密码子。所述起始密码子可位于编码序列的上游。所述起始密码子可在具有编码序列的框内。所述起始密码子可与一个或多个有效翻译起始所需的信号，例如但不限于核糖体结合位点相关。

[0169] (9) 终止密码子

[0170] 所述重组核酸序列构建体可包括一个或多个终止密码子 (termination or stop codon)。所述终止密码子可位于编码序列的下游。所述终止密码子可在具有编码序列的框内。所述终止密码子可与一个或多个有效翻译终止所需的信号相关。

[0171] (10) 多聚腺苷酸化信号

[0172] 所述重组核酸序列构建体可包括一个或多个多聚腺苷酸化信号。所述多聚腺苷酸化信号可包括转录物的有效多腺苷酸化所需的一个或多个信号。所述多聚腺苷酸化信号可位于编码序列的下游。所述多聚腺苷酸化信号可以是 SV40 多聚腺苷酸化信号、LTR 多聚腺苷酸化信号、牛生长激素 (bGH) 多聚腺苷酸化信号、人生长激素 (hGH) 多聚腺苷酸化信号或人 β-珠蛋白多聚腺苷酸化信号。所述 SV40 多聚腺苷酸化信号可以是来自 pCEP4 质粒 (Invitrogen, San Diego, CA) 的多聚腺苷酸化信号。

[0173] (11) 前导序列

[0174] 所述重组核酸序列构建体可包括一个或多个前导序列。所述前导序列可编码信号肽。所述信号肽可以是免疫球蛋白 (Ig) 信号肽，例如但不限于 IgG 信号肽和 IgE 信号肽。在一些实例中，所述前导序列是 IgE 前导 IgE 前导序列 SEQ ID NO:65 :atggactgga cttggattct gttcctggtc gccgccgcaa ctcgcgtgca tagc，其编码蛋白质 SEQ ID NO:66 :Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val His Ser。

[0175] b. 重组核酸序列构建体的排列

[0176] 如上所述，所述重组核酸序列可包括一个或多个重组核酸序列构建体，其中每一个重组核酸序列构建体可包括一个或多个组件。所述一个或多个组件在上文中进行了详细描述。所述一个或多个组件，当包括在重组核酸序列构建体中时，可相对彼此以任意顺序排列。在一些实施方案中，可如下所述，在重组核酸序列构建体中排列所述一个或多个组件。

[0177] (1) 排列 1

[0178] 在一个排列中，第一重组核酸序列构建体可包括编码重链多肽的异源核酸序列，并且第二重组核酸序列构建体可包括编码轻链多肽的异源核酸序列。

[0179] 所述第一重组核酸序列构建体可被置于载体中。所述第二重组核酸序列构建体可被置于第二或单独的载体中。在下文中更详细地描述了将重组核酸序列构建体置于载体中。

[0180] 所述第一重组核酸序列构建体还可包括启动子、内含子、转录终止区、起始密码子、终止密码子和 / 或多聚腺苷酸化信号。所述第一重组核酸序列构建体还可包括前导序

列，其中所述前导序列位于编码重链多肽的异源核酸序列的上游（或 5’）。因此，由前导序列编码的信号肽可通过肽键连接至重链多肽。

[0181] 所述第二重组核酸序列构建体还可包括启动子、起始密码子、终止密码子和多聚腺苷酸化信号。所述第二重组核酸序列构建体还可包括前导序列，其中所述前导序列位于编码轻链多肽的异源核酸序列的上游（或 5’）。因此，由前导序列编码的信号肽可通过肽键连接至轻链多肽。

[0182] 因此，排列 1 的一个实例包括编码包括 VH 和 CH1 的重链多肽的第一载体（和从而第一重组核酸序列构建体）和编码包括 VL 和 CL 的轻链多肽的第二载体（和从而第二重组核酸序列构建体）。排列 1 的第二实例可包括编码包括 VH、CH1、铰链区、CH2 和 CH3 的重链多肽的第一载体（和从而第一重组核酸序列构建体）和编码包括 VL 和 CL 的轻链多肽的第二载体（和从而第二重组核酸序列构建体）。

[0183] (2) 排列 2

[0184] 在第二排列中，所述重组核酸序列构建体可包括编码重链多肽的异源核酸序列和编码轻链多肽的异源核酸序列。所述编码重链多肽的异源核酸序列可被置于编码轻链多肽的异源核酸序列的上游（或 5’）。或者，所述编码轻链多肽的异源核酸序列可被置于编码重链多肽的异源核酸序列的上游（或 5’）。

[0185] 所述重组核酸序列构建体可被置于下文中更详细描述的载体中。

[0186] 所述重组核酸序列构建体可包括编码蛋白酶切割位点的异源核酸序列和 / 或接头序列。如果包括在所述重组核酸序列构建体中，则编码蛋白酶切割位点的异源核酸序列可位于编码重链多肽的异源核酸序列与编码轻链多肽的异源核酸序列之间。因此，所述蛋白酶切割位点允许重链多肽和轻链多肽在表达后分离为不同的多肽。在其它实施方案中，如果将接头序列包括在重组核酸序列构建体中，那么可将所述接头序列置于编码重链多肽的异源核酸序列与编码轻链多肽的异源核酸序列之间。

[0187] 所述重组核酸序列构建体还可包括启动子、内含子、转录终止区、起始密码子、终止密码子和 / 或多聚腺苷酸化信号。所述重组核酸序列构建体可包括一个或多个启动子。所述重组核酸序列构建体可包括 2 个启动子，以便一个启动子可与编码重链多肽的异源核酸序列相联，并且第二启动子可与编码轻链多肽的异源核酸序列相联。在其它实施方案中，所述重组核酸序列构建体可包括一个与编码重链多肽的异源核酸序列和编码轻链多肽的异源核酸序列相联的启动子。.

[0188] 所述重组核酸序列构建体还可包括 2 个前导序列，其中第一前导序列位于编码重链多肽的异源核酸序列的上游（或 5’），并且第二前导序列位于编码轻链多肽的异源核酸序列的上游（或 5’）。因此，由第一前导序列编码的第一信号肽可通过肽键连接于重链多肽并且由第二前导序列编码的第二信号肽可通过肽键连接于轻链多肽。

[0189] 因此，排列 2 的一个实例可包括编码包括 VH 和 CH1 的重链多肽和包括 VL 和 CL 的轻链多肽的载体（和从而重组核酸序列构建体），其中接头序列位于编码重链多肽的异源核酸序列与编码轻链多肽的异源核酸序列之间。

[0190] 排列 2 的第二实例可包括编码包括 VH 和 CH1 的重链多肽和包括 VL 和 CL 的轻链多肽的载体（和从而重组核酸序列构建体），其中编码蛋白酶切割位点的异源核酸序列位于编码重链多肽的异源核酸序列与编码轻链多肽的异源核酸序列之间。

[0191] 排列 2 的第三实例可包括编码包括 VH、CH1、铰链区、CH2 和 CH3 的重链多肽和包括 VL 和 CL 的轻链多肽的载体（和从而重组核酸序列构建体），其中接头序列位于编码重链多肽的异源核酸序列与编码轻链多肽的异源核酸序列之间。

[0192] 排列 2 的第四实例可包括编码包括 VH、CH1、铰链区、CH2 和 CH3 的重链多肽和包括 VL 和 CL 的轻链多肽的载体（和从而重组核酸序列构建体），其中编码蛋白酶切割位点的异源核酸序列位于编码重链多肽的异源核酸序列与编码轻链多肽的异源核酸序列之间。

[0193] c. 来自重组核酸序列构建体的表达

[0194] 如上所述，所述重组核酸序列构建体可，在一个或多个组分当中，包括编码重链多肽的异源核酸序列和 / 或编码轻链多肽的异源核酸序列。因此，所述重组核酸序列构建体可有利重链多肽和 / 或轻链多肽的表达。

[0195] 当使用如上所述的排列 1 时，所述第一重组核酸序列构建体可促进重链多肽的表达，并且所述第二重组核酸序列构建体可促进轻链多肽的表达。当使用如上所述的排列 2 时，所述重组核酸序列构建体可促进重链多肽和轻链多肽的表达。

[0196] 在例如但不限于细胞、生物体或哺乳动物中表达后，所述重链多肽和轻链多肽可装配成合成抗体。具体地，所述重链多肽和轻链多肽可彼此相互作用，以便装配产生能够结合抗原的合成抗体。在其它实施方案中，所述重链多肽和轻链多肽可彼此相互作用，以便装配产生相较于未如本文中所述装配的抗体更具免疫原性的合成抗体。在其它实施方案中，所述重链多肽和轻链多肽可彼此相互作用，以便装配导致能够消除或诱发针对抗原的免疫应答的合成抗体。

[0197] d. 载体

[0198] 可将上述重组核酸序列构建体置于一个或多个载体中。所述一个或多个载体可含有复制起始点。所述一个或多个载体可以是质粒、噬菌体、细菌人工染色体或酵母人工染色体。所述一个或多个载体可以是自主复制的染色体外载体，或整合进宿主基因组的载体。

[0199] 所述一个或多个载体可以是异源表达构建体，其通常是用于将特定基因引入靶细胞的质粒。一旦表达载体在细胞内，由所述重组核酸序列构建体编码的重链多肽和 / 或轻链多肽由细胞转录和翻译机器核糖体复合物产生。所述一个或多个载体可表达大量稳定的信使 RNA，从而表达蛋白质。

[0200] (1) 表达载体

[0201] 所述一个或多个载体可以是环状质粒或线性核酸。所述环状质粒和线性核酸能够在适当的主题细胞中指导特定核苷酸序列的表达。所述一个或多个包含重组核酸序列构建体的载体可以是嵌合的，这意味着其组件的至少一个对于其至少一个其它组件是异源的。

[0202] (2) 质粒

[0203] 所述一个或多个载体可以是质粒。所述质粒可用于用重组核酸序列构建体转染细胞。所述质粒可用于将所述重组核酸序列构建体引入受试者。所述质粒还可包含调控序列，其可非常适合用于在施用了质粒的细胞中进行基因表达。.

[0204] 所述质粒还可包含哺乳动物复制起始点以在染色体外维持质粒并在细胞中产生多个拷贝的质粒。所述质粒可以是来自 Invitrogen(San Diego,CA) 的 pVAXI、pCEP4 或 pREP4，所述质粒可包含爱泼斯坦 - 巴尔病毒复制起始点和细胞核抗原 EBNA-1 编码区，其可产生高拷贝附加型复制而无需整合。质粒的主链可以是 pAV0242。所述质粒可以是复制缺

陷型腺病毒 5 型 (Ad5) 质粒。

[0205] 所述质粒可以是 pSE420(Invitrogen,San Diego,Calif.),其可用于在大肠杆菌 (Escherichia coli,E.coli) 中产生蛋白质。所述质粒还可以是 p YES2(Invitrogen,San Diego,Calif.),其可用于在酵母的酿酒酵母 (Saccharomyces cerevisiae) 菌株中产生蛋白质。所述质粒还可以是 MAXBAC™完全杆状病毒表达系统 (Invitrogen,San Diego,Calif.),其可用于在昆虫细胞中产生蛋白质。所述质粒还可以是 pcDNAI 或 (pcDNA3Invitrogen,San Diego,Calif.),其可用于在哺乳动物细胞诸如中国仓鼠卵巢 (CHO) 细胞中产生蛋白质。

[0206] (3) 环状和线性载体

[0207] 所述一个或多个载体可以是环状质粒,其可通过整合进细胞基因组转化靶细胞或存在于染色体外 (例如,具有复制起始点的自主复制质粒)。所述载体可以是 pVAX、pcDNA3.0 或 provax,或能够表达由所述重组核酸序列构建体编码的重链多肽和 / 或轻链多肽的任何其它表达载体。

[0208] 本文中还提供了能够经由电穿孔被有效地递送至受试者,并且表达由所述重组核酸序列构建体编码的重链多肽和 / 或轻链多肽的线性核酸或线性表达盒 (“LEC”)。所述 LEC 可以是不存在任何磷酸主链的任何线性 DNA。所述 LEC 可以不含任何抗生素抗性基因和 / 或磷酸主链。所述 LEC 可以不含与期望的基因表达无关的其它核酸序列。

[0209] 所述 LEC 可来源于能够被线性化的任何质粒。所述质粒可以能够表达由所述重组核酸序列构建体编码的重链多肽和 / 或轻链多肽。所述质粒可以是 pNP(Puerto Rico/34) 或 pM2(New Caledonia/99)。所述质粒可以是 WLV009、pVAX、pcDNA3.0 或 provax,或能够表达由所述重组核酸序列构建体编码的重链多肽和 / 或轻链多肽的任何其它表达载体。

[0210] 所述 LEC 可以是 pcrM2。所述 LEC 可以是 pcrNP。pcrNP 和 pcrMR 可分别来源于 pNP(Puerto Rico/34) 和 pM2(New Caledonia/99)。

[0211] (4) 制备载体的方法

[0212] 本文中提供了用于制备一个或多个其中已放置了所述重组核酸序列构建体的载体的方法。在最终的亚克隆步骤后,可使用本领域已知的方法将所述载体用在大规模发酵罐中接种细胞培养物。

[0213] 在其它实施方案中,在最终的亚克隆步骤后,可将所述载体与一个或多个电穿孔 (EP) 装置一起使用。在下文中更详细地描述了 EP 装置。

[0214] 可使用已知的装置和技术的组合配制或制造所述一个或多个载体,但优选使用 2007 年 5 月 23 日提交的批准的、共同未决的美国临时申请美国序列 No.60/939,792 中描述的质粒制造技术来制造它们。在一些实例中,可以以大于或等于 10mg/mL 的浓度配制本文中描述的 DNA 质粒。所述制造技术除了美国序列 No.60/939792 中描述的那些装置和方案 (包括 2007 年 7 月 3 日提交的批准的专利、美国专利 No.7,238,522 中描述的那些装置和方案) 之外,还包括或包含对于本领域普通技术人员来说是公知的各种装置和方案。以上提及的申请和专利 (分别为美国序列 No.60/939,792 和美国专利 No.7,238,522) 在此整体并入。

[0215] 4. 抗体

[0216] 如上所述,所述重组核酸序列可编码抗体、其片段、其变体或其组合。所述抗体可

结合在下文中更详细地描述的抗原或与其反应。

[0217] 所述抗体可包含分别插入重链和轻链框架（“FR”）组之间的重链和轻链互补决定区（“CDR”）组，所述框架组为CDR提供支持并且限定CDR相对于彼此的空间关系。所述CDR组可含有重链或轻链V区的3个高变区。从重链或轻链的N末端开始，这些区域被分别命名为“CDR1”、“CDR2”和“CDR3”。抗原结合位点从而可包含6个CDR，包括来自每一个重链和轻链V区的CDR组。

[0218] 蛋白水解酶木瓜蛋白酶优先切割IgG分子以产生几个片段，所述片段中的两个(F(ab)片段）各自包含共价异二聚体，所述共价异二聚体包含完整抗原结合位点。所述酶胃蛋白酶能够切割IgG分子以提供几个片段，包括包含两个抗原结合位点的F(ab')2片段。因此，所述抗体可以是Fab或F(ab')2。所述Fab可包括重链多肽和轻链多肽。所述Fab的重链多肽可包括VH区和CH1区。所述Fab的轻链可包括VL区和CL区。

[0219] 所述抗体可以是免疫球蛋白(Ig)。所述Ig可以是例如IgA、IgM、IgD、IgE和IgG。所述免疫球蛋白可包括重链多肽和轻链多肽。所述免疫球蛋白的重链多肽可包括VH区、CH1区、铰链区、CH2区和CH3区。所述免疫球蛋白的轻链多肽可包括VL区和CL区。

[0220] 所述抗体可以是多克隆抗体或单克隆抗体。所述抗体可以是嵌合抗体、单链抗体、亲和力成熟抗体、人抗体、人源化抗体或完全人抗体。所述人源化抗体可以是来自非人物种的结合期望的抗原的抗体，所述抗体具有一个或多个来自非人物种的互补决定区(CDR)和来自人免疫球蛋白分子的框架区。

[0221] 5.抗原

[0222] 所述合成抗体针对抗原或其片段或变体。所述抗原可以是核酸序列、氨基酸序列或其组合。所述核酸序列可以是DNA、RNA、cDNA、其变体、其片段或其组合。所述氨基酸序列可以是蛋白质、肽、其变体、其片段或其组合。

[0223] 所述抗原可来自许多生物体，例如病毒、寄生虫、细菌、真菌或哺乳动物。所述抗原可与自身免疫性疾病、过敏症或哮喘相关。在其它实施方案中，所述抗原可与癌症、疱疹、流感、乙型肝炎、丙型肝炎、人乳头状瘤病毒(HPV)或人免疫缺陷病毒(HIV)相关。

[0224] 在一些实施方案中，所述抗原是外来的。在一些实施方案中，所述抗原是自体抗原。

[0225] a.外来抗原

[0226] 在一些实施方案中，所述抗原是外来的。外来抗原是当被引入体内时，能够刺激免疫应答的任何非自身物质（即，来源于受试者外部）。

[0227] (1)病毒抗原

[0228] 外来抗原可以是病毒抗原或其片段或其变体。所述病毒抗原可来自来自下列科之一的病毒：腺病毒科(Adenoviridae)、沙粒病毒科(Arenaviridae)、布尼亚病毒科(Bunyaviridae)、杯状病毒科(Caliciviridae)、冠状病毒科(Coronaviridae)、丝状病毒科(Filoviridae)、嗜肝DNA病毒科(Hepadnaviridae)、疱疹病毒科(Herpesviridae)、正粘病毒科(Orthomyxoviridae)、乳多空病毒科(Papovaviridae)、副粘病毒科(Paramyxoviridae)、细小病毒科(Parvoviridae)、微小核糖核酸病毒科(Picornaviridae)、痘病毒科(Poxviridae)、呼肠孤病毒科(Reoviridae)、逆转录病毒科(Retroviridae)、弹状病毒科(Rhabdoviridae)或披膜病毒科(Togaviridae)。所述病毒

抗原可来自人免疫缺陷病毒 (HIV)、基孔肯雅热病毒 (CHIKV)、登革热病毒、乳头状瘤病毒，例如人类乳头状瘤病毒 (HPV)、脊髓灰质炎病毒、肝炎病毒，例如甲型肝炎病毒 (HAV)、乙型肝炎病毒 (HBV)、丙型肝炎病毒 (HCV)、丁型肝炎病毒 (HDV) 和戊型肝炎病毒 (HEV)、天花病毒 (重型天花和类天花)、牛痘病毒、流感病毒、鼻病毒、马脑炎病毒、风疹病毒、黄热病毒、诺沃克病毒 (Norwalk virus)、甲型肝炎病毒、人类 T 细胞白血病病毒 (HTLV-I)、多毛细胞白血病病毒 (HTLV- Ⅱ)、加利福尼亚脑炎病毒、汉坦病毒 (Hanta virus，出血热)、狂犬病病毒、埃博拉出血热病毒 (Ebola fever virus)、马尔堡病毒 (Marburg virus)、麻疹病毒、腮腺炎病毒、呼吸道合胞病毒 (RSV)、单纯疱疹 1(口腔疱疹)、单纯疱疹 2(生殖器疱疹)、带状疱疹 (水痘 - 带状疱疹，又名，水痘)、巨细胞病毒 (CMV)，例如人 CMV、爱泼斯坦 - 巴尔病毒 (EBV)、黄病毒、口蹄疫病毒、拉沙病毒、沙粒病毒或致癌病毒。

[0229] (a) 人免疫缺陷病毒 (HIV) 抗原

[0230] 所述病毒抗原可来自人免疫缺陷病毒 (HIV) 病毒。在一些实施方案中，所述 HIV 抗原可以是亚型 A 包膜蛋白，亚型 B 包膜蛋白，亚型 C 包膜蛋白，亚型 D 包膜蛋白，亚型 B Nef-Rev 蛋白，Gag 亚型 A、B、C 或 D 蛋白，Mpol 蛋白，Env A、Env B、Env C、Env D、B Nef-Rev、Gag 的核酸或氨基酸序列或其任意组合。

[0231] 对于 HIV 是特异性的合成抗体可包括 Fab 片段，所述 Fab 片段包含由 SEQ ID NO:3 的核酸序列编码的 SEQ ID NO:48 的氨基酸序列，和由 SEQ ID NO:4 的核酸序列编码的 SEQ ID NO:49 的氨基酸序列。所述合成抗体可包含由 SEQ ID NO:6 的核酸序列编码的 SEQ ID NO:46 的氨基酸序列，和由 SEQ ID NO:7 的核酸序列编码的 SEQ ID NO:47 的氨基酸序列。所述 Fab 片段包含由 SEQ ID NO:50 的核酸序列编码的 SEQ ID NO:51 的氨基酸序列。所述 Fab 可包含由 SEQ ID NO:52 的核酸序列编码的 SEQ ID NO:53 的氨基酸序列。

[0232] 对于 HIV 是特异性的合成抗体可包括包含 SEQ ID NO:5 的氨基酸序列的 Ig。所述 Ig 可包含由 SEQ ID NO:62 的核酸序列编码的 SEQ ID NO:1 的氨基酸序列。所述 Ig 可包含由 SEQ ID NO:63 的核酸序列编码的 SEQ ID NO:2 的氨基酸序列。所述 Ig 可包含由 SEQ ID NO:54 的核酸序列编码的 SEQ ID NO:55 的氨基酸序列，和由 SEQ ID NO:56 的核酸序列编码的 SEQ ID NO:57 的氨基酸序列。

[0233] (b) 基孔肯雅热病毒

[0234] 所述病毒抗原可来自基孔肯雅热病毒。基孔肯雅热病毒属于披膜病毒科的 α 病毒属。基孔肯雅热病毒通过感染的蚊子 (例如伊蚊属 (Aedes)) 叮咬传播给人。

[0235] 对于 CHIKV 是特异性的合成抗体可包括 Fab 片段，所述 Fab 片段包含由 SEQ ID NO:58 的核酸序列编码的 SEQ ID NO:59 的氨基酸序列，和由 SEQ ID NO:60 的核酸序列编码的 SEQ ID NO:61 的氨基酸序列。

[0236] (c) 登革热病毒

[0237] 所述病毒抗原可来自登革热病毒。所述登革热病毒抗原可以是形成病毒颗粒的 3 种蛋白或多肽 (C、prM 和 E) 之一。所述登革热病毒抗原可以是参与病毒的复制的 7 种其它蛋白或多肽 (NS1、NS2a、NS2b、NS3、NS4a、NS4b、NS5) 之一。所述登革热病毒可以是所述病毒的 5 个菌株或血清型之一，包括 DENV-1、DENV-2、DENV-3 和 DENV-4。所述抗原可以是多种登革热病毒抗原的任意组合。

[0238] 对于登革热病毒是特异性的合成抗体可包括包含由 SEQ ID NO:44 的核酸序列编

码的 SEQ ID NO:45 的氨基酸序列的 Ig。

[0239] (d) 肝炎抗原

[0240] 所述病毒抗原可包括肝炎病毒抗原（即，肝炎抗原）或其片段或其变体。所述肝炎抗原可以是来自甲型肝炎病毒 (HAV)、乙型肝炎病毒 (HBV)、丙型肝炎病毒 (HCV)、丁型肝炎病毒 (HDV) 和 / 或戊型肝炎病毒 (HEV) 的一种或多种的抗原或免疫原。

[0241] 所述肝炎抗原可以是来自 HAV 的抗原。所述肝炎抗原可以是 HAV 衣壳蛋白、HAV 非结构蛋白、其片段、其变体或其组合。

[0242] 所述肝炎抗原可以是来自 HCV 的抗原。所述肝炎抗原可以是 HCV 核衣壳蛋白（即，核心蛋白）、HCV 包膜蛋白（例如，E1 和 E2）、HCV 非结构蛋白（例如，NS1、NS2、NS3、NS4a、NS4b、NS5a 和 NS5b）、其片段、其变体或其组合。

[0243] 所述肝炎抗原可以是来自 HDV 的抗原。所述肝炎抗原可以是 HDV δ 抗原、其片段或其变体。

[0244] 所述肝炎抗原可以是来自 HEV 的抗原。所述肝炎抗原可以是 HEV 衣壳蛋白、其片段或其变体。

[0245] 所述肝炎抗原可以是来自 HBV 的抗原。所述肝炎抗原可以是 HBV 核心蛋白、HBV 表面蛋白、HBV DNA 聚合酶、由基因 X 编码的 HBV 蛋白、其片段、其变体或其组合。所述肝炎抗原可以是 HBV 基因型 A 核心蛋白、HBV 基因型 B 核心蛋白、HBV 基因型 C 核心蛋白、HBV 基因型 D 核心蛋白、HBV 基因型 E 核心蛋白、HBV 基因型 F 核心蛋白、HBV 基因型 G 核心蛋白、HBV 基因型 H 核心蛋白、HBV 基因型 A 表面蛋白、HBV 基因型 B 表面蛋白、HBV 基因型 C 表面蛋白、HBV 基因型 D 表面蛋白、HBV 基因型 E 表面蛋白、HBV 基因型 F 表面蛋白、HBV 基因型 G 表面蛋白、HBV 基因型 H 表面蛋白、其片段、其变体或其组合。

[0246] 在一些实施方案中，所述肝炎抗原可以是来自 HBV 基因型 A、HBV 基因型 B、HBV 基因型 C、HBV 基因型 D、HBV 基因型 E、HBV 基因型 F、HBV 基因型 G 或 HBV 基因型 H 的抗原。

[0247] (e) 人乳头状瘤病毒 (HPV) 抗原

[0248] 所述病毒抗原可包括来自 HPV 的抗原。所述 HPV 抗原可来自 HPV 型 16、18、31、33、35、45、52 和 58，其引起宫颈癌、直肠癌和 / 或其它癌症。所述 HPV 抗原可来自 HPV 6 型和 11 型，其可引起生殖器疣，并且已知是头颈癌的病因。

[0249] 所述 HPV 抗原可以是来自每一个 HPV 型的 HPV E6 或 E7 结构域。例如，对于 HPV 16 型 (HPV16)，所述 HPV16 抗原可包括 HPV16 E6 抗原、HPV16 E7 抗原、其片段、变体或组合。类似地，所述 HPV 抗原可以是 HPV 6 E6 和 / 或 E7、HPV 11 E6 和 / 或 E7、HPV 18 E6 和 / 或 E7、HPV 31 E6 和 / 或 E7、HPV 33 E6 和 / 或 E7、HPV 52 E6 和 / 或 E7 或 HPV 58 E6 和 / 或 E7、其片段、变体或组合。

[0250] (f)RSV 抗原

[0251] 所述病毒抗原可包括 RSV 抗原。所述 RSV 抗原可以是人 RSV 融合蛋白（在本文中也被称为“RSV F”、“RSV F 蛋白”和“F 蛋白”）或其片段或变体。所述人 RSV 融合蛋白在 RSV 亚型 A 与 B 之间可能是保守的。RSV 抗原可以是来自 RSV Long 株 (GenBank AAX23994.1) 的 RSV F 蛋白或其片段或变体。RSV 抗原可以是来自 RSV A2 株 (GenBank AAB59858.1) 的 RSV F 蛋白，或其片段或变体。RSV 抗原可以是 RSV F 蛋白或其片段或变体的单体、二聚体或三聚体。

[0252] 所述 RSV F 蛋白可以融合前形式或融合后形式存在。RSV F 的融合后形式在经免疫的动物中引发高滴度中和抗体，并且保护动物免受 RSV 攻击。

[0253] 所述 RSV 抗原还可以是人 RSV 附着糖蛋白（在本文中也被称为“RSV G”、“RSV G 蛋白”和“G 蛋白”），或其片段或变体。所述人 RSV G 蛋白在 RSV 亚型 A 与 B 之间不同。所述抗原可以是来自 RSV Long 株 (GenBank AAX23993) 的 RSV G 蛋白，或其片段或变体。所述 RSV 抗原可以是来自 RSV 亚型 B 分离株 H5601、RSV 亚型 B 分离株 H1068、RSV 亚型 B 分离株 H5598、RSV 亚型 B 分离株 H1123 的 RSV G 蛋白，或其片段或变体。

[0254] 在其它实施方案中，RSV 抗原可以是人 RSV 非结构蛋白 1（“NS1 蛋白”），或其片段或变体。例如，RSV 抗原可以是来自 RSV Long 株 (GenBank AAX23987.1) 的 RSV NS1 蛋白，或其片段或变体。人 RSV 抗原还可以是 RSV 非结构蛋白 2（“NS2 蛋白”），或其片段或变体。例如，RSV 抗原可以是来自 RSV Long 株 (GenBank AAX23988.1) 的 RSV NS2 蛋白，或其片段或变体。RSV 抗原还可以是人 RSV 核衣壳（“N”）蛋白，或其片段或变体。例如，RSV 抗原可以是来自 RSV Long 株 (GenBank AAX23989.1) 的 RSV N 蛋白，或其片段或变体。RSV 抗原可以是人 RSV 磷蛋白（“P”）蛋白，或其片段或变体。例如，RSV 抗原可以是来自 RSV Long 株 (GenBank AAX23990.1) 的 RSV P 蛋白，或其片段或变体。RSV 抗原还可以是人 RSV 基质蛋白（“M”）蛋白，或其片段或变体。例如，RSV 抗原可以是来自 RSV Long 株 (GenBank AAX23991.1) 的 RSV M 蛋白，或其片段或变体。

[0255] 在其它实施方案中，RSV 抗原可以是人 RSV 小疏水（“SH”）蛋白，或其片段或变体。例如，RSV 抗原可以是来自 RSV Long 株 (GenBank AAX23992.1) 的 RSV SH 蛋白，或其片段或变体。RSV 抗原还可以是人 RSV 基质蛋白 2-1（“M2-1”）蛋白，或其片段或变体。例如，RSV 抗原可以是来自 RSV Long 株 (GenBank AAX23995.1) 的 RSV M2-1 蛋白，或其片段或变体。RSV 抗原还可以是人 RSV 基质蛋白 2-2（“M2-2”）蛋白，或其片段或变体。例如，RSV 抗原可以是来自 RSV Long 株 (GenBank AAX23997.1) 的 RSV M2-2 蛋白，或其片段或变体。人 RSV 抗原可以是 RSV 聚合酶 L（“L”）蛋白，或其片段或变体。例如，RSV 抗原可以是来自 RSV Long 株 (GenBank AAX23996.1) 的 RSV L 蛋白，或其片段或变体。

[0256] 在其它实施方案中，RSV 抗原可具有 NS1、NS2、N、P、M、SH、M2-1、M2-2 或 L 蛋白的优化的氨基酸序列。RSV 抗原可以是人 RSV 蛋白或重组抗原，诸如由人 RSV 基因组编码的蛋白的任一种。

[0257] 在其它实施方案中，RSV 抗原可以是但不限于来自 RSV Long 株的 RSV F 蛋白、来自 RSV Long 株的 RSV G 蛋白、优化的氨基酸 RSV G 氨基酸序列、RSV Long 株的人 RSV 基因组、优化的氨基酸 RSV F 氨基酸序列、来自 RSV Long 株的 RSV NS1 蛋白、来自 RSV Long 株的 RSV NS2 蛋白、来自 RSV Long 株的 RSV N 蛋白、来自 RSV Long 株的 RSV P 蛋白、来自 RSV Long 株的 RSV M 蛋白、来自 RSV Long 株的 RSV SH 蛋白、来自 RSV Long 株的 RSV M2-1 蛋白、来自 RSV Long 株的 RSV M2-2 蛋白、来自 RSV Long 株的 RSV L 蛋白、来自 RSV 亚型 B 分离株 H5601 的 RSV G 蛋白、来自 RSV 亚型 B 分离株 H1068 的 RSV G 蛋白、来自 RSV 亚型 B 分离株 H5598 的 RSV G 蛋白、来自 RSV 亚型 B 分离株 H1123 的 RSV G 蛋白或其片段或其变体。

[0258] (g) 流感抗原

[0259] 病毒抗原可包括来自流感病毒的抗原。流感抗原是能够引发哺乳动物中的针对一个或多个流感血清型的免疫应答的那些抗原。所述抗原可包括全长翻译产物 HA0、亚单位

HA1、亚单位 HA2、其变体、其片段或其组合。流感血凝素抗原可来源于甲型流感血清型 H1、血清型 H2 的多个病毒株、来源于不同组的甲型流感血清型 H1 的多个病毒株或来源于乙型流感的多个病毒株的杂交序列。流感血凝素抗原可来自乙型流感。

[0260] 流感抗原还可含有至少一种抗原表位，所述表位可有效地抗可针对其引发免疫应答的特定流感免疫原。所述抗原可提供存在于完整流感病毒中的免疫原性位点和表位的完整库。所述抗原可来源于来自一个血清型的多个甲型流感病毒株（诸如血清型 H1 或血清型 H2 的多个甲型流感病毒株）的血凝素抗原序列。所述抗原可以是通过合并两个不同血凝素抗原序列或其部分衍生的杂交血凝素抗原序列。两个不同血凝素抗原序列的每一个可来源于不同组的一个血清型的多个甲型流感病毒株诸如血清型 H1 的多个甲型流感病毒株。所述抗原可以是来源于来自多个乙型流感病毒株的血凝素抗原序列的血凝素抗原序列。

[0261] 在一些实施方案中，流感抗原可以是 H1 HA、H2 HA、H3 HA、H5 HA 或 BHA 抗原。

[0262] (h) 埃博拉病毒

[0263] 病毒抗原可来自埃博拉病毒。埃博拉病毒病 (EVD) 或埃博拉出血热 (EHF) 包括 5 种已知的埃博拉病毒中的 4 种的任一种，包括本迪布焦病毒 (Bundibugyo virus, BDBV)、埃博拉病毒 (EBOV)、苏丹病毒 (Sudan virus, SUDV) 及塔伊病毒 (Taï Forest virus, TAFV，也被称为科特迪瓦埃博拉病毒（象牙海岸埃博拉病毒，CIEBOV))。

[0264] (2) 细菌抗原

[0265] 外来抗原可以是细菌抗原或其片段或变体。所述细菌可来自下列门的任一个：酸杆菌门 (Acidobacteria)、放线菌门 (Actinobacteria)、产水菌门 (Aquificae)、拟杆菌门 (Bacteroidetes)、嗜热丝菌门 (Caldiserica)、衣原体门 (Chlamydiae)、绿菌门 (Chlorobi)、绿弯菌门 (Chloroflexi)、产金菌门 (Chrysiogenetes)、蓝藻门 (Cyanobacteria)、脱铁杆菌门 (Deferribacteres)、异常球菌-栖热菌门 (Deinococcus-Thermus)、网团菌门 (Dictyoglomi)、迷踪菌门 (Elusimicrobia)、纤维杆菌门 (Fibrobacteres)、厚壁菌门 (Firmicutes)、梭杆菌门 (Fusobacteria)、芽单胞菌门 (Gemmatimonadetes)、黏胶球形菌门 (Lentisphaerae)、硝化螺旋菌门 (Nitrospira)、浮霉菌门 (Planctomycetes)、变形菌门 (Proteobacteria)、螺旋体门 (Spirochaetes)、互养菌门 (Synergistetes)、软壁菌门 (Tenericutes)、热脱硫杆菌门 (Thermodesulfobacteria)、热袍菌门 (Thermotogae) 和疣微菌门 (Verrucomicrobia)。

[0266] 细菌可以是革兰阳性细菌或革兰阴性细菌。细菌可以是好氧细菌或厌氧细菌。细菌可以是自养细菌或异养细菌。细菌可以是嗜温菌、嗜中性菌、嗜极菌、嗜酸菌、嗜碱菌、嗜热菌、嗜冷菌、嗜盐菌或嗜高渗菌。

[0267] 细菌可以是炭疽杆菌 (anthrax bacterium)、耐抗生素细菌、致疾菌、食物中毒细菌、感染性细菌、沙门菌属 (Salmonella) 细菌、葡萄球菌属 (Staphylococcus) 细菌、链球菌属 (Streptococcus) 细菌或破伤风 (tetanus) 细菌。该细菌可以是分枝杆菌属生物 (mycobacteria)、破伤风梭菌 (Clostridium tetani)、鼠疫耶尔森氏菌 (Yersinia pestis)、炭疽芽孢杆菌 (Bacillus anthracis)、耐甲氧西林金黄色葡萄球菌 (Staphylococcus aureus)(MRSA) 或艰难梭菌 (Clostridium difficile)。细菌可以结核分枝杆菌 (Mycobacterium tuberculosis)。

[0268] (a) 结核分枝杆菌抗原

[0269] 细菌抗原可以是结核分枝杆菌抗原（即，TB 抗原或 TB 免疫原），或其片段或其变体。TB 抗原可来自 TB 抗原的 Ag85 家族，例如，Ag85A 和 Ag85B。TB 抗原可来自 TB 抗原的 Esx 家族，例如，EsxA、EsxB、EsxC、EsxD、EsxE、EsxF、EsxH、EsxO、EsxQ、EsxR、EsxS、EsxT、EsxU、EsxV 和 EsxW。

[0270] (3) 寄生虫抗原

[0271] 外来抗原可以是寄生虫抗原，或其片段或变体。寄生虫可以是原生动物、蠕虫或体外寄生虫。蠕虫 (helminth)（即，蠕虫 (worm)）可以是扁虫（例如，吸虫和绦虫）、棘头蠕虫或蛔虫（例如，蛲虫）。体外寄生虫可以是虱子、跳蚤、蜱和螨虫。

[0272] 寄生虫可以是引起下列疾病的任一种的任何寄生虫：棘阿米巴角膜炎、阿米巴病、蛔虫病、巴贝虫病、小袋虫病、浣熊蛔虫病 (Baylisascariasis)、南美锥虫病、华支睾吸虫病、锥蝇 (Cochliomyia)、隐孢子虫病、裂头绦虫病、麦地那龙线虫病、棘球蚴病、象皮肿、蛲虫病、片形吸虫病、姜片虫病、丝虫病、贾第虫病、颚口线虫病、膜壳绦虫病、等孢子球虫病、片山热、利什曼病、莱姆病 (Lyme disease)、疟疾、后殖吸虫病、蝇蛆病、盘尾丝虫病、虱病、疥疮、血吸虫病、昏睡病、类圆线虫病、绦虫病、弓蛔虫病、弓形体病、旋毛虫病和鞭虫病。

[0273] 寄生虫可以是棘阿米巴属 (Acanthamoeba)、异尖属 (Anisakis)、蛔虫 (Ascaris lumbricoides)、马蝇 (Botfly)、结肠小袋纤毛虫 (Balantidium coli)、臭虫 (Bedbug)、多节绦虫亚纲 (Cestoda)（绦虫））、恙螨 (Chiggers)、锥形蛆蝇 (Cochliomyia hominivorax)、痢疾阿米巴 (Entamoeba histolytica)、肝片形吸虫 (Fasciola hepatica)、兰伯贾第虫 (Giardia lamblia)、钩虫、利什曼原虫属 (Leishmania)、锯齿状舌形虫 (Linguatula serrata)、肝吸虫 (Liver fluke)、罗阿丝虫 (Loa loa)、并殖吸虫属 (Paragonimus)- 肺吸虫、蛲虫、恶性疟原虫 (Plasmodium falciparum)、血吸虫属、粪类圆线虫 (Strongyloides stercoralis)、螨、绦虫、刚地弓形虫 (Toxoplasma gondii)、锥虫属 (Trypanosoma)、鞭虫或班氏吴策线虫 (Wuchereria bancrofti)。

[0274] (a) 疟疾抗原

[0275] 外来抗原可以是疟疾抗原（即，PF 抗原或 PF 免疫原）、或其片段或其变体。所述抗原可来自引发疟疾的寄生虫。引发疟疾的寄生虫可以是恶性疟原虫 (Plasmodium falciparum)。恶性疟原虫抗原可包括环子孢子 (CS) 抗原。

[0276] 在一些实施方案中，疟疾抗原可以是恶性疟原虫 (P. falciparum) 免疫原 CS、LSA1、TRAP、CelTOS 和 Ama1 之一。免疫原可以是全长蛋白的全长或免疫原性片段。

[0277] 在其它实施方案中，疟疾抗原可以是也被称为 SSP2 的 TRAP。在其它实施方案中，疟疾抗原可以是也被称为 Ag2 并且是高度保守的疟原虫属抗原的 CelTOS。在其它实施方案中，疟疾抗原可以是为高度保守的疟原虫属抗原的 Ama1。在一些实施方案中，疟疾抗原可以是 CS 抗原。

[0278] 在其它实施方案中，疟疾抗原可以是包含本文中所示的 PF 蛋白的两种或更多种的组合的融合蛋白。例如，融合蛋白可包含彼此直接邻接或通过之间的间隔子或一个或多个氨基酸连接的 CS 免疫原、ConLSA1 免疫原、ConTRAP 免疫原、ConCelTOS 免疫原和 ConAma1 免疫原的两个或更多个免疫原。在一些实施方案中，融合蛋白包含 2 个 PF 免疫原；在一些实施方案中，融合蛋白包含 3 个 PF 免疫原，在一些实施方案中融合蛋白包含 4 个 PF 免疫原，以及在一些实施方案中融合蛋白包含 5 个 PF 免疫原。具有 2 个 PF 免疫原的融合蛋白可包

含 :CS 和 LSA1 ;CS 和 TRAP ;CS 和 CelTOS ;CS 和 Ama1 ;LSA1 和 TRAP ;LSA1 和 CelTOS ;LSA1 和 Ama1 ;TRAP 和 CelTOS ;TRAP 和 Ama1 ;或 CelTOS 和 Ama1。具有 3 个 PF 免疫原的融合蛋白可包含 :CS、LSA1 和 TRAP ;CS、LSA1 和 CelTOS ;CS、LSA1 和 Ama1 ;LSA1、TRAP 和 CelTOS ;LSA1、TRAP 和 Ama1 ;或 TRAP、CelTOS 和 Ama1。具有 4 个 PF 免疫原的融合蛋白可包含 :CS、LSA1、TRAP 和 CelTOS ;CS、LSA1、TRAP 和 Ama1 ;CS、LSA1、CelTOS 和 Ama1 ;CS、TRAP、CelTOS 和 Ama1 ;或 LSA1、TRAP、CelTOS 和 Ama1。具有 5 个 PF 免疫原的融合蛋白可包含 CS 或 CS-alt、LSA1、TRAP、CelTOS 和 Ama1。

[0279] (4) 真菌抗原

[0280] 外来抗原可以是真菌抗原,或其片段或变体。真菌可以是曲霉菌属 (Aspergillus) 各种、皮炎芽生菌 (Blastomyces dermatitidis)、念珠菌属 (Candida) 酵母 (例如,白色念珠菌 (Candida albicans))、球孢子菌属 (Coccidioides)、新型隐球菌 (Cryptococcus neoformans)、格特隐球菌 (Cryptococcus gattii)、表皮寄生菌 (dermatophyte)、镰刀菌属 (Fusarium) 各种,荚膜组织胞浆菌 (Histoplasma capsulatum)、毛霉菌亚门 (Mucoromycotina)、杰氏肺囊虫肺炎 (Pneumocystis jirovecii)、申克氏孢子丝菌 (Sporothrix schenckii)、突脐蠕孢属 (Exserohilum) 或枝孢属 (Cladosporium)。

[0281] b. 自体抗原

[0282] 在一些实施方案中,抗原是自体抗原。自体抗原可以是受试者自己身体的能够刺激免疫应答的成分。在一些实施方案中,除非受试者处于疾病状态,例如自身免疫性疾病,否则自体抗原不激发免疫应答。

[0283] 自体抗原可包括但不限于细胞因子、抗病毒诸如上文中所列的那些病毒 (包括 HIV 和登革热) 的抗体、影响癌症进展或发展的抗原以及细胞表面受体或跨膜蛋白。

[0284] (1) WT-1

[0285] 自体抗原可以是维尔姆斯肿瘤 (Wlim's tumor) 抑制基因 1(WT1)、其片段、其变体或其组合。WT1 是在 N 末端上含有富含脯氨酸 / 谷氨酰胺的 DNA 结合结构域和在 C 末端上含有 4 个锌指基序的转录因子。WT1 在泌尿生殖系统的正常发育中起着作用,并且与许多因子例如,p53(一种已知的肿瘤抑制基因) 和丝氨酸蛋白酶 HtrA2(其可在用细胞毒性药物处理后在多个位点上切割 WT1) 相互作用。WT1 的突变可导致肿瘤或癌症形成,例如,维尔姆斯肿瘤或表达 WT1 的肿瘤。

[0286] (2) EGFR

[0287] 自体抗原可包括表皮生长因子受体 (EGFR) 或其片段或变体。EGFR(也被称为 ErbB-1 和 HER1) 是细胞外蛋白配体的表皮生长因子家族 (EGF- 家族) 的成员的细胞表面受体。EGFR 是受体的 ErbB 家族的成员,其包括 4 个密切相关的受体酪氨酸激酶 : EGFR(ErbB-1)、HER2/c-neu(ErbB-2)、Her3(ErbB-3) 和 Her 4(ErbB-4)。影响 EGFR 表达或活性的突变可导致癌症。

[0288] 抗原可包括 ErbB-2 抗原。Erb-2(人表皮生长因子受体 2) 也被称为 Neu、HER2、CD340(分化簇 340) 或 p185,并且由 ERBB2 基因编码。已显示该基因的扩增或过表达在某些侵袭性类型的乳腺癌的发展和进展中起作用。在大约 25-30％的患有乳腺癌的妇女中,在 ERBB2 基因中发生遗传改变,这导致在肿瘤细胞表面上增加的量的 HER2 的产生。HER2 的该过表达促进快速细胞分裂,从而 HER2 标志肿瘤细胞。

[0289] 对于 HER2 是特异性的合成抗体可包括 Fab 片段，所述 Fab 片段包含由 SEQ ID NO:40 的核酸序列编码的 SEQ ID NO:41 的氨基酸序列，和由 SEQ ID NO:42 的核酸序列编码的 SEQ ID NO:43 的氨基酸序列。

[0290] (3) 可卡因

[0291] 自体抗原可以是可卡因受体抗原。可卡因受体包括多巴胺转运蛋白。

[0292] (4) PD-1

[0293] 自体抗原可包括程序化死亡 1(PD-1)。程序化死亡 1(PD-1) 及其配体 PD-L1 和 PD-L2，递送调控 T 细胞活化、耐受性与免疫病理学之间的平衡的抑制性依赖。PD-1 为 288 个氨基酸的细胞表面蛋白分子，其包括细胞外 IgV 结构域，随后的跨膜区和细胞内尾。

[0294] (5) 4-1BB

[0295] 自体抗原可包括 4-1BB 配体。4-1BB 配体是属于 TNF 超家族的 2 型跨膜糖蛋白。4-1BB 配体可在活化的 T 淋巴细胞上表达。4-1BB 是活化诱导的 T 细胞共刺激分子。经由 4-1BB 的信号传导上调存活基因，增强细胞分裂，诱发细胞因子产生，和阻止 T 细胞中活化诱发的细胞死亡。

[0296] (6) CTLA4

[0297] 自体抗原可包括 CTLA-4(细胞毒性 T 淋巴细胞抗原 4)，也称为 CD152(分化簇 152)。CTLA-4 是在 T 细胞表面上发现的蛋白质受体，其导致对抗原的细胞免疫攻击。所述抗原可以是 CTLA-4 的片段，诸如细胞外 V 结构域、跨膜结构域和细胞质尾或其组合。

[0298] (7)IL-6

[0299] 自体抗体可包括白细胞介素 6(IL-6)。IL-6 刺激许多疾病包括但不限于糖尿病、动脉粥样硬化、抑郁症、阿尔茨海默氏病、全身性红斑狼疮、多发性骨髓瘤、癌症、白塞氏病 (Behcet's) 和类风湿性关节炎的炎性和自身免疫过程。

[0300] (8)MCP-1

[0301] 自体抗原可包括单核细胞趋化蛋白 -1(MCP-1)。MCP-1 也被称为趋化因子 (C-C 基序) 配体 2(CCL2) 或小可诱导细胞因子 A2。MCP-1 是属于 CC 趋化因子家族的细胞因子。MCP-1 招募单核细胞、记忆 T 细胞和树突状细胞至由组织损伤或感染产生的炎症位点。

[0302] (9) 淀粉样蛋白 β

[0303] 自体抗原可包括淀粉样蛋白 β (Aβ) 或其片段或变体。Aβ 抗原可包含 Aβ (X-Y) 肽，其中所述氨基酸序列来自人序列 Aβ 蛋白的氨基酸位置 X 至氨基酸 Y(包括 X 和 Y)，特别是氨基酸序列 DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVI 的氨基酸位置 X 至氨基酸位置 Y(对应于氨基酸位置 1 至 47 ;人查询序列) 的氨基酸序列或其变体。Aβ 抗原可包含 Aβ (X-Y) 多肽的 Aβ 多肽，其中 X 可以是 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 或 32 并且 Y 可以是 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 或 15。Aβ 多肽可包含为至少 15、至少 16、至少 17、至少 18、至少 19、至少 20、至少 21、至少 22、至少 23、至少 24、至少 25、至少 30、至少 35、至少 36、至少 37、至少 38、至少 39、至少 40、至少 41、至少 42、至少 43、至少 44、至少 45 或至少 46 个氨基酸的片段。

[0304] (10)IP-10

[0305] 自体抗原可包括干扰素 (IFN)-γ- 诱发的蛋白 10(IP-10)。IP-10 也被称为小可

诱导的细胞因子 B10 或 C-X-C 基序趋化因子 10(CXCL10)。CXCL10 由几个细胞类型诸如单核细胞、内皮细胞和成纤维细胞响应 IFN-γ 而分泌。

[0306] (11)PSMA

[0307] 自体抗原可包括前列腺特异性膜抗原 (PSMA)。PSMA 也被称为谷氨酸羧肽酶 II(GCPII)、N- 乙酰基 -L- 天冬氨酰基 -L- 谷氨酸肽酶 I(NAALAD 酶 I)、NAAG 肽酶或叶酸水解酶 (FOLH)。PMSA 是由前列腺癌细胞高度表达的膜内在蛋白。

[0308] c. 其它抗原

[0309] 在一些实施方案中，抗原是除外来抗原和 / 或自体抗原外的抗原。

[0310] (a)HIV-1 VRC01

[0311] 另一种抗原可以是 HIV-1 VRC01。HIV-1 VCR01 是 HIV 的中和 CD4- 结合位点 - 抗体。HIV-1 VCR01 接触包括在 HIV-1 的 gp120 环 D、CD4 结合环和 V5 区域内的 HIV-1 的部分。

[0312] (b)HIV-1 PG9

[0313] 另一种抗原可以是 HIV-1 PG9。HIV-1 PG9 是聚糖依赖性人抗体的扩大的家族的创立成员，其优先结合 HIV(HIV-1) 包膜 (Env) 糖蛋白 (gp) 三聚体并且广泛地中和病毒。

[0314] (c)HIV-1 4E10

[0315] 另一种抗原可以是 HIV-1 4E10。HIV-1 4E10 是中和抗 -HIV 抗体。HIV-1 4E10 针对定位至 HIV-1 的近膜端外部区 (MPER) 的线性表位，该表位位于 gp41 胞外域的 C 末端。

[0316] (d)DV-SF1

[0317] 另一种抗原可以是 DV-SF1。DV-SF1 是结合 4 种登革热病毒血清型的包膜蛋白的中和抗体。

[0318] (e)DV-SF2

[0319] 另一种抗原可以是 DV-SF2。DV-SF2 是结合登革热病毒表位的中和抗体。DV-SF2 对于 DENV4 血清型可以是特异性的。

[0320] (f)DV-SF3

[0321] 另一种抗原可以是 DV-SF3。DV-SF3 是结合登革热病毒包膜蛋白的 EDIII A 链的中和抗体。

[0322] 6. 组合物的赋形剂和其它组分

[0323] 组合物还可包含药学上可接受的赋形剂。药学上可接受的赋形剂可以是功能性分子诸如媒介物、载体或稀释剂。药学上可接受的赋形剂可以是转染促进剂，其可包括表面活性剂，诸如免疫刺激复合物 (ISCOMS)、弗氏不完全佐剂 (Freunds incomplete adjuvant)，LPS 类似物，包括单磷酰脂质 A、胞壁酰肽、醌类似物、囊泡诸如角鲨烯和角鲨烯、透明质酸、脂质、脂质体、钙离子、病毒蛋白、聚阴离子、聚阳离子或纳米颗粒，或其它已知的转染促进剂。

[0324] 转染促进剂是多聚阴离子、多聚阳离子，包括聚 -L- 谷氨酸 (LGS) 或脂质。转染促进剂是聚 -L- 谷氨酸，并且所述聚 -L- 谷氨酸可以以低于 6mg/ml 的浓度存在于组合物中。转染促进剂还可包括表面活性剂诸如免疫刺激复合物 (ISCOMS)、弗氏不完全佐剂，LPS 类似物，包括单磷酰脂质 A、胞壁酰肽、醌类似物和囊泡诸如角鲨烯和角鲨烯，并且还可将透明质酸与组合物结合施用。所述组合物还可包括转染促进剂，诸如脂质、脂质体，包括卵磷脂

脂质体或本领域中已知的其它脂质体，作为 DNA- 脂质体混合物（参见例如 WO9324640）、钙离子、病毒蛋白质、聚阴离子、聚阳离子或纳米颗粒，或其它已知的转染促进剂。转染促进剂是聚阴离子、聚阳离子，包括聚 -L- 谷氨酸 (LGS) 或脂质。疫苗中的转染剂的浓度低于 4mg/ml、低于 2mg/ml、低于 1mg/ml、低于 0.750mg/ml、低于 0.500mg/ml、低于 0.250mg/ml、低于 0.100mg/ml、低于 0.050mg/ml 或低于 0.010mg/ml。

[0325] 组合物还可包含 1994 年 4 月 1 日提交的美国序列 No.021,579（其通过引用完全并入）中描述的基因促进剂。

[0326] 组合物可以以约 1 纳克至 100 毫克、约 1 微克至约 10 毫克，或优选地约 0.1 微克至约 10 毫克，或更优选约 1 毫克至约 2 毫克的量包含 DNA。在一些优选实施方案中，根据本发明的组合物包含约 5 纳克至约 1000 微克的 DNA。在一些优选实施方案中，组合物可含有约 10 纳克至约 800 微克 DNA。在一些优选实施方案中，组合物可含有约 0.1 至约 500 微克的 DNA。在一些优选实施方案中，组合物可含有约 1 至约 350 微克的 DNA。在一些优选实施方案中，组合物可含有约 25 至约 250 微克、约 100 至约 200 微克、约 1 纳克至 100 毫克、约 1 微克至约 10 毫克、约 0.1 微克至约 10 毫克、约 1 毫克至约 2 毫克、约 5 纳克至约 1000 微克、约 10 纳克至约 800 微克、约 0.1 至约 500 微克、约 1 至约 350 微克、约 25 至约 250 微克、约 100 至约 200 微克的 DNA。

[0327] 可按照待使用的施用模式配制组合物。可注射药物组合物可以是无菌、无热原和无颗粒的。可使用等渗制剂或溶液。用于等渗性的添加剂可包括氯化钠、葡萄糖、甘露醇、山梨醇和乳糖。所述组合物可包含血管收缩剂。等渗溶液可包括磷酸盐缓冲盐溶液。组合物还可包含稳定剂，包括明胶和白蛋白。稳定剂可使得制剂在室温或环境温度长时间稳定，包括 LGS 或聚阳离子或聚阴离子。

[0328] 7. 产生合成抗体的方法

[0329] 本发明还涉及产生合成抗体的方法。所述方法可包括通过使用下文中更详细地描述的递送方法向有此需要的受试者施用组合物。因此，在向受试者施用组合物后在受试者中或体内产生合成抗体。

[0330] 所述方法还可包括将组合物引入一个或多个细胞，从而可在一个或多个细胞中产生或生成合成抗体。所述方法还可包括将组合物引入一个或多个组织，例如但不限于皮肤和肌肉，从而可在一个或多个组织中产生或生成合成抗体。

[0331] 8. 鉴定或筛选抗体的方法

[0332] 本发明还涉及鉴定和筛选上述抗体的方法，所述抗体与本文中描述的抗原反应或结合。鉴定或筛选抗体的方法可使用本领域技术人员已知的鉴定和筛选抗体的方法中的抗原。这类方法可包括但不限于从文库选择抗体（例如，噬菌体展示）和免疫动物，随后分离和 / 或纯化抗体。参见例如可在 Rajan,S. 和 Sidhu,S.,Methods in Enzymology，第 502 卷，第 1 章“Simplified Synthetic Antibody Libraries(2012)（其整体并入本文）中获得的方法。

[0333] 9. 组合物的递送方法

[0334] 本发明还涉及将组合物递送至有此需要的受试者的方法。所述递送方法可包括向受试者施用组合物。施用可包括但不限于通过和不通过体内电穿孔的 DNA 注射、脂质体介导的递送和纳米颗粒促进的递送。

[0335] 接受组合物的递送的哺乳动物可以是人、灵长类动物、非人灵长类动物、母牛、牛、绵羊、山羊、羚羊、野牛、水牛、野牛、牛科动物、鹿、刺猬、大象、骆驼、羊驼、小鼠、大鼠和鸡。

[0336] 组合物可以通过不同的途径（包括口服、肠胃外、舌下、经皮、经直肠、经粘膜、局部、经由吸入、经颊施用、胸膜内、静脉内、动脉内、腹膜内、皮下、肌内、鼻内鞘内和关节内或其组合）施用。对于兽医学用途，可按照正常兽医实践以适当地可接受的制剂施用组合物。兽医可以容易地确定最适合于特定动物的给药方案和施用途径。组合物可通过常规注射器、无针注射装置、“微弹轰击基因枪”或其它物理方法诸如电穿孔（“EP”）、“流体动力法”或超声波进行施用。

[0337] a. 电穿孔

[0338] 可使用电穿孔装置来实现经由电穿孔的组合物的施用，所述电穿孔装置被构造来向哺乳动物的期望的组织递送有效地在细胞膜中引起可逆孔形成的能量的脉冲，并且优选的能量脉冲是与由用户预设的电流输入相似的恒定电流。电穿孔装置可包括电穿孔组件和电极部件或操作部件。电穿孔组件可包括和包含电穿孔装置的各种元件的一个或多个，包括：控制器、电流波形发生器、阻抗测试器、波形记录器、输入元件、状态报告元件、通信端口、记忆组件、电源和电源开关。电穿孔可使用体内电穿孔装置例如CELLECTRA EP系统(VGX Pharmaceuticals,Blue Bell,PA)或Elgen电穿孔器(Genetronics,San Diego,CA)促进质粒对细胞的转染来实现。

[0339] 电穿孔组件可作为电穿孔装置的一个元件起作用，并且其它元件是与所述电穿孔组件通信的分开的元件（或组件）。电穿孔组件可作为电穿孔装置的多于一个元件起作用，其可以与同所述电穿孔组件分开的所述电穿孔设备的另外的其它元件通信。作为机电或机械装置的部分存在的电穿孔装置的元件可以不受限制，因为所述元件可作为一个装置或作为彼此相互通信的分开的元件起作用。电穿孔组件可以能够递送在期望的组织中产生恒定电流的能量脉冲，并且包括反馈机制。电极部件可包括在空间排列上具有多个电极的电极阵列，其中所述电极组件接收来自电穿孔组件的能量脉冲并将其通过电极递送至期望的组织。多个电极的至少一个在能量脉冲的递送过程中是中性的，并且测量期望的组织的阻抗，以及与将所述阻抗传达给电穿孔组件。反馈机制可接收测量的阻抗，并可调整通过电穿孔组件递送的能量脉冲以维持恒定电流。

[0340] 多个电极可以以分散模式递送能量脉冲。众多的电极可通过在程序化顺序下控制电极以分散模式递送能量脉冲，并且所述程序化顺序由用户输入至电穿孔组件中。程序化顺序可包含多个按顺序递送的脉冲，其中多个脉冲的每一个脉冲通过至少两个有源电极（其中一个中性电极测量阻抗）来递送，并且其中多个脉冲的随后脉冲通过至少两个有源电极（一个中性电极测量阻抗）的不同的一个电极来递送。

[0341] 可通过硬件或软件来进行反馈机制。可通过模拟闭环电路来进行反馈机制。反馈每50μs、20μs、10μs或1μs产生一次，但优选地是实时反馈或瞬时反馈（即，基本上同时的，如通过可获得的用于测定反应时间的技术测定）。中性电极可测量期望的组织中的阻抗，并将所述阻抗传达到反馈机制，并且所述反馈机制响应所述阻抗并调整能量脉冲以将恒定电流维持在与预设电流相似的值上。反馈机制可在能量脉冲递送过程中连续和瞬时地维持恒定电流。

[0342] 可促进本发明的组合物的递送的电穿孔装置和电穿孔方法的实例包括在

Draghia-Akli 等人的美国专利 No.7,245,963、由 Smith 等人提交的美国专利公开 2005/0052630（其内容在此通过引用整体并入）中描述的那些电穿孔装置和方法。可用于促进组合物的递送的其它电穿孔装置和电穿孔方法包括 2007 年 10 月 17 日提交的共同未决和共同拥有的美国专利申请序列 No.11/874072 中提供的那些电穿孔装置和方法，所述专利申请序列要求 2006 年 10 月 17 日提交的美国临时申请序列 No.60/852,149 和 2007 年 10 月 10 日提交的 60/978,982（其全都在此整体并入）在 35 USC 119(e) 下的权益。

[0343] Draghia-Akli 等人的美国专利 No.7,245,963 描述了模块化电极系统及它们用于促进将生物分子引入至身体或植物的选择的组织的细胞中的用途。模块化电极系统可包含多种针电极；皮下针头；提供从可编程恒定电流脉冲控制器至多个针电极的导电连接的电连接器；和电源。操作者可抓住多个安装在支持结构上的针电极，并将它们牢固地插入身体或植物的选择的组织中。随后经由皮下针头将生物分子递送至选择的组织。激活可编程恒定电流脉冲控制器，并将恒定电流电极脉冲施加于多个针电极。施加的恒定电流电脉冲促进将生物分子引入至多个电极之间的细胞中。美国专利 No.7,245,963 的整个内容在此通过引用并入。

[0344] 由 Smith 等人提交的美国专利公开 2005/0052630 描述了可用于有效地促进将生物分子引入至身体或植物的选择的组织的细胞中的电穿孔装置。所述电穿孔装置包含其操作通过软件或固件来指定的电动力学装置（"EKD 装置"）。EKD 装置基于脉冲参数的用户控制和输入在阵列中的电极之间产生一系列可编程的恒定电流脉冲波形，并且允许存储和获取电流波形数据。电穿孔装置还包含具有一个阵列的针电极的可替换电极盘、用于注射针的中央注射通道和可移去的引导盘。美国专利公开 2005/0052630 的整个内容在此通过引用并入。

[0345] 美国专利 No.7,245,963 和美国专利公开 2005/0052630 中描述的电极阵列和方法可适用于不仅至组织诸如肌肉，而且还至其它组织或器官内的深度穿透。由于电极阵列配置的原因，还可将注射针（以递送选择的生物分子）完全插入靶器官，并且在利用电极预先描绘的区域中垂直于靶组织施用注射剂。美国专利 No.7,245,963 和美国专利公开 2005/005263 中描述的电极优选为 20mm 长和 21 规格。

[0346] 此外，在一些包括电穿孔装置及其用途的实施方案中，设想存在电穿孔装置，其为在下列专利：1993 年 12 月 28 日颁布的美国专利 5,273,525、2000 年 8 月 29 日颁布的美国专利 6,110,161、2001 年 7 月 17 日颁布的 6,261,281 和 2005 年 10 月 25 日颁布的 6,958,060，以及 2005 年 9 月 6 日颁布的美国专利 6,939,862 中描述的那些电穿孔装置。此外，本文设想了针对注射 DNA 的方法设计的涵盖 2004 年 2 月 24 日颁布的美国专利 6,697,669（其涉及使用各多装置的任一种递送 DNA）和 2008 年 2 月 5 日颁布的美国专利 7,328,064 中提供的主题的专利。上述专利通过引用整体并入。

[0347] 10. 治疗方法

[0348] 本文中还提供了通过在受试者中产生合成抗体来治疗有此需要的受试者的疾病、保护其免受疾病侵害和 / 或预防所述疾病的方法。所述方法可包括向受试者施用组合物。向受试者施用组合物可使用上述递送方法来进行。

[0349] 在于受试者中产生合成抗体后，所述合成抗体可结合至抗原或与其反应。这样的结合可中和抗原、阻断另一分子例如蛋白质或核酸对抗原的识别，以及引发或诱发对所述

抗原的免疫应答，从而治疗受试者的与所述抗原相关的疾病，保护受试者免受所述疾病侵害和 / 或预防所述疾病。

[0350] 组合物剂量可以为 1μg 至 10mg 活性组分 /kg 体重 / 时间，以及可以为 20μg 至 10mg 组分 /kg 体重 / 时间。可以每 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 或 31 天施用组合物一次。用于有效治疗的组合物的剂量数可以为 1、2、3、4、5、6、7、8、9 或 10 次。

[0351] 在一个治疗方法中，可向需要抗感染（无论是病毒或细菌，还是癌性细胞）治疗的受试者施用合成抗体或其功能性片段。本文中描述的合成抗体的施用，在体内表达后，可提供可在身体的患病区域快速呈递其自己并且激发对靶（所述抗体被设计来结合所述靶，并且优选中和所述靶）的中和响应的功能性抗体。该快速呈递对于相当快的和 / 或在不具有现有记忆免疫的个体中的疾病病状可以是非常重要的。一些其中快速中和对于被感染的受试者是至关重要的特殊病例存在于热带疾病（诸如登革热、基孔肯雅热和埃博拉病）中。这样的感染需要在病毒感染时立即快速中和。实施例 5 和图 6A 及 6B 展示了使用利用描述的方法产生的表达构建体快速产生抗体。图 6A 显示在质粒 DNA 构建体施用的当天内表达抗体；而在图 6B 中，蛋白质 / 抗原的施用在约 8 天内导致抗体表达。

[0352] 该治疗方法可以是单独的，或可将其与利用抗原的正常接种组合，这随后可使受试者产生抗所述靶的宿主免疫应答。组合疫苗可提供抗期望的靶的两相免疫应答的益处：1) 如由编码合成抗体和其功能性片段的核苷酸序列提供的第一快速应答，和 2) 由常规疫苗（其可包括 DNA 疫苗或合成免疫原）触发的第二宿主免疫应答，在宿主可激发其自身的针对所述靶的免疫应答之前，所述第二宿主免疫应答可具有迟滞期。

[0353] 本发明具有通过下列非限定性实施例说明的多个方面。

[0354] 11. 实施例

[0355] 本发明将在下面的实施例中进一步说明。应理解，这些实施例，虽然指示了本发明的优选实施方案，但仅是以说明性方式给出的。根据上面的讨论和这些实施例，本领域普通技术人员可确定本发明的本质特征，并且在不背离其精神和范围的情况下，可以对本发明进行各种改变和改进，以使其适应各种用途和条件。因此，根据前述描述，除了本文中显示和描述的那些改进之外的本发明的各种改进对于本领域技术人员来说将显而易见。此类改进也意欲落入所附权利要求的范围内。

[0356] 实施例 1

[0357] 构建用于体内免疫球蛋白 (Ig) 产生的高表达系统。具体地，修饰 Ig 重链和轻链序列以提高完全装配的 Ig 分子的体内表达，所述分子包括 2 个重链和 2 个轻链多肽。产生 gp120IgG- 重链和轻链分子的构建体，并将其单独地插入 pVAX1 载体 (Life Technologies, Carlsbad, CA)。该抗体具有确定的性质，所述性质允许其用于如下所述的表征研究中。当产生构建体时可包括几种修饰来优化 Ig 在体内的表达。优化包括密码子优化和 kozak 序列 (GCC ACC) 的引入。Ig 的重链和轻链的优化构建体的核酸序列分别示于 SEQ ID NO:6 和 SEQ ID NO:7（分别是图 1 和 2）中。在图 1 和 2 中，加以下划线和加以双下划线标示用于将构建体克隆进 pVAX1 载体的 BamHI (GGA TCC) 和 XhoI (CTC GAG) 限制性酶位点，而粗体标示起始 (ATG) 和终止 (TGA TAA) 密码子。SEQ ID NO:6 编码 SEQ ID NO:46 中所示的氨基酸序列，即 IgG 重链的氨基酸序列（图 42）。SEQ ID NO:7 编码 SEQ ID NO:47

中所示的氨基酸序列，即 IgG 轻链的氨基酸序列（图 43）。

[0358] 用天然 Ig 构建体（即，未优化的）或含有 SEQ ID NO:6 和 7 的构建体（即，优化的）转染细胞。转染后，测量来自转染的细胞的 IgG 分泌，并且 IgG 合成的动力学示于图 3 中。如图 3 中所示，未优化的和优化的构建体都表达 Ig 的重链和轻链以形成 IgG，但优化的构建体导致 IgG 抗体更快积累。用含有 SEQ ID NO:6 和 7（即，优化的 Ig 序列）的质粒转染的细胞显示比用含有非优化的 Ig 序列的质粒转染的细胞产生更多的完全装配的 Ig 分子。因此，构建体的优化或修饰显著增加 Ig 表达。换句话说，由于用于产生 SEQ ID NO:6 和 7 的优化或修饰，含有 SEQ ID NO:6 和 7 的构建体提供了相较于天然构建体显著更高的 Ig 表达。这些数据还表明可在体内从质粒系统高效地装配 Ig 的重链和轻链。

[0359] 为了进一步检查含有 SEQ ID NO:6 和 7 的构建体，向小鼠施用含有 SEQ ID NO:6 和 7 所示的序列的质粒。具体地，使用电穿孔施用质粒。施用后，通过蛋白质印迹（即，将小鼠的血清用于检测 gp120 抗原）评价对经免疫的小鼠的免疫应答（即，IgG 水平）的诱发。如图 4 中所示，因为在蛋白质印迹分析中观察到抗体的结合，因此施以含有 SEQ ID NO:6 和 7 的质粒的小鼠导致强抗体产生。只需一次施用就可观察到该抗体产生。

[0360] 总之，这些数据表明编码 Ig 重链和轻链的核酸序列，当包括在表达载体诸如 pVAX1 中时，在转染的细胞和施以所述表达载体的小鼠中导致装配的 IgG（即，重链和轻链一起形成结合其抗原的抗体）表达。这些数据还表明编码 Ig 重链和轻链的核酸序列的优化或修饰显著增加 Ig 产量。

[0361] 实施例 2

[0362] 用于实施例 3-7 的材料和方法

[0363] 细胞和试剂。将 293T 和 TZM-Bl 细胞维持在补充有 10％胎牛血清 (FBS) 和抗生素的达尔伯克改良伊格尔培养基 (Dulbecco’s Modified Eagle’s medium, DMEM；Gibco-Invitrogen, CA) 中，并且在汇合后进行传代。重组 HIV-1 p24 和 gp120 Env (rgp120) 蛋白获自 Protein Science Inc.，并且缀合有过氧化物酶的链霉抗生物素蛋白来自 Jackson Laboratory。所列的细胞系和其它试剂获自 AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH。

[0364] 动物和蛋白质以及质粒施用和递送。雌性 BALB/c 小鼠 (8 周龄) 购自 Taconic Farms (Germantown, NY)。对于这些施用，肌内注射 (IM) 25μg 的 50μl 体积中的质粒 DNA (pVax1 或 pHIV-1Env-Fab)，随后 MID-EP 系统（CELLECTRA®；Inovio Pharmaceuticals, Blue Bell, PA) 进行 EP 介导的增强递送。用于递送的脉冲参数为：3 个脉冲的 0.5Amp 恒定电流（间隔 1 秒，长度为 52ms)。每一只动物接受实验性或对照质粒制剂的单次施用。对于蛋白质免疫分析，使用来自 JRFL 株（购自 Immune Technology Corp, NY) 的 HIV-1 重组 gp120 (rgp120)。在蛋白质免疫研究中，将单次 25μg 剂量的 rgp120 与 TiterMax 佐剂混合，随后进行皮下注射。根据具体的分析，在不同的时间点收集来自施以 pHIV-1 Env Fab 或 rgp120 的小鼠的血清。

[0365] HIV-1Env-Fab 质粒 DNA 的构建。通过使用具有几个修饰的合成寡核苷酸产生来自抗 -Env VRC01 人 mAb 的 HIV-1 Env-Fab（序列 VH 和 VL)。重链 (VH-CH1) 由 SEQ ID NO:3 所示的核酸序列编码，并且轻链 (VL-CL) 由 SEQ ID NO:4 所示的核酸序列编码（分别地，图 9 和 10)。在图 9 和 10 中，加以下划线和加以双下划线标示用于将编码核酸序列克隆进

pVAX1 的 HindIII(AAG CTT) 和 XhoI(CTC GAG) 限制性酶位点，而粗体标示起始 (ATG) 和终止 (TGA 或 TAA) 密码子。SEQ ID NO:3 编码 SEQ ID NO:48 中所示的氨基酸序列，即 HIV-1 Env-Fab 的 VH-CH1 的氨基酸序列 (图 44)。SEQ ID NO:4 编码 SEQ ID NO:49 所示的氨基酸序列，即 HIV-1 Env-Fab 的 VL-CL 的氨基酸序列 (图 45)。

[0366] 将有效 IgE 前导序列 (编码 SEQ ID NO:66 蛋白的 SED ID NO:65 核苷酸) 掺入 Env 抗原基因序列以增加表达。对所得的经修饰的和增强的 HIV-1Env-Fab DNA 免疫原进行密码子优化和 RNA 优化，然后利用 GenScript(Piscataway,NJ) 将其克隆进 pVax1 表达载体，随后大规模产生这些构建体。将 VH 和 VL 基因 (分别地，SEQ ID NO:3 和 4) 插入在 BamH1 与 Xho1 限制位点之间。随后在水中配制纯化的质粒 DNA，以用于随后施用至小鼠体内。对于阴性对照质粒，使用产生“无关”/ 对照 Ig 的 pIgG-E1M2。

[0367] HIV-1Env-Fab 表达和免疫印迹分析。使用由制造商推荐的方法，将 293T 细胞系用于使用非脂质体 FuGENE6 转染试剂 (Promega,WI) 的表达分析中。简而言之，以 50-70％汇合 (1-3x10^5个细胞 /2mL/ 孔，于 35mm 培养皿中) 接种细胞 24 小时，之后用 5μg pVax1 对照或 pHIV-1Env-Fab 进行转染。在长达 70 小时的不同时间点收集上清液，并通过标准 ELISA 法评估其特定 Fab 分子的水平。将来自 pVax1 转染的细胞的上清液用作阴性对照。此外，用 HIV gp160 Env 蛋白的基因转染 293T 细胞。

[0368] 通过免疫印迹分析进一步确认产生的 Fab 对天然 HIV-1 Env 蛋白的识别。对于本研究，将上述 rgp120 在 12％ SDS-PAGE 上进行电泳。将凝胶印迹于硝酸纤维素膜 (Millipore,Bedford,MA) 上，并用 5％ w/v 的于 PBS-T(0.05％) 中的脱脂奶粉进行封闭。随后将硝酸纤维素切成单条以用于分析。以 1:100 将施用后 48 小时收集的施以 pHIV-1 Env Fab 的小鼠的血清于 PBS 中进行稀释，并将所述血清与单个硝酸纤维素条反应 1 小时。随后，用 Tris- 缓冲盐溶液 -0.2％ Tween 洗涤条 4 次，将其与偶联有过氧化物酶的针对小鼠 IgG 的抗血清 (Jackson Laboratories,ME) 反应，并用二氨基联苯胺底物 (Sigma,St.Louis,MO) 进行孵育，从而使得能够显现产生的 HIV-1 Env Fab 对 gp120 的正确结合。

[0369] Ig 结合分析 - ELISA。通过 ELISA 评价 DNA 质粒产生的 Fab 或抗 -rgp120 抗体对 rgp120 的结合的确认。用来自施以 pHIV-1 Env Fab、pVax1 或 rgp120 蛋白的单个动物的血清进行 Ig 结合测定。再次地，对于该基础 Ig 免疫测定分析，在单次 DNA 质粒施用后 48 小时收集血清样品。简而言之，用于 PBS 中稀释的进化枝 B HIV MN rgp120(2μg/mL) 在 4℃涂覆 96 孔高结合聚苯乙烯板 (Corning,NY) 过夜。第 2 天，用 PBS-T(PBS,0.05％ Tween 20) 洗涤板，用 3％于 PBS-T 中的 BSA 封闭 1 小时，及在 37℃用来自已免疫的小鼠和首次接触实验的小鼠的血清以 1:100 的稀释度孵育 1 小时。按 1:5,000 的稀释度使用山羊抗 - 小鼠 IgG-HRP(Research Diagnostics,NJ) 检测结合的 IgG。通过添加色原体底物溶液 TMB(R&D Systems) 检测结合的酶，并在 Biotek EL312e Bio-Kinetics 读数器上于 450nm 处读数。以一式二份测试所有血清样品。进行额外的免疫测定分析，所述分析使用商业 IgG1 定量 ELISA 试剂盒定量来自施以 pHIV-1 Env Fab 的小鼠的血清中的 Fab 浓度。该分析按照制造商的说明书进行。

[0370] 流式细胞分析 (FACS)。对于流式细胞分析 (FACS)，用共有进化枝 A Env 质粒 (pCon-Env-A) 或表达来自原代病毒分离株 (Q23Env17) 的 Env 的优化的进化枝 A 质粒 (pOpt-Env-A) 转染 293T 细胞。通过标准方法进行转染。在确认转染后，利用冰冷缓冲液

A(PBS/0.1％ BSA/0.01％ NaN3)洗涤细胞，并在4℃下用第一Ig(纯化的VRC01或在质粒施用后48小时收集的，来自用pHIV-1 Env Fab或对照pIgG-E1M2质粒注射的小鼠的血清)的1:100稀释物孵育20分钟。随后洗涤，并用50μl的1:100稀释的荧光标记的缀合于藻红蛋白(PE)的第二Ig再孵育20分钟。随后洗涤细胞，并立即在流式细胞仪(Becton Dickinson FACS)上进行分析。用冰冷缓冲液A在4℃下进行所有孵育和洗涤。针对单重态和活细胞对细胞进行门控。为了评估GFP表达，用FACS-LSR仪，使用CellQuest软件(BD Bioscience)进行GFP阳性细胞分析。利用Flow Jo软件分析数据。

[0371] 单循环HIV-1中和测定。用基于TZM-BI(HeLa细胞衍生的)的测定测量Fab介导的HIV-1中和分析，其中在于实验性血清或对照血清存在或不存在的情况下利用Env-假型病毒进行单轮感染后，将荧光素酶基因表达的减少用作中和的终点。将TZM-B1细胞工程化以表达CD4和CCR5，并且所述细胞含有萤火虫荧光素酶的报告基因。在该测定中，将仅施以pVax1或施以pHIV-1Env Fab的小鼠的血清在各孔中以1:50稀释，随后以0.01的感染复数(MOI)添加假型HIV-1 Bal26、Q23Env17、SF162S或ZM53M无细胞病毒。Bal26和SF162S都是进化枝B tier 1病毒，该tier状态表明所述病毒具有高的对中和的敏感性或高于平均敏感性的敏感性。Q23Env17和ZM53M分别是进化枝A(Tier 1)和进化枝C(Tier 2)病毒。Tier 2的状态表明所述病毒具有平均或适度的对中和的敏感性。随后在该测定中，将10^4 TZM-BL细胞添加至每一个孔，孵育48小时，裂解，之后随后添加100μl的Bright-Glo底物(Luciferase Assay System,Promega,WI)，随后使用光度计进行荧光素酶定量。该测定的读出是RLU(相对光单位)。将RLU减少的百分比计算为(1-(实验样品的平均RLU-对照的平均RLU)/来自对照孔的平均RLU-无添加对照孔的平均RLU))x100。随后将HIV-1中和表达为RLU的百分比减少，其表示感染的百分比抑制。

[0372] 实施例3

[0373] 抗-HIV-1 Env-Fab表达构建体的产生

[0374] 通过NIH AIDS Research and Reference Reagent Program从VRC(疫苗研究中心，NIH)获得编码广泛中和人mAb VRC01的抗-HIV-1包膜的VH和VL-Ig(免疫球蛋白)链的cDNA，随后将其克隆进pVax1载体。将如上文实施例2中指定的几个修饰掺入表达载体以最大化和优化生物活性Ig分子的产生。具体地，这些修饰包括密码子和RNA优化和稳定化、增强的前导序列效用、以高浓度进行的质粒产生，以及通过EP促进的体内质粒递送。将产生的构建体置于来自人巨细胞病毒(CMV)的立即早期启动子的控制之下，这对于在哺乳动物细胞和组织中的适当且高效表达是非常重要的。本研究中使用的构建体的示意性图谱示于表5A和5B中。

[0375] 此外，由pHIV-Env-Fab制备抗-HIV-1 Env Fab，并将其用于对用编码HIV Env的质粒转染的细胞进行染色。pVAX1用作对照。如图11中所示，免疫荧光染色显示载体pHIV-Env-Fab允许制备抗-HIV-1 Env Fab，因为抗-HIV-1 Env Fab染色用编码HIV Env的质粒转染的细胞。因此，抗-HIV-1 Env Fab对于与HIV Env糖蛋白的结合是特异性。

[0376] 实施例4

[0377] 通过转染的细胞进行的Ig产生

[0378] 为了评价pHIV-1Env-Fab的表达，将所述构建体转染进293T细胞中。使用共有HIV-1进化枝B gp120蛋白的ELISA免疫测定确认抗-HIV-1 Env-Fab存在于来自早至转染

后 24 小时的转染的 293T 细胞的上清液中（图 5C）。在来自转染后 24 至 72 小时的细胞提取物中检测到高 OD450nm 值（即范围从约 0.5 至 0.8），并且该值随后达到峰值并在 48 小时达到平台。这些结果确认了抗 -HIV-1 Env Fab 对于 HIV Env 糖蛋白的特异性。图 5C 中提供的数据的统计分析如下：来自注射 pHIV-1 Env-Fab 的小鼠的血清的 OD450nm 值从 22 至 72 小时的时间点测量相较于 pVax1 对照是显著的（$p<0.05$，学生 t 检验）。

[0379] 实施例 5

[0380] HIV-1 Env Fab 的体内表征

[0381] 为了证明 Fab 在体内从 DNA 质粒体产生，通过肌内途径，随后进行通过 EP 的增强递送向小鼠施用 pHIV-1 Env Fab。递送 DNA 质粒的单次注射，并在施用后第 12 小时和第 1、2、3、4、7 和 10 天收集血清。随后通过 ELISA 分析评价血清（以 1:100 的稀释度）的 Ig/Fab 水平，如图 6A 中显示的。将图 6A 中的数据呈现（来自 pVax1 和 HIV-1 Env-Fab 组中的单个小鼠）为 OD450nm，所述 OD450nm 与 Ig/Fab 的水平成正比。这些数据表明，pHIV-1Env-Fab 的单次施用后的 Fab 的相对水平在第 1 天变得可检测，并且随后随时间提高。为了进行比较，对 Balb/C 小鼠进行 rgp120 的单次施用 / 免疫（如上文实施例 2 中描述的），随后随时间收集血清并通过 ELISA 进行分析（以 1:100 的稀释度），以测定特异性抗 -gp120 抗体的水平的程度和寿命。图 6B 显示结果。

[0382] 在该蛋白质递送研究中，仅在免疫后 10 天才可检测到高于本底的抗原特异性 Ig 水平。这与通过 pHIV-1 Env Fab 施用（图 6A）引发的 Fab 水平形成对比，其中 OD450nm 值在施用后第 1 天达到至少 0.1 OD450nm 单位，并在第 10 天达到平台，水平为 0.28 至 0.35 OD 单位。因此，pHIV-1 Env Fab 的递送导致比常规蛋白质免疫更快速的特定 Fab 的产生。该发现强调了该 DNA 质粒递送法用于产生生物活性 Ig 的潜在临床效用。

[0383] 进行另外的分析以确保通过 DNA 递送技术产生的重组 Fab 的质量及数量。具体地，使用经电泳和印迹的重组 HIV-1 gp120 蛋白进行免疫印迹分析，并用来自施用后 48 小时的 pHIV-1Env-Fab 小鼠的血清进行探测（图 6C）。印迹指示适合 gp120 蛋白的分子量的条带，从而确认其具有功能性并且能够结合 gp120。同样地，通过 ELISA 进行人 Fab 定量，并将其表示为质粒施用后时间的函数（即天）（图 6D）。结果表明产生的 Fab 的水平在 2-3 μg/ml 达到峰值。这些结果表明产生的基于 VRC01 的 Fab 的 VH 和 VL 链的正确多肽装配以及识别并特异性结合 HIV-1 Env 蛋白的能力。

[0384] 图 6 中提供的数据的统计分析如下。对于图 6A 中概述的数据，来自注射 pHIV-1 Env-Fab 的小鼠的血清的 OD450nm 值从第 1 天至第 10 天测量时间点相较于来自注射 pVax1 的小鼠的血清在统计上升高（$p<0.05$，学生 t 检验）。对于图 6B 中概述的数据，来自 rpg120 组的 OD450nm 值从第 10 天至第 14 天时间点测量相较于 PBS 对照显著升高（$p<0.05$，学生 t 检验）。对于图 6D 中概述的数据，来自注射 pHIV-1 Env-Fab 的小鼠的 OD450nm 值从第 2 天至第 10 天时间点测量显著升高（$p<0.05$，学生 t 检验）。

[0385] 实施例 6

[0386] Fab/Ig 对表达不同 HIV-1 Env 蛋白的细胞的结合：基于 FACS 的分析

[0387] 来自施以 pHIV-1Env-Fab 的小鼠的血清也用于测试产生的 Fab 与由 293T 细胞瞬时表达的不同 HIV-Env 蛋白的结合。将天然形式的 VRC01-mAb 用作阳性对照，以确保 Env 蛋白在细胞表面上的正确表达和检测。如更早指示的，“无关 / 不相关”Ig(Ig-E1M2) 用作阴

性对照。如图 7A 和 7B 中显示的，对于 pVax1（即缺乏 Env 插入物）转染的细胞，只存在使用不同 Ig/Fab 进行的本底染色。然而，对于纯化的 VRC01 mAb 和来自施用 pHIV-1Env-Fab 的小鼠的血清，都存在表达共有进化枝 A Env 质粒 (pCon-Env-A) 以及优化的进化枝 C 质粒 (pOpt-Env-A) 和表达来自原代 HIV-1 分离株 pQ23Env17 的 Env 的转染细胞的显著阳性染色。此外，来自施用 pIg-E1M2 的小鼠的血清未能显示高于本底水平的任何 HIV1 Env 转染的细胞的染色。显示这些结果的 FACS 分析提供于图 7A 中。显示该实验的 FACS 分析（即，图 7A) 的数据的代表性图提供于图 7B 中。

[0388] 图 7B 中呈现的数据的统计分析如下。在天然 VRC01 抗体与来自注射 pHIV-1 Env-Fab 的小鼠的血清之间对由 pCon-Env-A 产生的包膜糖蛋白的特异性结合上不存在显著差异 ($p<0.05$，学生 t 检验）。然而，VRC01 抗体与由 pOpt-Env-A 产生的包膜糖蛋白的结合比通过来自注射 pHIV-1 Env-Fab 的小鼠的血清的结合显著更强 ($p<0.05$，学生 t 检验）。

[0389] 实施例 7

[0390] 由 pHIV-1 Env Fab 产生的 Ig 的 HIV 中和活性

[0391] 将来自施以 pHIV-1Env-Fab 的小鼠的血清用于测定 HIV-Env Fab 与被瞬时转染至 293T 细胞中表达的 HIV-1 Env 蛋白的结合。在 pHIV-1Env-Fab 施用后 6 天从小鼠获得血清。具体地，用从其表达来自进化枝 A、B 或 C 株的 HIV-1 Env 的质粒转染细胞。进化枝 A、B 和 C 株是 92RW020、SF162 和 ZM197。如图 12 中所示，来自施以 pHIV-1Env-Fab 的小鼠的血清结合来自进化枝 A、B 和 C HIV-1 株的 HIV-1 Env，从而表明所述血清含有与来自多个亚型的 HIV-1 的 HIV-1 Env 交叉反应的抗体（即，HIV-Env Fab)。

[0392] 为了评估本研究中产生的 HIV-Env Fab 的潜在 HIV-1 中和活性，进行基于使用 TZM-B1 靶细胞的基于发光的中和测定。如上文实施例 2 中所述，在实验血清和对照不存在或存在的情况下用 4 种不同的假型 HIV 病毒分离株感染 TZM-B1 靶细胞。

[0393] 图 8 描绘来自注射 pHIV-1 Env Fab 的小鼠的血清针对 HIV 假型病毒的中和曲线。具体地测试 HIV-1 tier 1 病毒 Bal26 和 SF162S（两者都是进化枝 B) 以及 Q23Env（进化枝 A)。此外，还针对 HIV-1 进化枝 C tier 2 病毒 ZM53M 测试血清。数据呈现为 HIV 感染的百分比中和 / 抑制。图中的阴影水平线指示测定中 50％的中和 / 抑制水平。将阳性中和对照 mAb（数据未显示）在本研究中用于确认该测定法的实用性和有效性。简而言之，所述中和 mAb 的阳性对照能够抑制所有 4 种病毒假型的感染至少 50％。

[0394] 来自施以 pHIV-1 Env Fab 的小鼠的血清显示在质粒施用后随时间的 HIV 中和活性增强，对于 Bal25、Q23Env17 和 SF162S，百分比中和到第 2 天达到 50％。同样地，这 3 种病毒的平台百分比中和分别约为 62、60 和 70％。对于 ZM53M，直至第 3 天才达到 50％的中和阈值，并且平台中和未超过 50％。相较于测试的另外 3 种病毒，该不太强的中和特征可能反映其为不太可中和的 Tier 2 病毒。总之，本研究中产生的 Fab 能够有效地中和一系列 HIV 分离株。图 8 中呈现的数据的统计分析如下。基于 Kruskal-Wallis 非参数分析，仅由来自注射 pHIV-1 Env-Fab 的小鼠的血清诱发的 ZM53M 进化枝 C 病毒（图 8D) 的 HIV 中和水平与其它测试的病毒显著不同（图 8A、8B 和 8C)。该差异在于达到 50％中和所需的时间（天数）以及最大获得的中和水平。

[0395] 总结实施例 3-7，施以 pHIV-1 Env Fab 的小鼠中的 VRC01 Fab 的血清浓度在注射后第 12 天达到峰值 2-3 μg/mL。该范围可与目前由 FDA 批准的单克隆抗体的数目相当，这

表明我们的抗体法在该小动物模型中产生显著的和生物学相关水平的抗体。具体地，优特克单抗（商品名称：Stelara）和戈利木单抗（Simponi），被指定用于抗自身免疫性疾病诸如斑块状银屑病和关节炎的两种抗体，分别具有 0.31±0.33μg/mL 和 1.8±1.1μg/mL 的平均 ±SD 血清浓度。此外，TNF 抑制剂阿达木单抗（Humira）具有约 6μg/mL 的平均血清谷浓度（mean rough serum concentration）。在这点上，实施例 4-8 中描述的数据显示编码抗体的 DNA 递送至生物体导致在体内被组装，使得显著的和生物学相关水平的抗体存在于生物体中。

[0396] 这些数据还显示在 pHIV-1Env Fab 的单次 EP 增强施用后，相较于由常规蛋白质施用产生的 Ig，在体内更快速地产生 Fab 的能力（图 6A 和 6B）。此外，还阐明了产生针对不同疫苗靶的功能性保护 Ig 样分子的能力。迄今为止，在主动接种后诱发 HIV-1 中和抗体一直以来非常困难，并且在初次感染过程中，中和抗体直至传染后数年才产生。借助于该 DNA 质粒法，在递送后 1-2 天内观察到中和滴度，而在施用后 1 周出现峰值中和 Fab 血清浓度（3.31±0.13μg/mL）（图 6D）。该 Ig 水平与在最近的研究中已被证明提供免受感染的完全保护的 8.3μg/mL 浓度相对相似。这些数据证明了生物活性 Ig 片段的快速诱发。

[0397] 这些数据还显示中和抗体滴度和通过 HIV-1Env-Fab DNA 施用引发的针对 HIV-1 原代分离株的应答。针对一小组不同的病毒 tier 1 和 2 病毒分离株测试血清，所述病毒分离株代表了来自进化枝 A、B 和 C 的实例。结果表明产生针对这些病毒的强效中和活性（图 8）。

[0398] 因此，该基于 DNA 质粒方法在体内产生特异性和生物活性 Fab 或 Ig 分子，避开使用常规基于抗原的接种来进行抗体产生的需要，以及避免在体外产生 Ig 和纯化产生的 Ig 的需要。

[0399] 实施例 8

[0400] 编码人 Ig 抗体的质粒的构建

[0401] 如上所述，从 VRC01 抗体产生 Fab，即在对受试者施用所述编码核酸后在体内产生的 HIV-Env Fab。为了进一步扩展这些研究，产生编码来源于 VRC01 抗体的 IgG1 抗体的核酸序列。如图 13 中的示意图中所示，该核酸序列编码被费林蛋白酶切割位点和编码 P2A 肽序列的核酸序列分隔的 IgG 重链和轻链。P2A 肽序列增强蛋白酶的切割效率，从而在切割后导致分开的多肽。

[0402] 所述 IgG 重链包括可变重（VH）区、恒定重 1（CH1）区、铰链区、恒定重 2（CH2）区和恒定重 3（CH3）区。所述 IgG 轻链包括可变轻（VL）区和恒定轻（CL）区。将该构建体例如在表达载体 pVAX1 中置于巨细胞病毒（CMV）启动子的控制下。该构建体导致为反应性 gp120（即，被 VRC01 抗体识别的抗原）的完全装配的 IgG 抗体产生（如图 14 中显示的）。该完全装配的 IgG 在本文中被称为 VRC01 IgG。VRC01 IgG 的氨基酸序列（在被费林蛋白酶切割前）示于图 15 中，并且示于 SEQ ID NO:5 中，其由编码 SEQ ID NO:64 的核酸序列编码（参见图 62）。

[0403] 具体地，VRC01 IgG 的氨基酸序列（在被费林蛋白酶切割前；SEQ ID NO:5 和图 15，其由核苷酸序列 SEQ ID NO:64 编码）具有下列结构：免疫球蛋白 E1（IgE1）信号肽、可变重区（VH）、恒定重区 1（CH1）、铰链区、恒定重区 2（CH2）、恒定重区 3（CH3）、费林蛋白酶切割位点、GSG 接头、P2A 肽、IgE1 信号肽、可变轻区（VL）和恒定轻区（CL，具体地为 κ）。在下文

中提供了所述结构的每一个部分的序列（其全部序列以上述和图 13 中显示的顺序包含在 SEQ ID NO:15 内）。

[0404] VRC-1 IgG 的 IgE1 信号肽 -MDWTWILFLVAAATRVHS(SEQ ID NO:8)。

[0405] VRC01 IgG 的可变重区 -QVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMG WLKPRGGAVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSPSTKG (SEQ ID NO:9)。

[0406] VRC01 IgG 的恒定重区 1(CH1)-PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSC(SEQ ID NO:10)。

[0407] VRC01 IgG 的铰链区 EPKSCDKT HTCPPCP(SEQ ID NO:11)。

[0408] VRC01 IgG 的恒定重区 2(CH2)-APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK(SEQ ID NO:12)。

[0409] VRC01 IgG 的恒定重区 3(CH3)-GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:13)

[0410] VRC01 IgG 的费林蛋白酶切割位点 -RGRKRRS(SEQ ID NO:14)。

[0411] VRC01 IgG 的 GSG 接头和 P2A 肽 -GSGATNFSLLKQAGDVEENPGP(SEQ ID NO:15)。

[0412] VRC01 IgG 的 IgE1 信号肽 -MDWTWILFLVAAATRVHS(SEQ ID NO:8)。

[0413] VRC01 IgG 的可变轻区 (VL)-EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLV IYSGSTRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKR(SEQ ID NO:16)。

[0414] VRC01 IgG 的恒定轻区 (CL，κ)-TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGEC(SEQ ID NO:17)。

[0415] 实施例 9

[0416] 由两个质粒编码的 HIV-1 VRC01 IgG

[0417] 如上文实施例 2-8 中所描述的，从 VRC01 抗体产生 Fab（从单独的质粒表达的每一条链），即 HIV-Env Fab，并且从 VRC01 抗体产生 IgG（从单个质粒表达），即 VRC01 IgG。为了进一步扩展这些研究，从 VRC01 抗体产生 IgG，其中重链（即，可变重区 (VH)、恒定重区 1(CH1)、铰链区、恒定重区 2(CH2) 和恒定重区 3(CH3)）以及轻链（即，可变轻区 (VL) 和恒定轻区 (CL)）由单独的构建体编码（图 50 和 51）。该 IgG 在本文中被称为 HIV-1 VRC01 IgG。

[0418] 每一个构建体还包括前导序列以用于一旦抗体在体内产生就优化抗体的分泌。将每一个构建体克隆进 pVAX1 载体的 BamHI 和 XhoI 位点，从而将构建体置于巨细胞病毒 (CMV) 启动子的控制下（图 50 和 51）。因此，为了在体内形成或产生 VRC01 IgG，必须向受试者施用质粒的混合物，即含有编码重链的构建体的质粒和含有编码轻链的构建体的质粒。

[0419] 此外，进一步优化每一个构建体。优化包括 kozak 序列 (GCC ACC) 的添加和密码子优化。编码 HIV-1 VRC01 IgG 的 IgG1 重链的核酸序列示于 SEQ ID NO:54 和图 52 中。在图 52 中，加以下划线和加以双下划线标示用于将核酸序列克隆进 pVAX1 载体的 BamHI (GGA TCC) 和 XhoI(CTC GAG) 限制性酶位点，而粗体标示起始 (ATG) 和终止 (TGA TAA) 密码子。

SEQ ID NO:54 编码 SEQ ID NO:55 和图 53 中所示的氨基酸序列，即 HIV-1 VRC01 IgG 的 IgG1 重链的氨基酸序列。

[0420] 编码 HIV-1 VRC01 IgG 的 IgG 轻链的核酸序列示于 SEQ ID NO:56 和图 54 中。在图 54 中，加以下划线和加以双下划线标示用于将核酸序列克隆进 pVAX1 载体的 BamHI(GGA TCC) 和 XhoI(CTC GAG) 限制性酶位点，而粗体标示起始 (ATG) 和终止 (TGA TAA) 密码子。SEQ ID NO:56 编码 SEQ ID NO:57 和图 55 中所示的氨基酸序列，即 HIV-1 VRC01 IgG 的 IgG 轻链的氨基酸序列。

[0421] 实施例 10

[0422] HIV-1 Env-PG9 Ig

[0423] 除了 VRC01 IgG 以外，还可产生编码与 HIV-1 Env 反应的 IgG 的另一个构建体。该构建体是已被优化并且被克隆进表达载体的 HIV-1 Env-PG9（图 16A 和 16B）。优化包括引入 kozak 序列（例如，GCC ACC）、前导序列的引入和密码子优化。含有编码 HIV-1 Env-PG9 Ig 的核酸序列的表达载体的产生通过如图 16C 中显示的限制性酶消化来确认。在图 16C 中，泳道 1 是未消化的表达载体，泳道 2 是用 BamHI 和 Xho1 消化的表达载体，而泳道 M 是标记。

[0424] 编码 HIV-1 Env-PG9 Ig 的核酸序列示于 SEQ ID NO:63 和图 61 中。在图 61 中，加以下划线和加以双下划线标示用于将核酸序列克隆进 pVAX1 载体的 BamHI(GGA TCC) 和 XhoI(CTC GAG) 限制性酶位点，而粗体标示起点 (ATG) 和终止 (TGA TAA) 密码子。SEQ ID NO:63 编码 SEQ ID NO:2 和图 18 中所示的氨基酸序列，即 HIV-1 ENv-PG9 Ig 的氨基酸序列（在被费林蛋白酶切割之前）。

[0425] 在该氨基酸序列中，信号肽通过肽键连接至每一条重链和轻链以增加体内产生的抗体的分泌。此外，编码 P2A 肽的核酸序列位于编码重链与轻链的核酸序列之间，以允许将翻译的多肽更高效地切割成单独的含有重链或轻链的多肽。

[0426] 具体地，HIV-1 Env-PG9 Ig 的氨基酸序列（在被费林蛋白酶切割之前；SEQ ID NO:2 和图 18）具有下列结构：人 IgG 重链信号肽、可变重区 (VH)、恒定重区 1(CH1)、铰链区、恒定重区 2(CH2)、恒定重区 3(CH3)、费林蛋白酶切割位点、GSG 接头、P2A 肽、人 λ 轻链信号肽、可变轻区 (VL) 和恒定轻区 (CL，具体地 λ)。下文中提供了所述结构的每一个部分的序列（其全部序列以上述顺序包含在 SEQ ID NO:2 内）。

[0427] HIV-1 Env-PG9 Ig 的人 IgG 重链信号肽 -MDWTWRILFLVAAATGTHA(SEQ ID NO:18)。

[0428] HIV-1 Env-PG9 Ig 的可变重区 -EFGLSWVFLVAFLRGVQCQRLVESGGGVVQPGSSLRLSCAASGFDFSRQGMHWVRQAPGQGLEWVAFIKYDGSEKYHADSVWGRLSISRDNSKDTLYLQMNSLRVEDTATYFCVREAGGPDYRNGYNYYDFYDGYYNYHYMDVWGKGTTVTVSS(SEQ ID NO:19)。

[0429] HIV-1 Env-PG9 Ig 的恒定重区 1(CH1)-ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV(SEQ ID NO:20)。

[0430] HIV-1 Env-PG9 Ig 的铰链区 -EPKSCDKTHTCPPCP(SEQ ID NO:21)。

[0431] HIV-1 Env-PG9 Ig 的恒定重区 2(CH2)-APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK(SEQ ID NO:22)。

[0432] HIV-1 Env-PG9 Ig 的恒定重区 3(CH3) - GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:23)。

[0433] HIV-1 Env-PG9 Ig 的费林蛋白酶切割位点 - RGRKRRS(SEQ ID NO:24)。

[0434] HIV-1 Env-PG9 Ig 的 GSG 接头和 P2A 肽 - GSGATNFSLLKQAGDVEENPGP(SEQ ID NO:25)。

[0435] HIV-1 Env-PG9 Ig的人 λ 轻链信号肽 - MAWTPLFLFLLTCCPGGSNS(SEQ ID NO:26)。

[0436] HIV-1 Env-PG9 Ig的可变轻区 (VL) - QSALTQPASVSGSPGQSITISCNGTSNDVGGYESVSWYQQHPGKAPKVVIYDVSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEGDYYCKSLTSTRRRVFGTGTKLTVL(SEQ ID NO:27)。

[0437] HIV-1 Env-PG9 Ig 的恒定轻区 (CL, λ) - GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS(SEQ ID NO:28)。

[0438] 实施例 11

[0439] HIV-1 PG9 单链 Fab(scFab)

[0440] 除了上述HIV-1 Env-PG9 Ig以外,还可基于PG9抗体(在本文中被称为HIV-1 PG9 scFab) 产生单链 Fab(即,由被转录成单个转录物并被翻译成单个多肽的核酸序列编码的 VH/CH1 和 VL/CL)。编码 HIV-1 PG9 scFab 的核酸序列示于 SEQ ID NO:50 和图 46 中。在图 46 中,加以下划线和加以双下划线标示用于将该酸序列克隆进 pVAX1 载体的 BamHI(GGA TCC) 和 XhoI(CTC GAG),而粗体标示起始 (ATG) 和终止 (TGA TAA) 密码子。SEQ ID NO:50 中所示的核酸序列是优化的核酸序列,即包含 kozak 序列 (GCC ACC)、密码子优化和前导序列。前导序列位于构建体的 5' 末端,即在单链 Fab 之前,从而由接头序列编码的信号肽通过肽键连接于单链 Fab 的氨基末端。SEQ ID NO:50 中所示的核酸序列还包括位于编码 VH/CH1 的核酸序列与编码 VL/CL 的核酸序列之间的接头序列。因此,在由 SEQ ID NO:50 编码的多肽中,由接头序列编码的氨基酸序列使 VH/CH1 和 VL/CL 保持在一起。SEQ ID NO:50 编码 SEQ ID NO:51 和图 47 中所示的氨基酸序列,即 HIV-1 PG9 scFab 的氨基酸序列。

[0441] 实施例 12

[0442] HIV-1 Env-4E10 Ig

[0443] 除了 VRC01 IgG 和 HIV-1 Env-PG9 Ig 以外,还产生了编码与 HIV-1Env 反应的 IgG 的另一种构建体。该构建体是 HIV-1 Env-4E10,其已被优化并且被插入表达载体(图 17A 和 17B)。优化包括引入 kozak 序列 (例如,GCC ACC)、前导序列和密码优化。含有编码 HIV-1 Env-4E10 Ig 的核酸序列的表达载体的产生通过如图 17C 中显示的限制性酶消化来确认。在图 17C 中,泳道 1 是未被消化的表达载体,泳道 2 是用 BamHI 和 Xho1 消化的表达载体,且泳道 M 是标记。

[0444] 编码 HIV-1 Env-4E10 Ig 的核酸序列示于 SEQ ID NO:62 和图 60 中。在图 60 中,加以下划线和加以双下划线标示用于将核酸序列克隆进 pVAX1 载体的 BamHI(GGA TCC) 和 XhoI(CTC GAG) 限制性酶位点,而粗体标示起始 (ATG) 和终止 (TGA TAA) 密码子。SEQ ID NO:62 编码 SEQ ID NO:1 和图 19 中所示的氨基酸序列,即,HIV-1 ENv-4E10 Ig 的氨基酸序列 (在被费林蛋白酶切割前)。

[0445] 在该氨基酸序列中，信号肽通过肽键连接于重链和轻链的每一个以增加体内产生的抗体的分泌。此外，编码 P2A 肽的核酸序列位于编码重链与轻链的核酸序列之间，以允许翻译的多肽更高效地切割成含有重链或轻链的单独多肽。

[0446] 具体地，HIV-1 Env-4E10 Ig 的氨基酸序列（在被费林蛋白酶切割之前；SEQ ID NO:1 和图 19）具有下列结构：人 IgG 重链信号肽、可变重区 (VH)、恒定重区 1(CH1)、铰链区、恒定重区 2(CH2)、恒定重区 3(CH3)、费林蛋白酶切割位点、GSG 接头、P2A 肽、人 κ 轻链信号肽、可变轻区 (VL) 和恒定轻区 (CL，具体地 κ）。在下文中提供了所述结构的每一个部分的序列（其全部序列以上述顺序包含在 SEQ ID NO:1 中）。

[0447] HIV-1 Env-4E10 Ig 的人 IgG 重链信号肽 -MDWTWRILFLVAAATGTHA(SEQ ID NO:29)。

[0448] HIV-1 Env-4E10 Ig的可变重区 -QVQLVQSGAEVKRPGSSVTVSCKASGGSFSTYALSWVRQAPGRGLEWMGGVIPLLTITNYAPRFQGRITITADRSTSTAYLELNSLRPEDTAVYYCAREGTTGWGWLGKPIGAFAHWGQGTLVTVSS(SEQ ID NO:30)。

[0449] HIV-1 Env-4E10 Ig 的恒定重区 1(CH1)-ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV(SEQ ID NO:31)。

[0450] HIV-1 Env-4E10 Ig 的铰链区 -EPKSCDKTHTCPPCP(SEQ ID NO:32)。

[0451] HIV-1 Env-4E10 Ig的恒定重区 2(CH2)-APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK(SEQ ID NO:33)。

[0452] HIV-1 Env-4E10 Ig的恒定重区 3(CH3)-GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO:34)。

[0453] HIV-1 Env-4E10 Ig 的费林蛋白酶切割位点 -RGRKRRS(SEQ ID NO:35)。

[0454] HIV-1 Env-4E10 Ig 的 GSG 接头和 P2A 肽 -GSGATNFSLLKQAGDVEENPGP(SEQ ID NO:36)。

[0455] HIV-1 Env-4E10 Ig 的人 κ 轻链信号肽 -MVLQTQVFISLLLWISGAYG(SEQ ID NO:37)。

[0456] HIV-1 Env-4E10 Ig的可变轻区 (VL)-EIVLTQSPGTQSLSPGERATLSCRASQSVGNNKLAWYQQRPGQAPRLLIYGASSRPSGVADRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGQSLSTFGQGTKVE(SEQ ID NO:38)。

[0457] HIV-1 Env-4E10 Ig 的恒定轻区 (CL，κ)-KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE(SEQ ID NO:39)。

[0458] 实施例 13

[0459] HIV-1 4E10 ScFab

[0460] 除了上述 HIV-1 Env-PG9 Ig 以外，基于 4E10 抗体（在本文中被称为 HIV-1 4E10 scFab）产生单链 Fab（即，由被转录成单个转录物并被翻译成单个多肽的核酸序列编码的 VH/CH1 和 VL/CL）。编码 HIV-1 4E10 scFab 的核酸序列示于 SEQ ID NO:52 和图 48 中。在图

48 中，加以下划线和加以双下划线标示用于将该核酸序列克隆进 pVAX1 载体的 BamHI(GGA TCC) 和 XhoI(CTC GAG)，而粗体标示起始 (ATG) 和终止 (TGA TAA) 密码子。SEQ ID NO:52 中所示的核酸序列是优化的核酸序列，即包含 kozak 序列 (GCC ACC)、密码子优化和前导序列。前导序列位于构建体的 5’末端，即在单链 Fab 之前，从而由接头序列编码的信号肽通过肽键连接于单链 Fab 的氨基末端。SEQ ID NO:52 中所示的核酸序列还包括位于编码 VH/CH1 的核酸序列与编码 VL/CL 的核酸序列之间的接头序列。因此，在由 SEQ ID NO:52 编码的多肽中，由接头序列编码的氨基酸序列使 VH/CH1 和 VL/CL 保持在一起。SEQ ID NO:52 编码 SEQ ID NO:53 和图 49 中所示的氨基酸序列，即 HIV-1 4E10 scFab 的氨基酸序列。

[0461] 实施例 14

[0462] CHIKV-Env-Fab

[0463] 如上所述，在编码 HIV-1Env Fab 的重链 (VH-CH1) 和轻链 (VL-CL) 的核酸序列被递送至细胞或小鼠后，在体内装配或产生与 HIV-1 Env 反应的 Fab。为了确定与其它抗原反应的 Fab 是否可在编码核酸序列被递送至细胞或受试者后在体内产生，产生编码与基孔肯雅热病毒 (CHIKV) 的包膜蛋白 (Env) 反应的抗体的重链 (VH-CH1) 和轻链 (VL-CL，λ 型) 的构建体。每一个构建体包括如图 20A、20B 和 21 中显示的前导序列和 kozak 序列。将编码 VH-CH1 和 VL-CL 的构建体克隆进表达载体，从而将其置于巨细胞病毒 (CMV) 启动子的控制之下 (图 21)。含有编码 VH-CH1 和 VL-CL 的构建体的表达载体分别被称为 CHIKV-H 和 CHIV-L。综上所述，CHIKV-H 和 CHIKV-L 载体的混合物被称为 pCHIKV-Env-Fab，并且该混合物在体内 (即，在引入细胞或受试者后) 产生 CHIKV-Env-Fab。换句话说，需要两种载体来在体内产生 CHIKV-Env-Fab，如下文中更详细描述的。

[0464] 所述构建体也被优化以用于表达。具体地，将前导序列包含在每一个构建体中以在体内产生 CHIKV-Env-Fab 后提高 CHIKV-Env-Fab 的分泌效率。也对每一个构建体进行密码子优化，并且使其包含 kozak 序列 (GCC ACC)。编码 CHIKV-Env-Fab 的重链 (VH-CH1) 的核酸序列示于 SEQ ID NO:58 和图 56 中。在图 56 中，加以下划线和加以双下划线标示用于将核酸序列克隆进 pVAX1 载体的 BamHI(GGA TCC) 和 XhoI(CTC GAG) 限制性酶位点，而粗体标示起始 (ATG) 和终止 (TGA TAA) 密码子。SEQ ID NO:58 编码 SEQ ID NO:59 和图 57 中所示的氨基酸序列，即 CHIKV-Env-Fab 的重链 (VH-CH1) 的氨基酸序列。

[0465] 编码 CHIKV-Env-Fab 的轻链 (VL-CL) 的核酸序列示于 SEQ ID NO:60 和图 58 中。在图 58 中，加以下划线和加以双下划线标示用于将核酸序列克隆进 pVAX1 载体的 BamHI(GGA TCC) 和 XhoI(CTC GAG) 限制性酶位点，而粗体标示起始 (ATG) 和终止 (TGA TAA) 密码子。SEQ ID NO:60 编码 SEQ ID NO:61 和图 59 中所示的氨基酸序列，即 CHIKV-Env-Fab 的轻链 (VL-CL) 的氨基酸序列。

[0466] 为了测量 CHIKV-Env-Fab 体内产生的时间动力学，用 pVAX1、CHIKV-H、CHIKV-L 或 pCHIKV-Env-Fab 转染细胞。转染后，将 ELISA 用于测量随时间 CHIKV-Env-Fab 产生的水平。如图 22 中所示，用 pVAX1、CHIKV-H 或 CHIKV-L 转染的细胞不产生与 CHIKV Env 抗原反应的抗体。相反地，用 pCHIKV-Env-Fab 转染的细胞产生与 CHIKV Env 抗原反应的抗体 (即，CHIKV-Env-Fab，也被称为 CHIKV-Fab)。因此，这些数据表明编码 CHIKV-Env-Fab 的重链 (VH-CH1) 和轻链 (VL-CL) 的核酸序列的递送导致结合于 CHIKV-Env 抗原或与其反应的 Fab 的产生。

[0467] 此外，将 CHIKV-Env-Fab 用于获自用 pCHIKV-Env 转染的细胞的裂解物的蛋白质印迹，所述 pCHIKV-Env 是编码 CHIKV-Env 抗原的质粒。如图 23 中所示，经由 CHIKV-Env-Fab 检测到 CHIKV-Env 抗原，这表明该 Fab 结合于所述抗原。

[0468] 为了进一步研究 CHIKV-Env-Fab 在体内的产生或装配，向小鼠施用 pCHIKV-Env-Fab（即，12.5μg CHIKV-H 和 12.5μg CHIKV-L）。此外，向第 2、第 3 和第 4 组小鼠分别施用 25μg pVAX1、CHIKV-H 和 CHIKV-L，并将其用作对照。具体地，在获得前血样品的第 0 天，向各自组的小鼠施用所述质粒。在第 1 天、第 2 天、第 3 天、第 5 天、第 7 天和第 10 取血（图 24）。对这些血液进行 ELISA 测量以测定与 CHIKV-Env 抗原反应的抗体的水平。如图 25 中所示，施以 pCHIKV-Env-Fab 的小鼠导致与 CHIKV-Env 抗原反应的抗体（即，CHIKV-Env-Fab）的产生。施以 pVAX1、CHIKV-H 或 CHIKV-L 的小鼠不产生与 CHIKV-Env 抗原具有显著反应性的抗体。因此，这些数据还显示在递送编码 CHIKV-Env-Fab 的重（VH-CH1）链和轻（VL-CL）链的核酸序列后，在体内（即，在小鼠）中产生该 Fab，并且所述 Fab 与其抗原（即，CHIKV-Env）反应，从而证明所述 Fab 在体内被正确装配。

[0469] 为了确定 CHIKV-Env-Fab 是否可保护免受 CHIKV 感染，在第 0 天向 C57BL/6 小鼠（2-3 周龄；体重约 20-25 克）施用 pCHIKV-Env-Fab（50μg）或 pVAX1。施用 pCHIKV-Env-Fab 后 6 小时，通过鼻内途径用 7log 10PFU（于 25μl 的总体积中）接种每一只小鼠。在随后的每一天，测定每一只小鼠的体重，并且如果体重减轻超过 30％则处死小鼠。

[0470] 如图 26 中所示，约 75％的施以 pCHIKV-Env-Fab 的小鼠到研究的第 14 天为止幸免于 CHIKV 感染，然而到第 14 天，所有施以 pVAX1 的小鼠死亡。此外，施以 pCHIKV-Env-Fab 的小鼠相较于施以 pVAX1 的小鼠与更低的细胞因子水平相关（图 27 和 28）。测量获自小鼠的血清的 TNF-α 和 IL-6 水平。这些存活小鼠未表现病变体征、体重减轻，并且具有更低水平的细胞因子 TNF-α 和 IL-6。因此，这些数据表明 pCHIKV-Env-Fab 的施用保护小鼠免受 CHIKV 感染并且提高 CHIKV 感染的存活率。换句话说，CHIKV-Env-Fab 在小鼠中的体内产生保护免受 CHIKV 感染和提高所述感染的存活率。

[0471] 实施例 15

[0472] 抗 -Her-2 Fab

[0473] 如上所述，在编码 HIV-1Env Fab 或 CHIKV Env-Fab 的重（VH-CH1）链和轻（VL-CL）链的核酸序列被递送至细胞或小鼠后，在体内装配或产生与 HIV-1 Env 或 CHIKV Env 反应的 Fab（即，VH/CH1 和 VL/CL）。为了确定与自体抗原（即，对于待施用编码所述 Fab 的核酸序列的受试者是内源的抗原）反应的 Fab 是否可在编码核酸序列被递送至细胞或受试者后体内产生，创建了编码与人表皮生长因子受体 2（Her-2；也称为 Erb2）反应的抗体的重链（VH-CH1）和轻链（VL-CL，κ 型）的构建体。每一个构建体包括前导序列和 kozak 序列（GCC ACC），所述 kozak 序列位于编码图 28、30 和 31 中显示的抗 -Her-2 Fab 的 VH-CH1 或 VL-CL 的核酸序列之前。因此，这些构建体因前导序列和 kozak 序列的引入而被优化，并且还对其密码子使用进行优化。

[0474] 将编码 VH-CH1 和 VL-CL 的构建体克隆进 pVAX1 表达载体，即在 BamHI 与 XhoI 限制性位点之间，从而将其置于巨细胞病毒（CMV）启动子的控制之下。具体地，将编码 VH-CH1 和 VL-CL 的构建体克隆进两个单独的 pVAX1 载体，从而需要所得的两个质粒来在体内产生抗 -Her-2 Fab。

[0475] 编码抗 -Her-2 Fab 的 VH-CH1 的核酸序列示于 SEQ ID NO:40 和图 32 中。在图 32 中，加以下划线和加以双下划线标示用于将核酸序列克隆进 pVAX1 载体的 BamHI(GGA TCC) 和 XhoI(CTC GAG) 限制性酶位点，而粗体标示起始 (ATG) 和终止 (TGA TAA) 密码子。SEQ ID NO:40 编码 SEQ ID NO:41 中所示的氨基酸序列，即抗 -Her-2 Fab 的 VH-CH1 的氨基酸序列 (图 32 和 33)。

[0476] 编码抗 -Her-2 Fab 的 VL-CL 的核酸序列示于 SEQ ID NO:42 和图 34 中。在图 34 中，加以下划线和加以双下划线标示用于将核酸序列克隆进 pVAX1 载体的 BamHI(GGA TCC) 和 Xho(CTC GAG) 限制性酶位点，而粗体标示起始 (ATG) 和终止 (TGA TAA) 密码子。SEQ ID NO:42 编码 SEQ ID NO:43 中所示的氨基酸序列，即抗 -Her-2 Fab 的 VL-CL 的氨基酸序列 (图 34 和 35)。

[0477] 为了确定编码抗 -Her-2 Fab 的 VH-CH1 和 VL-CL 的质粒的混合物是否在体内产生抗 -Her-2 Fab，用编码抗 -Her-2 Fab 或 pVAX1 的重链 (VH-CH1) 和轻链 (VL 和 CL) 的质粒的混合物转染 293T 细胞。转染后，如图 36 中所示，测量总的 IgG 浓度。在图 36 中，误差条代表标准偏差。这些数据表明在引入所述两种质粒 (各自编码抗 -Her-2 Fab 的 VH-CH1 或 VL-CL) 后，在体内产生抗 -Her-2 Fab。

[0478] 实施例 16

[0479] 抗 - 登革热病毒人 IgG

[0480] 创建单质粒系统以在体内产生抗 - 登革热病毒 (DENV) 人 IgG 抗体。具体地，如图 37 的示意图中所示，产生构建体。具体地，将前导序列置于编码 IgG 重链 (即，可变重区 (VH)、恒定重区 1(CH1)、铰链区、恒定重区 2(CH2) 和恒定重区 3(CH3)) 的核酸序列的上游。反过来，编码蛋白酶切割位点的序列被置于编码 IgG 重链的核酸序列的下游。编码 IgG 轻链 (即，可变轻区 (VL) 和恒定轻区 (CL)) 的核酸序列位于编码蛋白酶切割位点 (即，费林蛋白酶切割位点) 的序列之后。由该构建体编码的信号肽是同源信号肽，从而在表达后提供了正确的抗体分泌。此外，在表达后，单个转录物被翻译成单个多肽，随后所述多肽被蛋白酶加工成对应于抗 -DENV 人 IgG 的重链和轻链的多肽。这些重链和轻链多肽随后装配成功能性抗 -DENV 人 IgG，即结合其同源抗原的抗体。

[0481] 将该构建体克隆进表达载体 pVAX1(即 BamHI 和 XhoI 位点)，从而将其置于启动子的控制之下。该编码抗 - 登革热病毒人 IgG 的构建体具有 SEQ ID NO:44(图 38) 中所示的核酸序列，所述核酸序列已被优化以用于表达。在图 38 中，加以下划线和加以双下划线标示用于将构建体克隆进 pVAX1 载体的 BamHI(GGA TCC) 和 XhoI(CTC GAG) 限制性酶位点，而粗体标示起始 (ATG) 和终止 (TGA TAA) 密码子。优化包括包含 kozak 序列 (例如，GCC ACC) 和密码子优化。SEQ ID NO:44 编码 SEQ ID NO:45 和图 39 中所示的氨基酸序列，即在被蛋白酶切割以将重链和轻链分离成 2 个单独的多肽之前的抗 -DENV 人 IgG 的氨基酸序列。

[0482] 向小鼠施用含有编码抗 - 登革热病毒人 IgG 的核酸序列的质粒以确定抗 - 登革热病毒人 IgG 是否在体内 (即，在小鼠中) 产生。在施用质粒后，从小鼠获取血清，并经由 ELISA 进行分析以确定所述血清是否含有与来自 4 种登革热病毒血清型即 DENV-1、DENV-2、DENV-3 和 DENV-4 的登革热 E 蛋白反应的抗体。如图 40 中所显示，来自施以含有编码抗 -DENV 人 IgG 的核酸序列的质粒的小鼠的血清与来自血清型 DENV-1、2、3 和 4 的 DENVE 蛋白反应。同种型抗体用作阳性对照。因此，这些数据表明在将所述质粒引入小鼠后，编码

抗 -DENV 人 IgG 的核酸序列被转录并被翻译成多肽，所述多肽经加工产生含有抗 -DENV 人 IgG 的重链和轻链。这些多肽装配成抗 -DENV 人 IgG，从而提供了结合于 DENV E 蛋白或与其反应的功能性抗体。

[0483] 为了进一步研究通过施用单个质粒进行的抗 -DENV 人 IgG 的体内产生，经由注射向小鼠施用含有编码抗 -DENV 人 IgG 的核酸序列的质粒。具体地，向小鼠施用 50μg 或 100μg 质粒，并且每组有 5 只小鼠。在注射后第 3 天和第 6 天，检测小鼠的血清转换。如图 41 中所示，来自两个组的小鼠对于抗 -DENV IgG 抗体都是血清阳性的。具体地，施以 50μg 质粒的小鼠具有约 110ng/mL 的人 IgG，并且施用 100μg 质粒的小鼠具有约 170ng/mL 的人 IgG。因此，这些数据进一步证明在施用编码抗 -DENV 人 IgG 的质粒后，在体内产生所述抗 -DENV 人 IgG。这些数据还证明抗 -DENV 人 IgG 抗体产生在 1 周内发生，从而允许抗 -DENV 人 IgG 快速产生。

[0484] 应理解，前文详述和所附实施例仅是例举性的并且无意被视为对本发明的范围的限制，所述范围仅由所附权利要求和它们的等同物确定。

[0485] 对公开的实施方案的各种改变和改进对于本领域技术人员来说是显而易见的。可在不背离其精神和范围的情况下进行这样的改变和改进，包括但不限于与化学结构、取代基、衍生物、中间体、合成、组合物、制剂或使用本发明的方法相关的那些改变和改进。

编码IgG重链的优化核酸序列

GGATCCGCCACCATGGAAACCGACACTCTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCCGGCTCAACAGGCGACGGC
GCTCAGGTCCAGCTGGTCCAGTCTGGAGCTGTGATCAAGACCCCTGGCAGCTCCGTCAAAATTTCTTGCAGAGCAAGTG
GCTACAACTTCCGGGACTATAGCATCCACTGGGTGCGGCTGATTCCTGATAAGGGATTTGAGTGGATCGGCTGGATCAA
GCCACTGTGGGGCGCTGTGTCCTACGCAAGGCAGCTGCAGGGGCGCGTCTCCATGACACGACAGCTGTCTCAGGACCC
AGACGATCCCGATTGGGGGGTGGCCTACATGGAGTTCAGTGGACTGACTCCCGCAGACACCGCCGAATATTTTTGCGTG
CGGAGAGGCTCCTGCGACTACTGTGGGGATTTCCCATGGCAGTATTGGTGTCAGGGAACTGTGGTCGTGGTCTCTAGTG
CATCAACCAAGGGCCCCAGCGTGTTTCCTCTGGCCCCATCAAGCAAAAGTACATCAGGAGGAACTGCAGCTCTGGGAT
GTCTGGTGAAGGATTACTTCCCCGAGCCTGTGACCGTCAGCTGGAACTCCGGAGCACTGACCTCCGGAGTGCACACATT
TCCCGCTGTCCTGCAGTCCTCTGGGCTGTACTCTCTGAGTTCAGTGGTCACAGTGCCTAGCTCCTCTCTGGGCACCCAGA
CATATATCTGCAACGTCAATCATAAGCCAAGTAATACTAAAGTGGACAAGAAAGTCGAACCCAAATCATGTTACCCCT
ATGACGTGCCTGATTATGCTTGATAACTCGAG (SEQ ID NO:6)

图 1

编码IgG轻链的优化核酸序列

GGATCCGCCACCATGGAGACTGATACACTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCTGGCTCAACCGGCGACGGG
GCTCAGGTCCAGATTGTGCTGACCCAGAGCCCTGGCATCCTGTCACTGAGCCCAGGAGAGACCGCAACACTGTTCTGCA
AGGCCTCCCAGGGCGGGAACGCTATGACATGGTACCAGAAACGGAGAGGACAGGTGCCCCGACTGCTGATCTATGACA
CTTCAAGGCGAGCAAGCGGAGTGCCTGATCGATTTGTCGGCAGCGGCTCTGGGACAGACTTCTTTCTGACTATTAATAA
GCTGGACAGAGAGGATTTCGCTGTGTACTATTGCCAGCAGTTTGAATTCTTTGGACTGGGCAGCGAGCTGGAAGTGCAC
AGGACCGTCGCCGCTCCAAGTGTGTTCATTTTTCCCCCTAGCGATGAGCAGCTGAAATCCGGGACAGCCTCTGTGGTCT
GTCTGCTGAACAATTTCTACCCCCGCGAAGCAAAGGTGCAGTGGAAAGTCGACAACGCCCTGCAGAGTGGCAATTCAC
AGGAGAGCGTGACCGAACAGGACTCCAAGGATTCTACATATAGTCTGAGCTCCACTCTGACCCTGTCTAAAGCTGATTA
CGAGAAGCACAAAGTGTATGCATGCGAAGTCACTCATCAGGGCCTGTCTAGTCCTGTGACCAAGAGCTTTAACCGAGG
GGAGTGTTACCCATATGACGTCCCCGATTACGCCTGATAACTCGAG (SEQ ID NO:7)

图 2

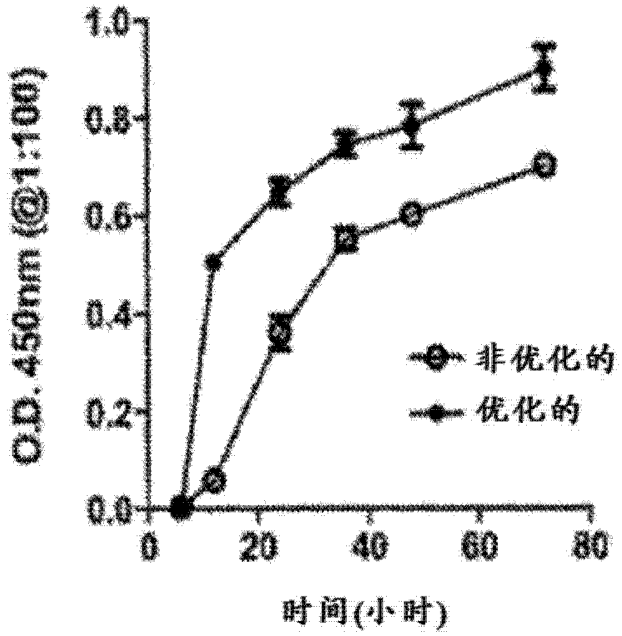

图 3

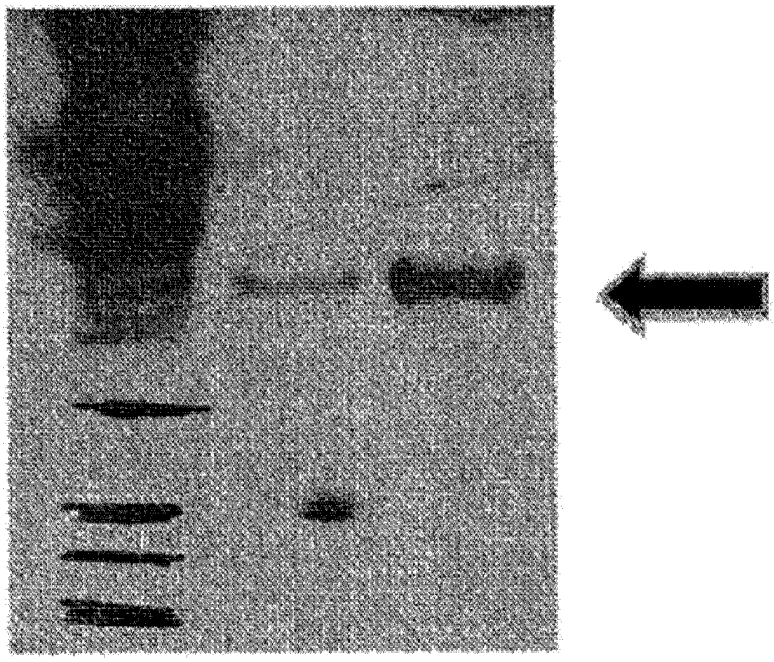

图 4

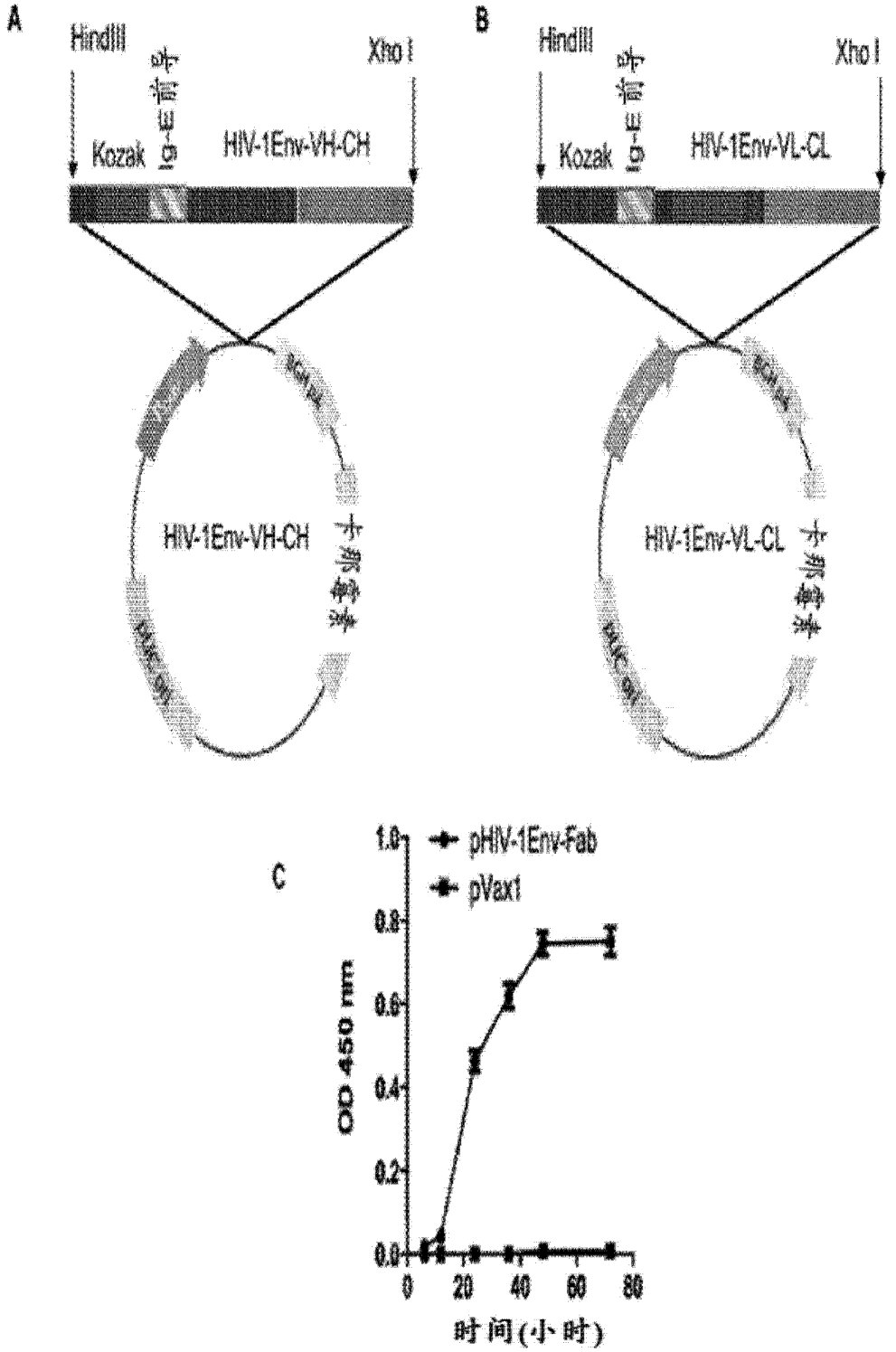
A
HindIII
Xho I
Kozak
HIV-1Env-VH-CH
HIV-1Env-VH-CH
B
HindIII
Xho I
Kozak
HIV-1Env-VL-CL
HIV-1Env-VL-CL
C
pHIV-1Env-Fab
pVax1
OD 450 nm
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
时间(小时)

图 5

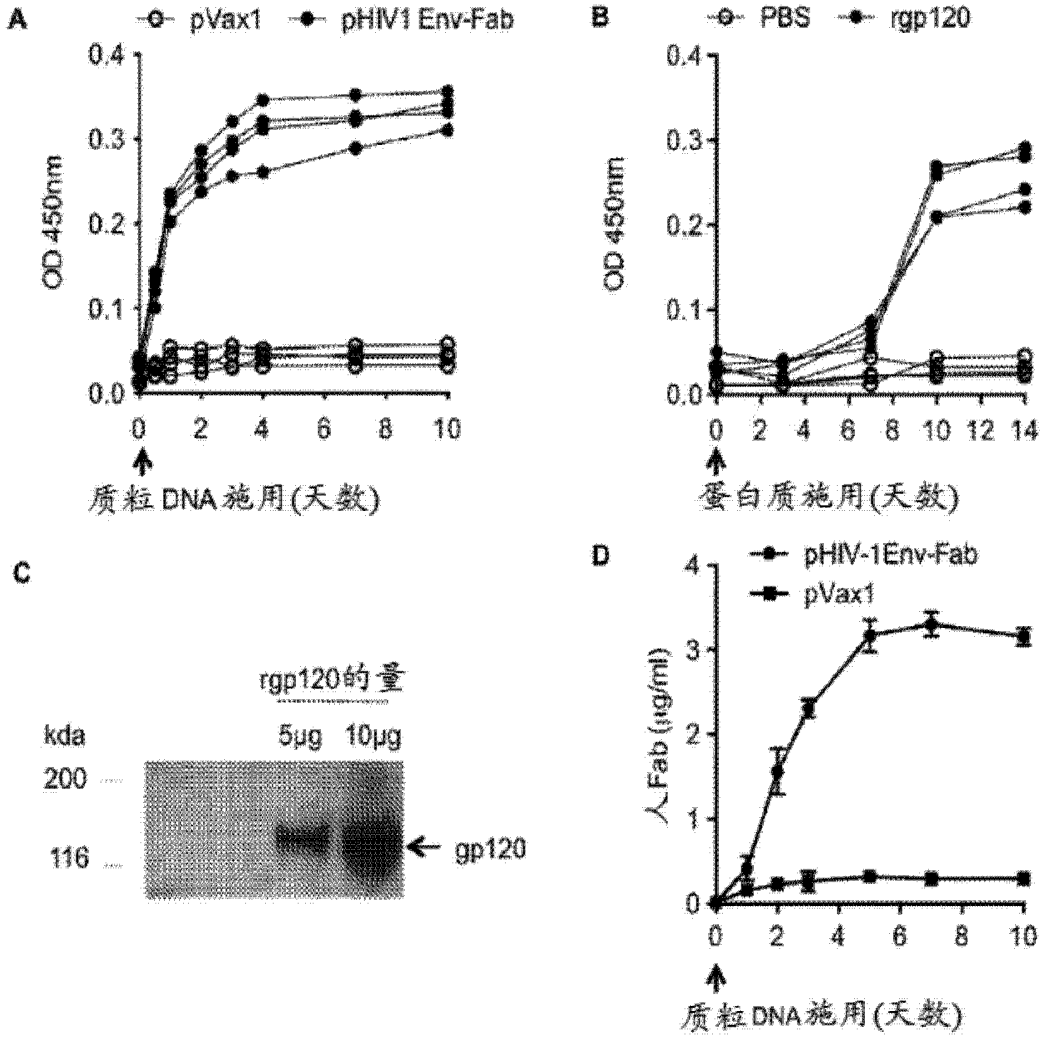
A
pVax1
pHIV1 Env-Fab
OD 450nm
0.4
0.3
0.2
0.1
0.0
0 2 4 6 8 10
质粒 DNA 施用(天数)
B
PBS
rgp120
OD 450nm
0.4
0.3
0.2
0.1
0.0
0 2 4 6 8 10 12 14
蛋白质施用(天数)
C
rgp120的量
kda
5μg 10μg
200
116
gp120
D
pHIV-1Env-Fab
pVax1
人Fab (μg/ml)
4
3
2
1
0
0 2 4 6 8 10
质粒 DNA 施用(天数)

图 6

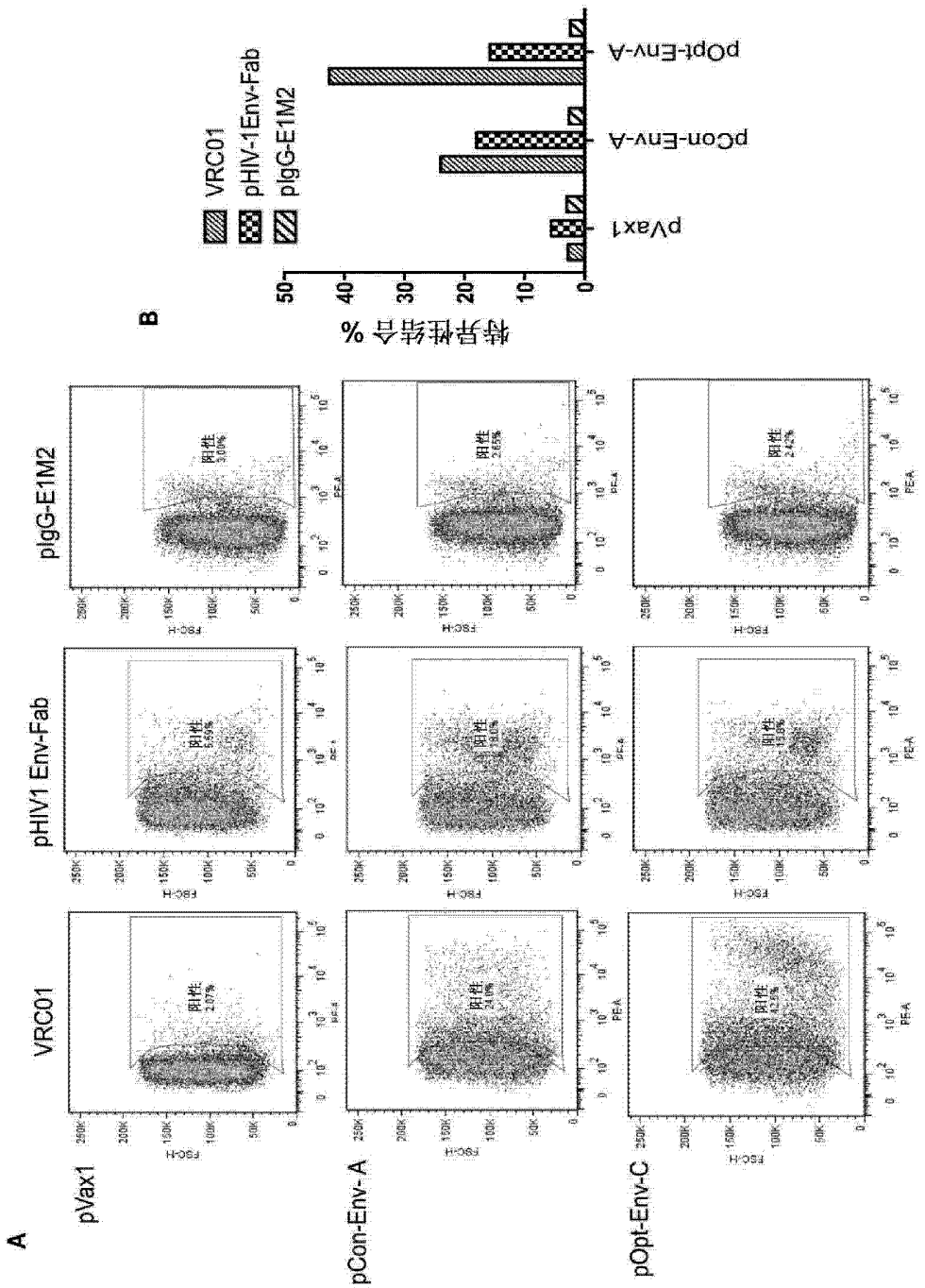
A
VRC01
pHIV1 Env-Fab
pIgG-E1M2
pVax1
pCon-Env- A
pOpt-Env-C
阳性
2.07%
阳性
5.69%
阳性
3.00%
阳性
24.0%
阳性
2.65%
阳性
43.5%
阳性
15.0%
阳性
2.42%
FSC-H
PE-A
B
特异性结合 %
0
10
20
30
40
50
pVax1
pCon-Env-A
pOpt-Env-A
VRC01
pHIV-1Env-Fab
pIgG-E1M2

图 7

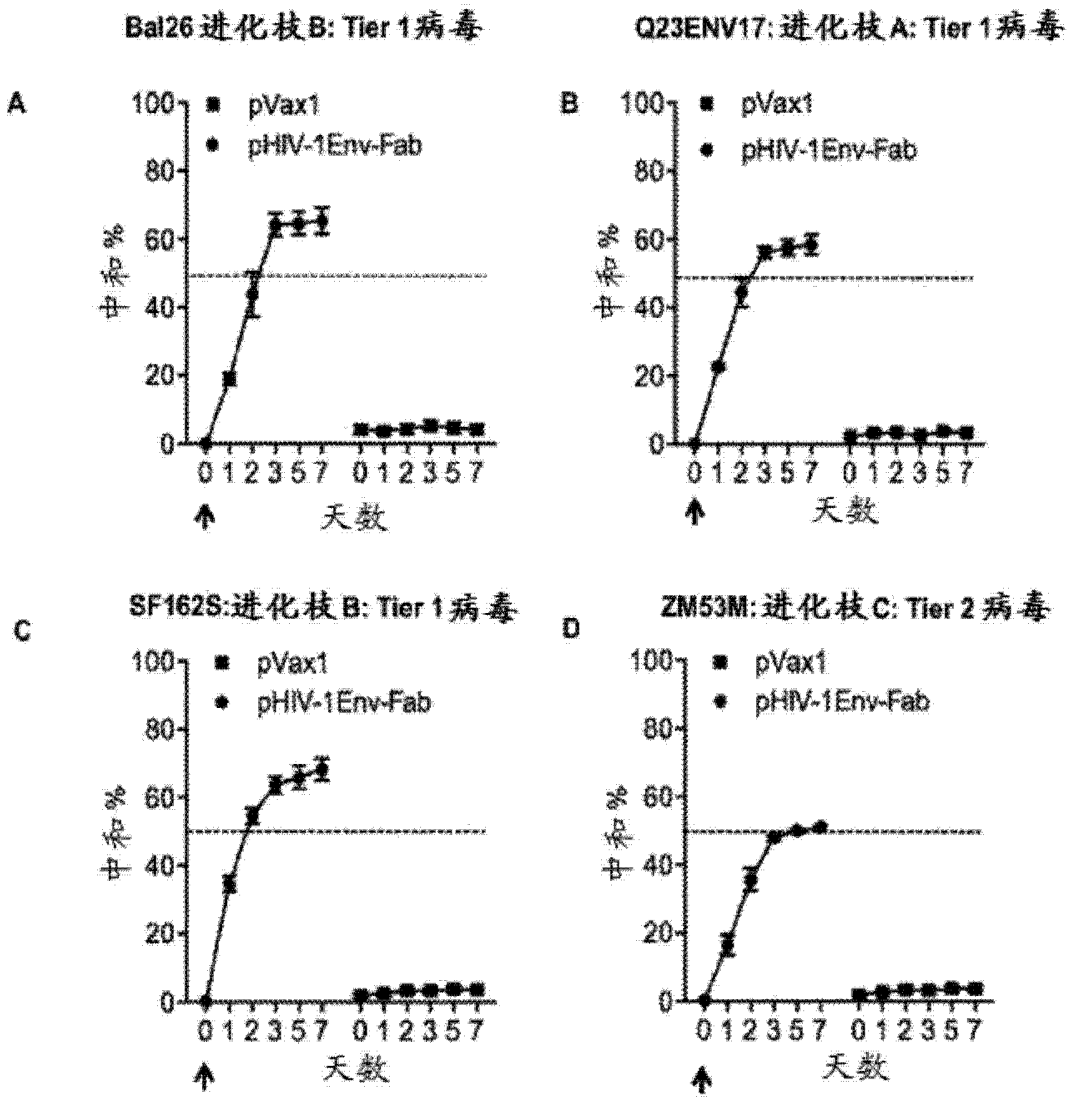
A
Bal26进化枝B: Tier 1病毒
pVax1
pHIV-1Env-Fab
中和%
天数
B
Q23ENV17:进化枝A: Tier 1病毒
C
SF162S:进化枝B: Tier 1病毒
D
ZM53M:进化枝C: Tier 2病毒

图 8

编码HIV-1 Env Fab的重链(VH-CH1)的核酸序列

AAGCTTGCCGCCACCATGGAGACTGATACACTGCTGCTGTGGGTGCTGCTGCTGTGG
GTGCCAGGGTCAACCGGAGATGGGGCTCAGGTCCAGCTGGTCCAGAGCGGCGGACA
GATGAAGAAACCCGGCGAGAGCATGAGGATCTCCTGCAGAGCATCTGGATACGAGT
TCATCGACTGTACCCTGAACTGGATTAGGCTGGCTCCTGGAAAGAGACCAGAGTGG
ATGGGGTGGCTGAAACCACGAGGGGGAGCAGTGAATTACGCCCGGCCCCTGCAGGG
ACGAGTGACCATGACCAGGGACGTGTACAGCGATACCGCCTTCCTGGAGCTGCGGT
CCCTGACAGTGGACGATACTGCTGTCTACTTCTGCACACGCGGAAAGAACTGTGACT
ATAATTGGGATTTTGAACACTGGGGCCGGGGAACACCCGTGATCGTCAGCTCCCCCA
GTACTAAGGGACCTTCAGTGTTTCCACTGGCCCCCTCTAGTAAATCCACCTCTGGAG
GGACAGCCGCTCTGGGATGCCTGGTGAAAGATTATTTCCCCGAACCTGTGACCGTCA
GTTGGAACTCAGGGGCTCTGACTTCTGGCGTGCACACCTTTCCTGCAGTCCTGCAGT
CAAGCGGGCTGTACAGTCTGTCCTCTGTGGTCACTGTGCCTAGTTCAAGCCTGGGCA
CTCAGACCTATATTTGTAACGTGAATCATAAGCCATCCAATACAAAAGTGGACAAA
AAAGCCGAACCCAAATCCTGTTACCCTTATGATGTGCCCGACTACGCCTGACTCGAG
(SEQ ID NO:3)

图 9

HIV-1 Env Fab的轻链(VL-CL)

AAGCTTGCCGCCACCATGGAAACCGATACACTGCTGCTGTGGGTGCTGCTGCTGTGG
GTGCCAGGAAGTACCGGGGATGGGGCTCAGGTCCAGATTGTGCTGACTCAGTCCCCT
GGGACCCTGTCTCTGAGTCCAGGCGAGACAGCTATCATTTCATGCCGAACTAGCCAG
TACGGCAGCCTGGCTTGGTATCAGCAGCGACCAGGACAGGCACCACGACTGGTCAT
CTACTCAGGCAGCACAAGGGCCGCTGGCATCCCCGACAGGTTCTCCGGCAGCAGGT
GGGGGCCTGATTACAACCTGACTATCTCTAATCTGGAGAGTGGGGACTTTGGCGTGT
ACTATTGCCAGCAGTATGAGTTCTTCGGCCAGGGAACTAAGGTGCAGGTGGACATC
AAAAGAACCGTGGCAGCCCCATCCGTCTTCATTTTTCCCCCTTCTGATGAGCAGCTG
AAGTCAGGCACCGCCAGCGTGGTCTGTCTGCTGAACAATTTCTACCCCCGGGAAGCC
AAGGTGCAGTGGAAAGTGGACAACGCTCTGCAGAGTGGAAATTCACAGGAGAGCGT
GACCGAACAGGACTCCAAGGATTCTACATATAGTCTGAGCAGCACCCTGACCCTGA
GTAAAGCAGATTACGAGAAGCACAAAGTGTATGCCTGTGAAGTCACACATCAGGGC
CTGAGGAGCCCCGTGACTAAAAGTTTCAACCGAGGAGAGTGCTACCCTTATGATGTG
CCCGACTACGCCTAACTCGAG (SEQ ID NO:4)

图 10

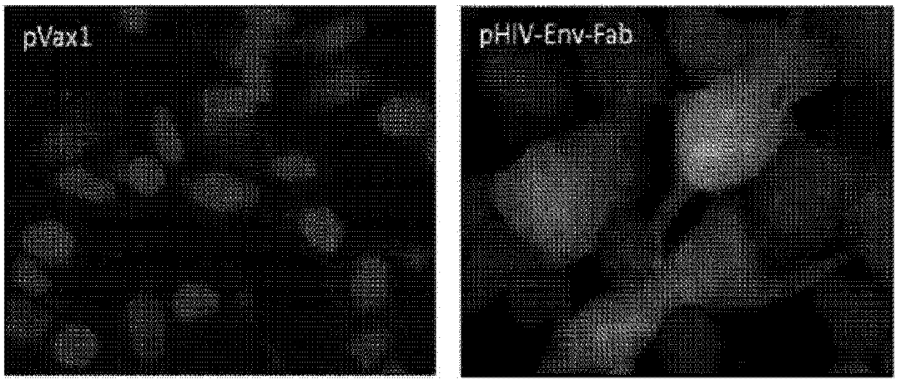
pVax1
pHIV-Env-Fab

图 11

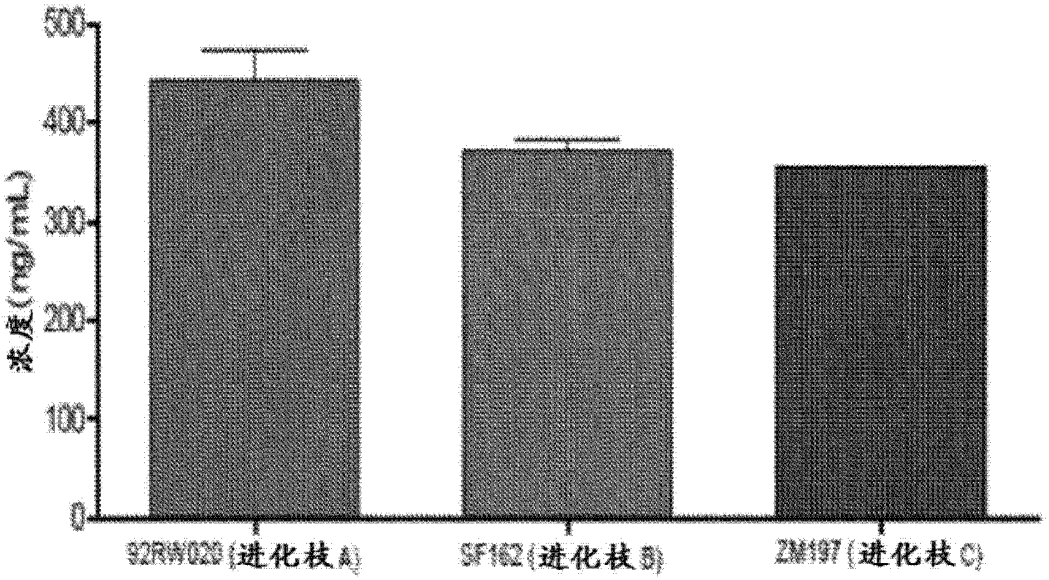
500
400
300
200
100
0
浓度(ng/mL)
92RW020(进化枝A)
SF162(进化枝B)
ZM197(进化枝C)

图 12

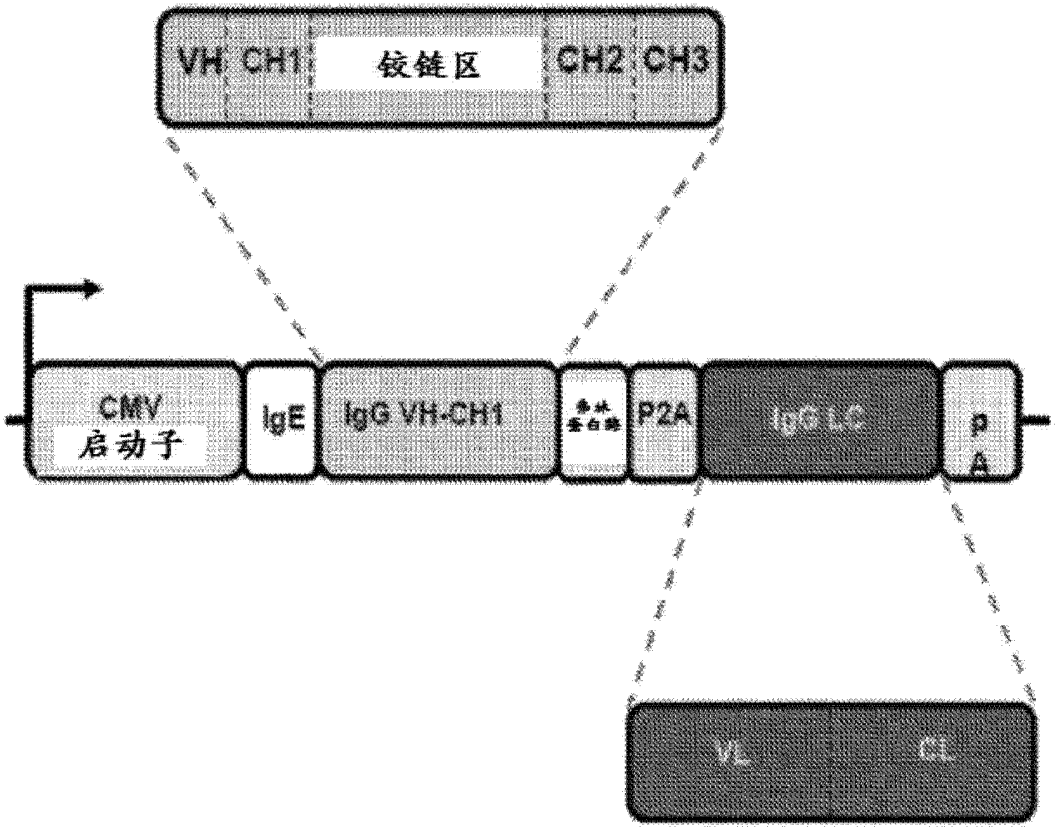

图 13

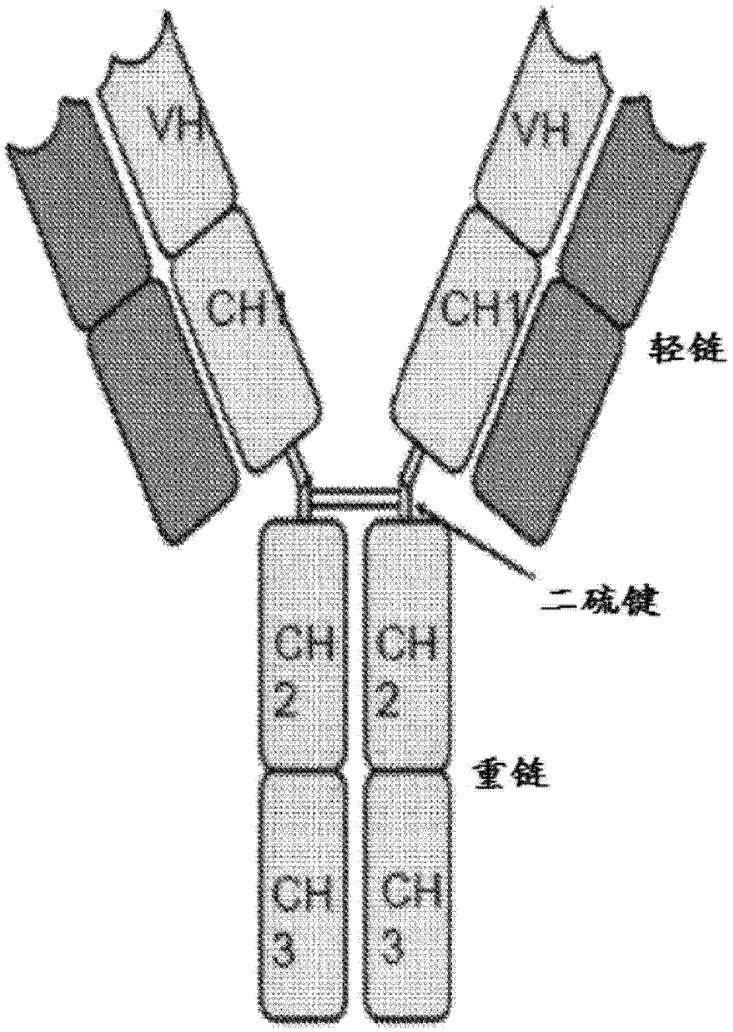

图 14

VRC01 IgG

MDWTWILFLVAAATRVHSQVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLA
PGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCT
RGKNCDYNWDFEHWGRGTPVIVSSPSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKAEPKSCEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKRGRKRRSGSGATNFSLLKQAGDVEENPGPMDWTWILFLVAAATRVHSEIVLTQ
SPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGP
DYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH
KVYACEVTHQGLRSPVTKSFNRGEC (SEQ ID NO:5)

图 15

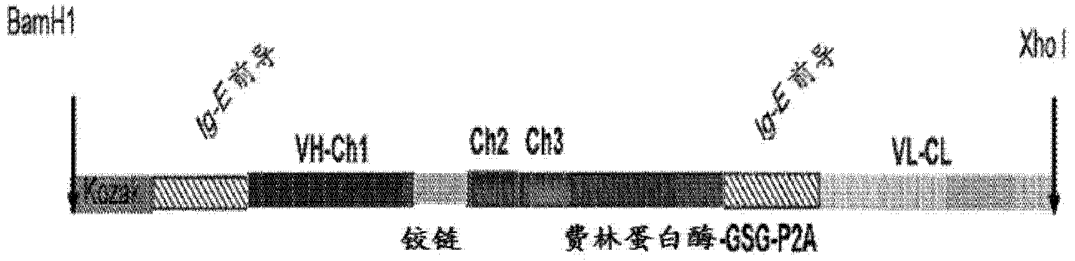

图 16A

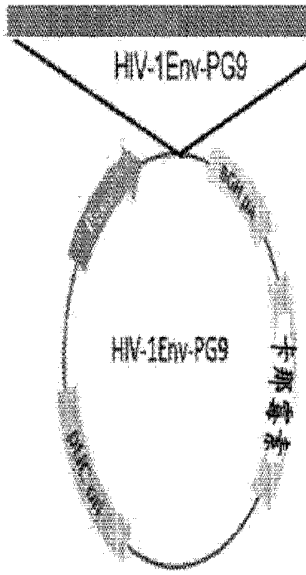

图 16B

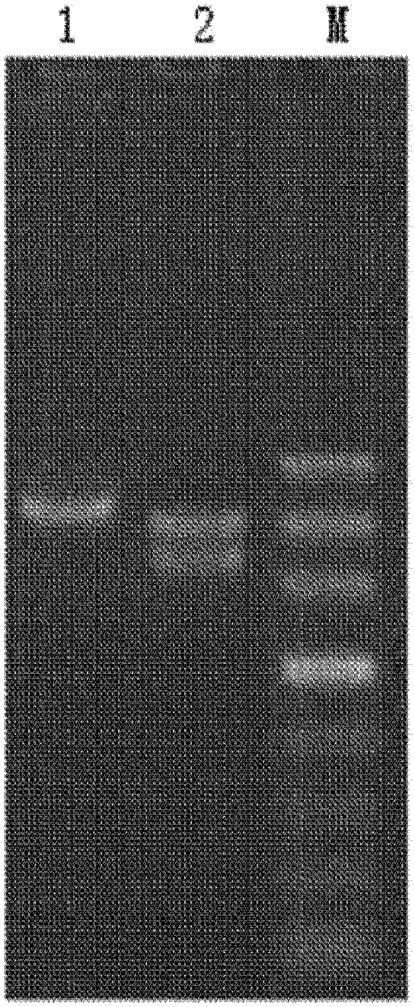

图 16C

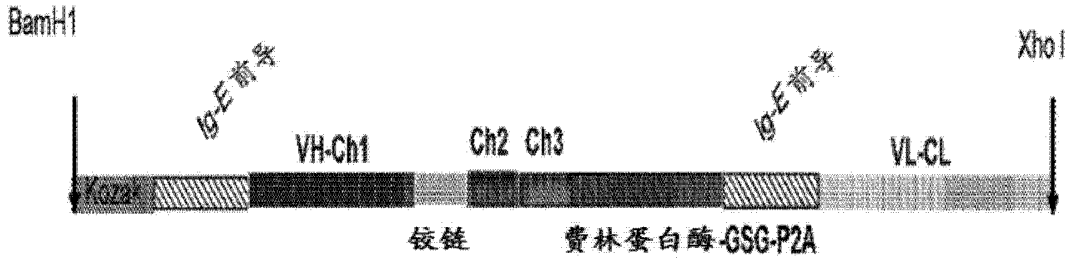

图 17A

图 17B

图 17C

HIV-1 Env-PG9 Ig的氨基酸序列(在蛋白酶切割之前)

MDWTWRILFLVAAATGTHAEFGLSWVFLVAFLRGVQCQRLVESGGGVVQPGSSLRLSC
AASGFDFSRQGMHWVRQAPGQGLEWVAFIKYDGSEKYHADSVWGRLSISRDNSKDTL
YLQMNSLRVEDTATYFCVREAGGPDYRNGYNYYDFYDGYYNYHYMDVWGKGTTVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKRGRKRRSGSGATNFSLLKQAGD
VEENPGPMAWTPLFLFLLTCCPGGSNSQSALTQPASVSGSPGQSITISCNGTSNDVGGYE
SVSWYQQHPGKAPKVVIYDVSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEGDYYCKS
LTSTRRRVFGTGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAW
KADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTV
APTECS (SEQ ID NO:2)

图 18

HIV-1 Env-4E10 Ig的氨基酸序列(在蛋白酶切割之前)

MDWTWRILFLVAAATGTHAQVQLVQSGAEVKRPGSSVTVSCKASGGSFSTYALSWVR
QAPGRGLEWMGGVIPLLTITNYAPRFQGRITITADRSTSTAYLELNSLRPEDTAVYYCAR
EGTTGWGWLGKPIGAFAHWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
LSLSPGKRGRKRRSGSGATNFSLLKQAGDVEENPGPMVLQTQVFISLLLWISGAYGEIVL
TQSPGTQSLSPGERATLSCRASQSVGNNKLAWYQQRPGQAPRLLIYGASSRPSGVADRF
SGSGSGTDFTLTISRLEPEDFAVYYCQQYGQSLSTFGQGTKVEKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK
ADYEKHKVYACEVTHQGLSSPVTKSFNRGE (SEQ ID NO:1)

图 19

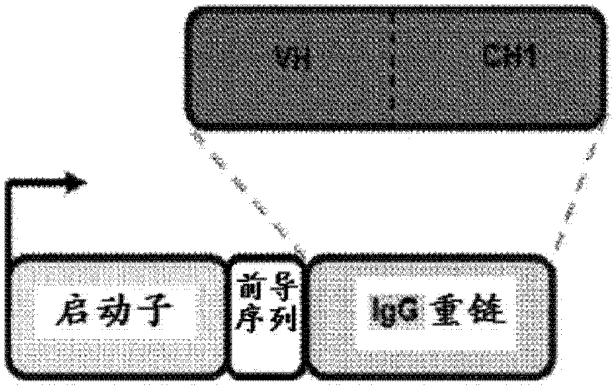

图 20A

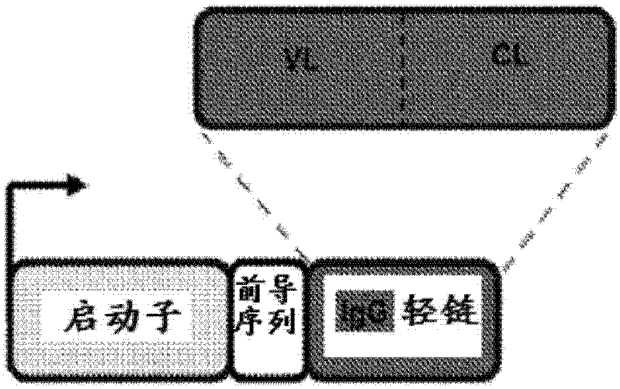
VL
CL
启动子
前导序列
IgG 轻链

图 20B

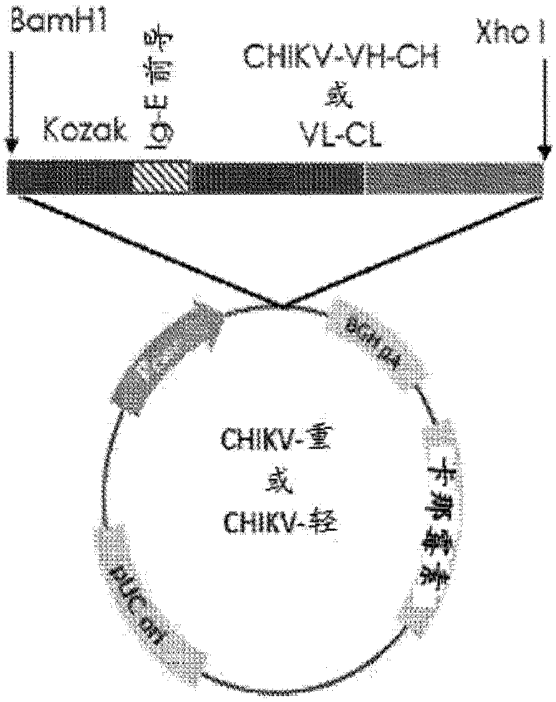
BamH1
Xho I
Kozak
CHIKV-VH-CH
或
VL-CL
CHIKV-重
或
CHIKV-轻

图 21

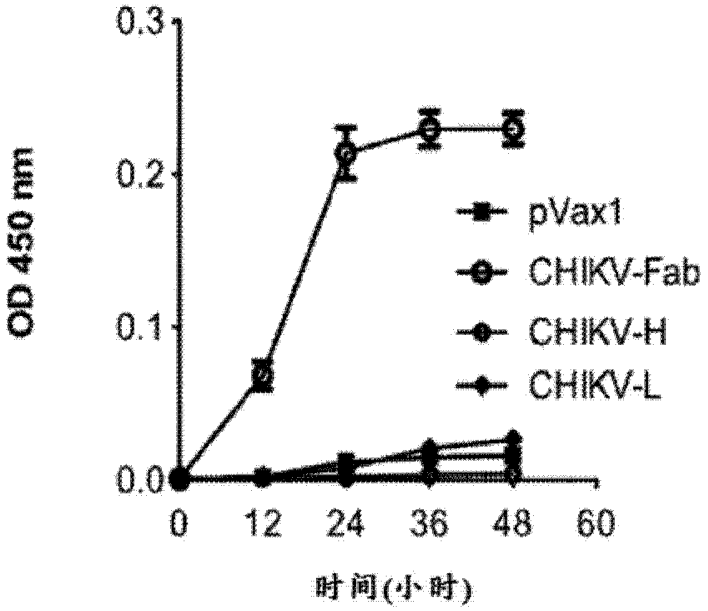
0.3
0.2
0.1
0.0
OD 450 nm
0
12
24
36
48
60
时间(小时)
pVax1
CHIKV-Fab
CHIKV-H
CHIKV-L

图 22

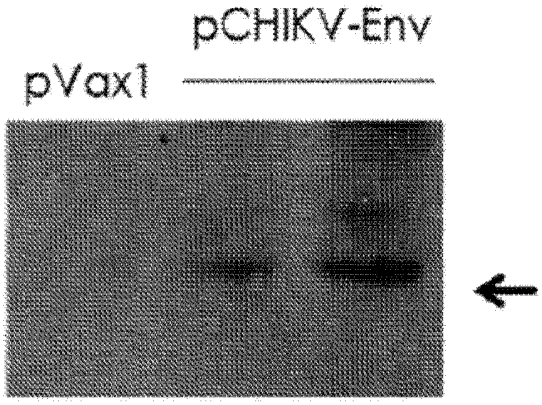
pCHIKV-Env
pVax1

图 23

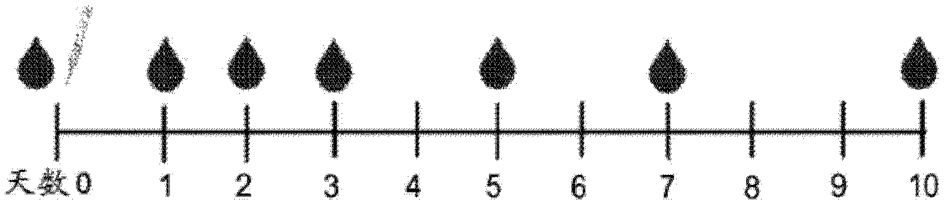
天数 0
1
2
3
4
5
6
7
8
9
10

图 24

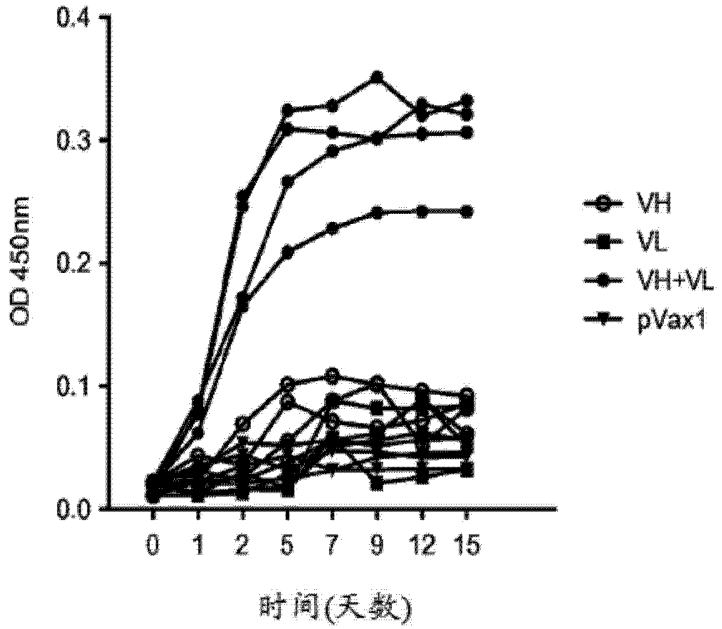
0.4
0.3
0.2
0.1
0.0
OD 450nm
0 1 2 5 7 9 12 15
时间(天数)
VH
VL
VH+VL
pVax1

图 25

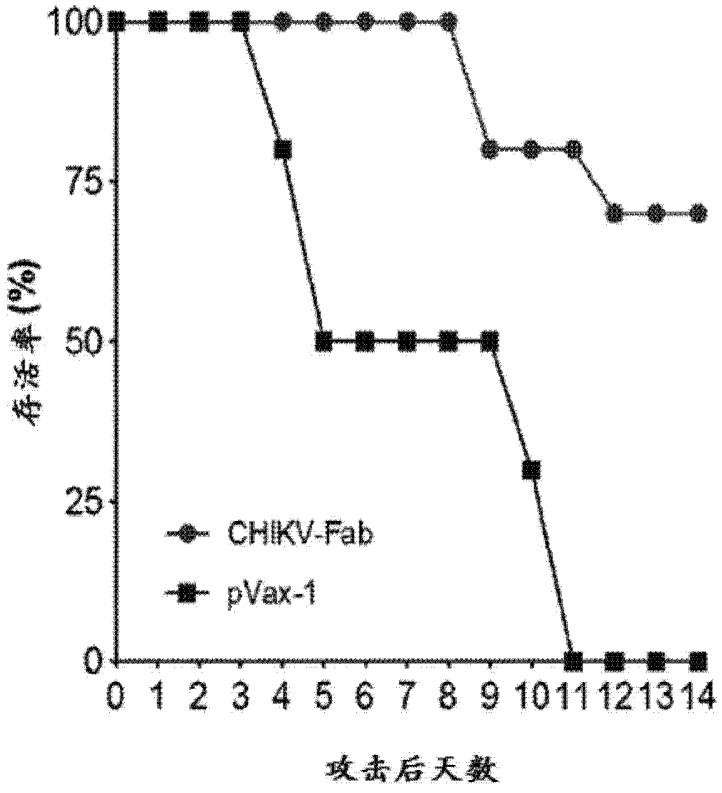
100
75
50
25
0
存活率(%)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
攻击后天数
CHIKV-Fab
pVax-1

图 26

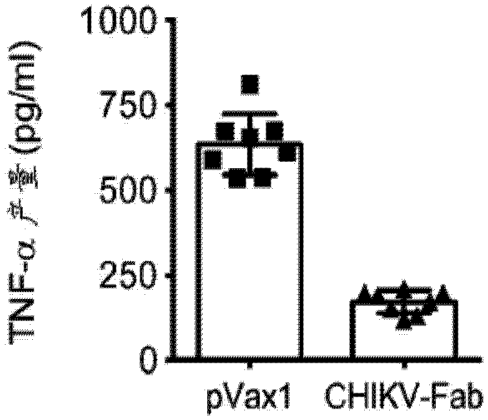
1000
750
500
250
0
TNF-α 产量 (pg/ml)
pVax1
CHIKV-Fab

图 27

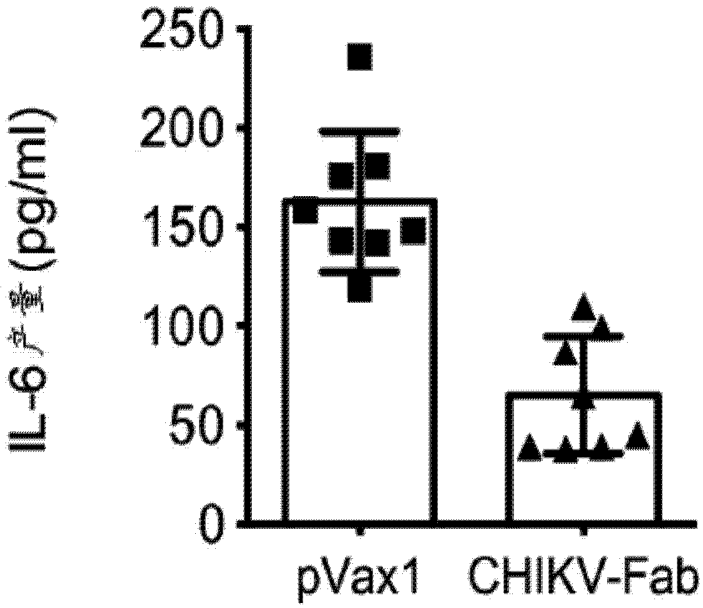
250
200
150
100
50
0
IL-6 产量 (pg/ml)
pVax1
CHIKV-Fab

图 28

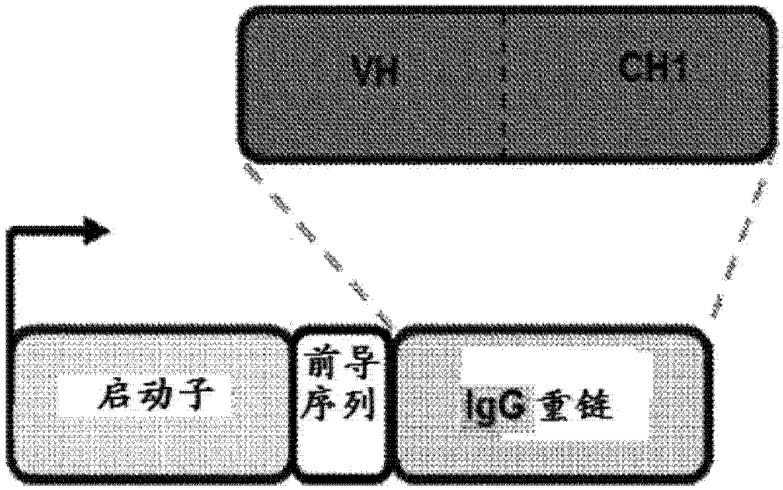

图 29

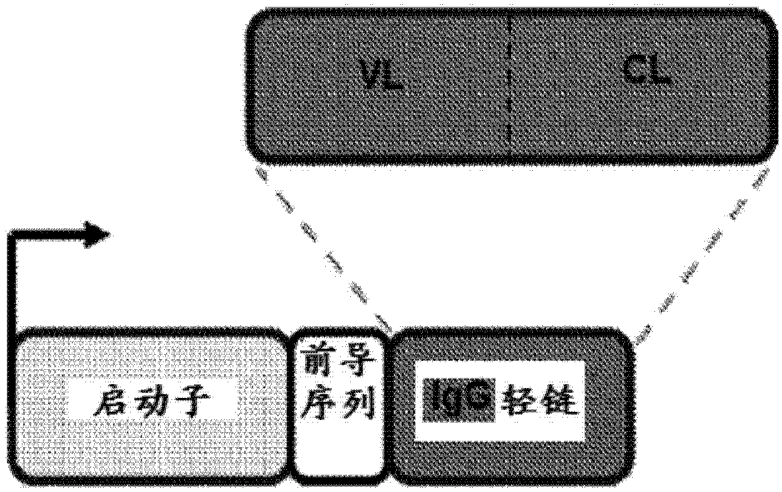

图 30

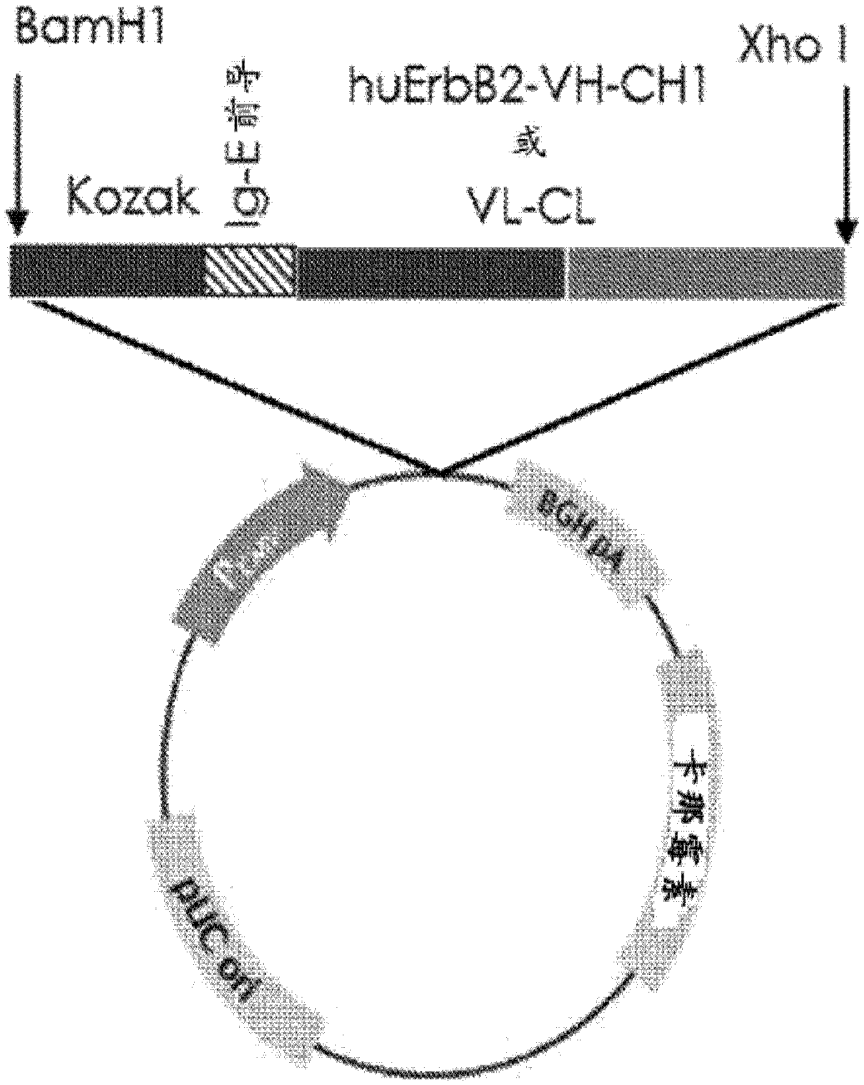

图 31

编码抗-Her-2 Fab的VH-CH1的核酸序列

GGATCCGCCACCATGGACTGGACATGGATTCTGTTTCTGGTCGCCGCCGCTACAAGAGTGCATTCCGAAGTGCAGCTGG
TCGAGAGTGGAGGGGGACTGGTGCAGCCCGGCGGATCTCTGCGACTGAGTTGCGCCGCTTCAGGCTTCACCTTTACAGA
CTACACCATGGATTGGGTGAGACAGGCACCTGGCAAGGGACTGGAGTGGGTGGCTGATGTCAACCCAAATAGTGGGGG
CTCAATCTACAACCAGAGGTTCAAGGGCAGGTTCACCCTGAGCGTGGACAGGTCCAAAAACACTCTGTATCTGCAGAT
GAATTCTCTGCGGGCTGAAGATACCGCAGTCTACTATTGCGCCCGCAATCTGGGCCCAAGCTTCTACTTTGACTATTGG
GGGCAGGGCACACTGGTGACTGTCAGCTCCGCTTCTACAAAGGGACCAAGCGTGTTCCCACTGGCACCCTCTAGTAAAT
CCACCTCTGGAGGGACAGCAGCCCTGGGCTGTCTGGTGAAAGACTATTTCCCCGAGCCTGTGACTGTCAGCTGGAACTC
CGGAGCACTGACTAGCGGAGTGCACACCTTTCCAGCCGTCCTGCAGTCAAGCGGCCTGTACTCCCTGTCCTCTGTGGTC
ACAGTGCCTAGTTCAAGCCTGGGAACTCAGACCTATATTTGTAATGTGAACCATAAACCAAGCAATACAAAGGTGGAC
AAGAAGGTGGAACCAAAATCCTGCTGATAACTCGAG (SEQ ID NO:40)

图 32

抗-Her-2 Fab的VH-CH1氨基酸序列

MDWTWILFLVAAATRVHSEVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYN
QRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
(SEQ ID NO:41)

图 33

编码抗-Her-2 Fab的VL-CL的核酸序列

GGATCCGCCACCATGGATTGGACTTGGATTCTGTTCCTGGTCGCCGCCGCTACCCGCGTGCATTCCGATATTCAGATGA
CTCAGAGCCCCTCCTCACTGTCAGCCAGCGTGGGCGACCGAGTCACCATCACATGCAAAGCTTCTCAGGATGTGAGTAT
TGGGGTCGCATGGTACCAGCAGAAGCCAGGCAAAGCACCCAAGCTGCTGATCTATTCCGCCTCTTACAGGTATACAGG
AGTGCCCAGCAGATTCAGTGGCTCAGGAAGCGGGACTGACTTTACTCTGACCATCAGCTCCCTGCAGCCTGAGGATTTC
GCTACCTACTATTGCCAGCAGTACTATATCTACCCATATACCTTTGGCCAGGGAACAAAAGTGGAGATCAAGCGGACCG
TGGCCGCTCCCTCCGTCTTCATTTTTCCCCCTTCTGACGAACAGCTGAAGAGCGGAACAGCAAGCGTGGTCTGTCTGCT
GAACAATTTCTACCCTCGCGAGGCCAAAGTGCAGTGGAAGGTCGATAACGCTCTGCAGTCCGGGAATTCTCAGGAGAG
TGTGACTGAACAGGACTCAAAAGATAGCACCTATTCCCTGTCTAGTACACTGACTCTGAGCAAGGCAGACTACGAAAA
GCACAAAGTGTATGCCTGTGAGGTCACCCACCAGGGGCTGTCAAGTCCCGTCACCAAGTCCTTCAATAGAGGCGAATG
CTGATAACTCGAG (SEQ ID NO:42)

图 34

抗-Her-2 Fab的VL-CL的氨基酸序列

MDWTWILFLVAAATRVHSDIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW
KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:43)

图 35

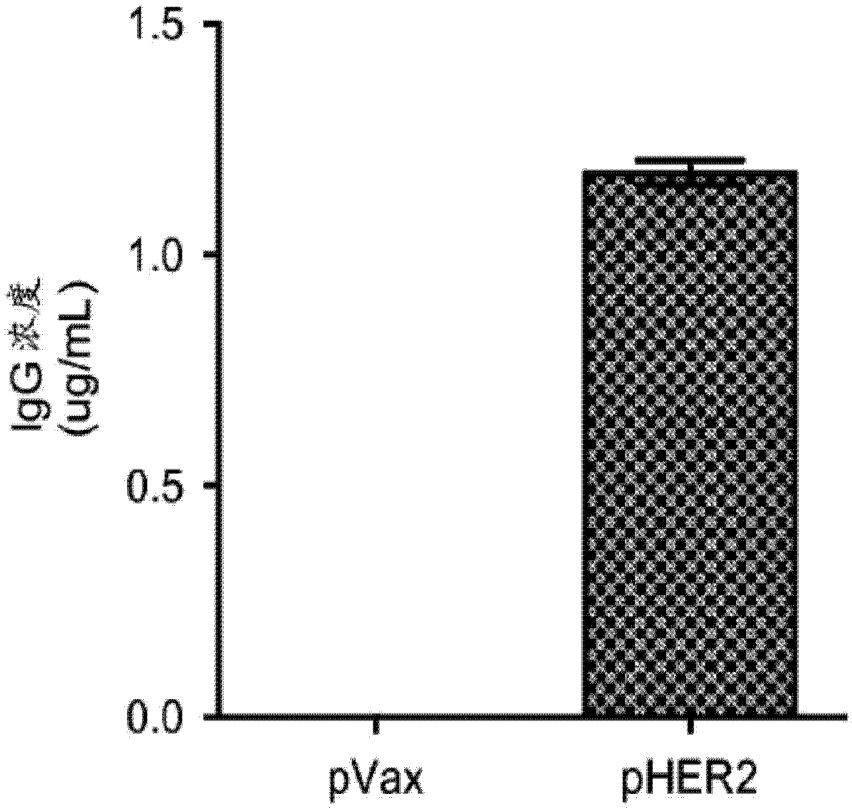

图 36

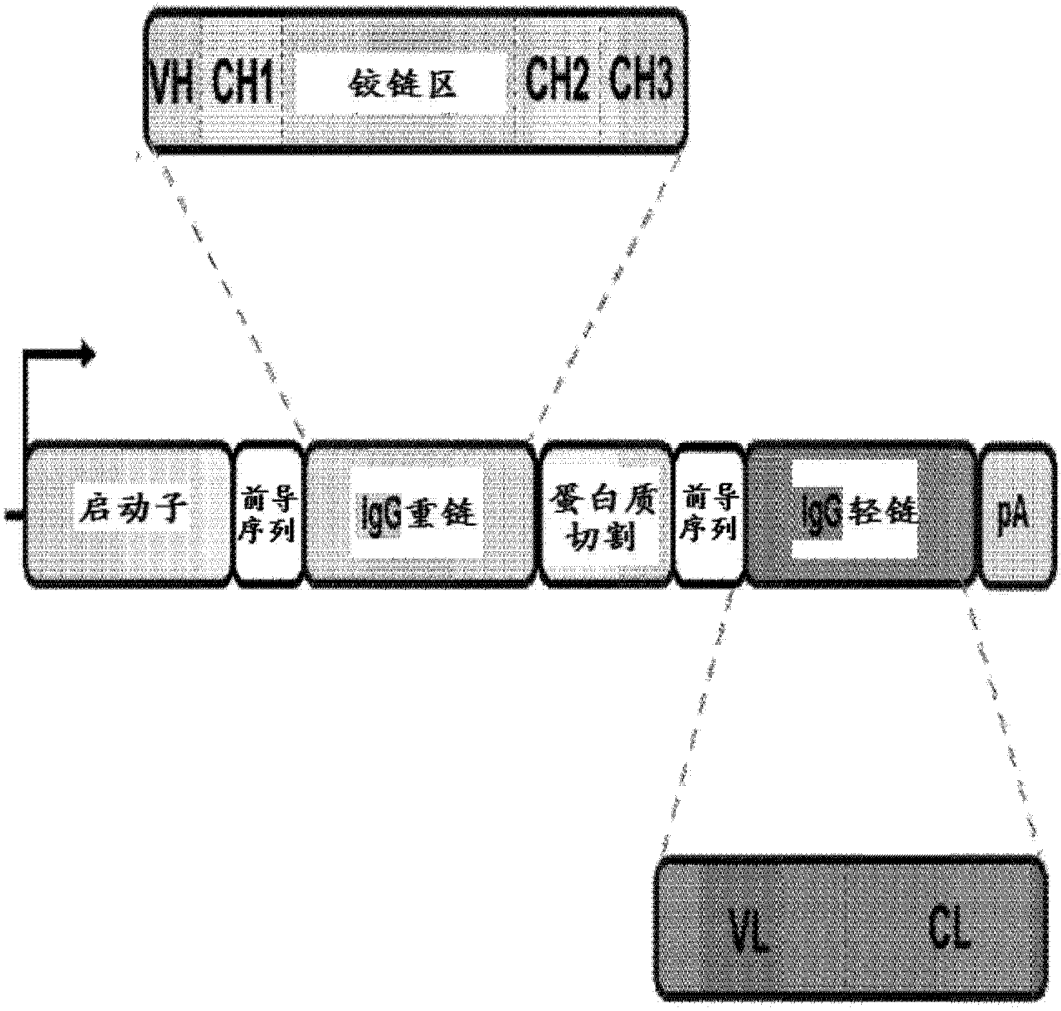

图 37

编码抗-DENV人IgG的核酸序列

GGATCCGCCACCATGGACTGGACTTGGAGGATTCTGTTTCTGGTCGCCGCCGCTACTGGGACTCACGCTCAGGCACATC
TGGTCGAATCTGGAGGAGGAGTGGTCCAGCCTGGCCGATCCCTGCGACTGTCTTGCGCAGCTAGCGCCTTCAACTTCAG
CACAAACGCAATGCACTGGGTGCGACAGGCACCAGGCAAGGGACTGGAGTGGGTCGCTGTGATCTCATACGACGGAA
GCCATAAGTACTATGCAGATTCTGTGAAAGGCCGGTTCACCATTTCCAGGGACAATTCTAAGAACACCCTGTATCTGCA
GATGAATAGCCTGCGCGCAGCCGATACCGCAGTGTACTATTGCGCAACTGTCGGCGTGCTGACCTGGCCAGTGAACGC
CGAATACTTTCACCATTGGGGACAGGGCAGTCTGGTCTCAGTGAGCTCCGCAAGTACTAAGGGACCATCAGTGTTCCCA
CTGGCACCCTCTAGTAAATCTACTAGTGGCGGGACCGCTGCACTGGGATGTCTGGTGAAGGACTATTTCCCCGAGCCTG
TCACCGTGAGCTGGAATTCCGGAGCCCTGACAAGCGGCGTCCACACTTTTCCCGCTGTGCTGCAGTCAAGCGGACTGTA
CTCCCTGTCCTCTGTGGTCACTGTGCCTAGTTCAAGCCTGGGCACTCAGACCTATATCTGCAATGTGAACCACAAGCCCT
CTAACACCAAAGTCGACAAGAAAGTGGAACCTAAGAGCTGTGATAAAACACATACTTGCCCACCTTGTCCAGCACCAG
AGCTGCTGGGAGGACCAAGCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACACTGATGATTAGCCGGACACCTGAAG
TCACTTGCGTGGTCGTGGACGTGTCCCACGAGGACCCCGAAGTCAAGTTTAATTGGTACGTGGATGGCGTCGAGGTGCA
TAACGCCAAGACCAAACCCCGGGAGGAACAGTACAATAGCACATATAGAGTCGTGTCCGTCCTGACTGTGCTGCATCA
GGATTGGCTGAATGGGAAGGAGTATAAGTGCAAAGTGTCTAACAAGGCTCTGCCTGCACCAATCGAGAAAACCATTAG
CAAGGCTAAAGGCCAGCCTAGGGAACCACAGGTGTACACACTGCCTCCAAGTCGCGACGAGCTGACCAAGAATCAGGT
CTCCCTGACATGTCTGGTGAAAGGCTTCTATCCATCAGATATCGCCGTGGAGTGGGAAAGCAACGGGCAGCCCGAAAA
CAATTACAAGACCACACCCCCTGTGCTGGACTCTGATGGCAGTTTCTTTCTGTATTCTAAGCTGACCGTGGACAAAAGT
AGATGGCAGCAGGGGAATGTCTTTTCATGTAGCGTGATGCACGAGGCCCTGCACAACCATTACACACAGAAGTCCCTG
TCTCTGAGTCCCGGAAAGAGGGGCCGCAAACGGAGATCAGGGAGCGGAGCTACTAATTTCAGCCTGCTGAAACAGGCA
GGGGATGTGGAGGAAAACCCCGGACCTATGGCTTGGACCCCACTGTTCCTGTTTCTGCTGACATGCTGTCCCGGGGGCA
GCAATTCTCAGAGTGTCCTGACACAGCCACCATCAGTGAGCGGAGCACCAGGACAGAGGGTGACCATCTCCTGCACAG
GCAGCAGCAGCAACATTGGCGCCGGGTACGACGTGCATTGGTATCAGCAGCTGCCCGGCACCGCTCCTAAGCTGCTGA
TCTGTGGCAACAATAACCGCCCATCTGGGGTGCCCGATCGATTCTCCGGCTCTAAAAGTGGGACTTCAGCCAGCCTGGC
TATTACCGGCCTGCAGGCCGAGGACGAAGCTGATTACTATTGCCAGAGCTACGACTCAAGCCTGACCGGAGTCGTGTTC
GGAGGAGGAACCAAGCTGACAGTCCTGGGACAGCCTAAAGCCGCTCCAAGCGTGACACTGTTTCCTCCATCCTCTGAG
GAACTGCAGGCAAACAAGGCCACCCTGGTGTGCCTGATTTCCGACTTCTACCCCGGGGCAGTCACTGTGGCTTGGAAG
GCAGATAGTTCACCTGTCAAAGCCGGAGTGGAGACTACCACACCATCAAAGCAGAGCAATAACAAATACGCAGCCAG
CTCCTATCTGTCCCTGACCCCTGAGCAGTGGAAGTCTCACAAATCCTATTCTTGCCAGGTCACTCACGAAGGAAGCACT
GTGGAGAAAACTGTCGCACCAACCGAATGTAGTTGATAACTCGAG (SEQ ID NO:44)

图 38

抗-DENV人 IgG 的氨基酸序列(在蛋白酶切割以分离开重链与轻链多肽之前)

MDWTWRILFLVAAATGTHAQAHLVESGGGVVQPGRSLRLSCAASAFNFSTNAMHWVRQAPGKGLEWVAVISYDGSHKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAADTAVYYCATVGVLTWPVNAEYFHHWGQGSLVSVSSASTKGPSVFPLAPSSKS
TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE
PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKRGRKRRSGSGATNF
SLLKQAGDVEENPGPMAWTPLFLFLLTCCPGGSNSQSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPK
LLICGNNNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLTGVVFGGGTKLTVLGQPKAAPSVTLFPPSSEEL
QANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTV
APTECS (SEQ ID NO:45)

图 39

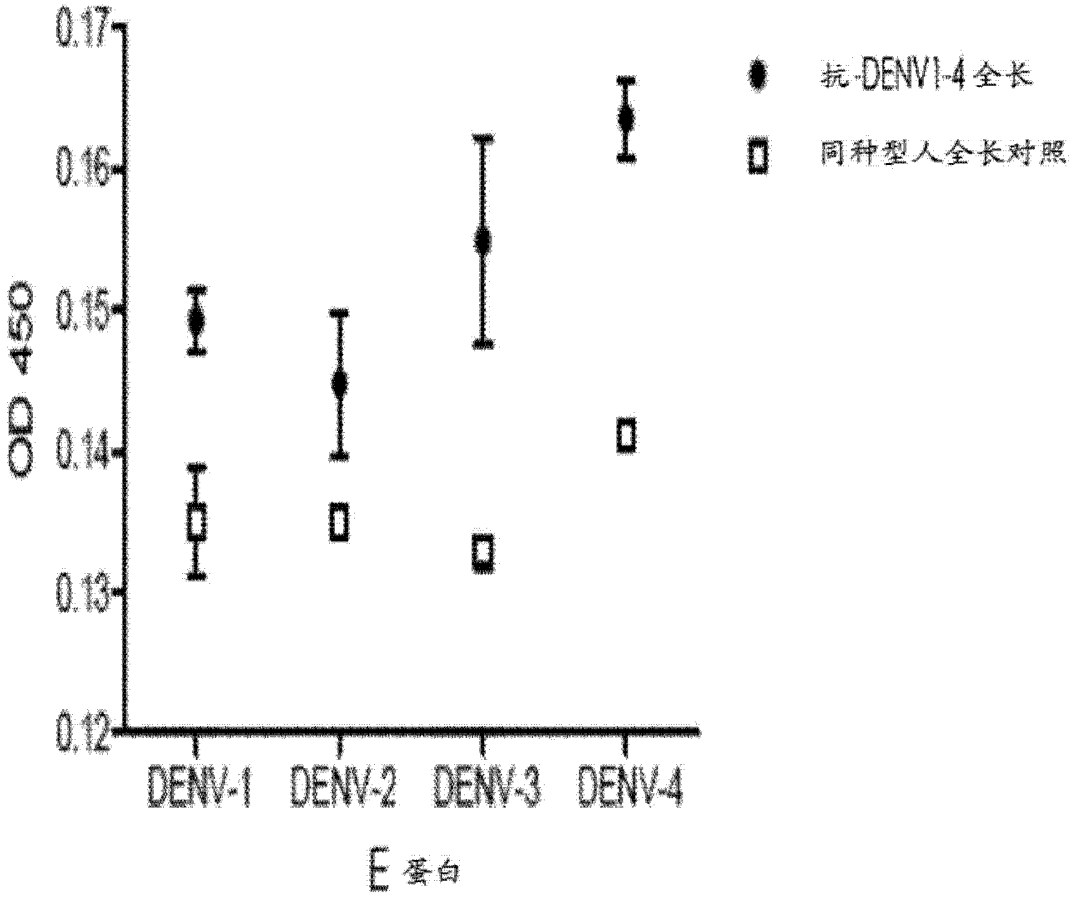

图 40

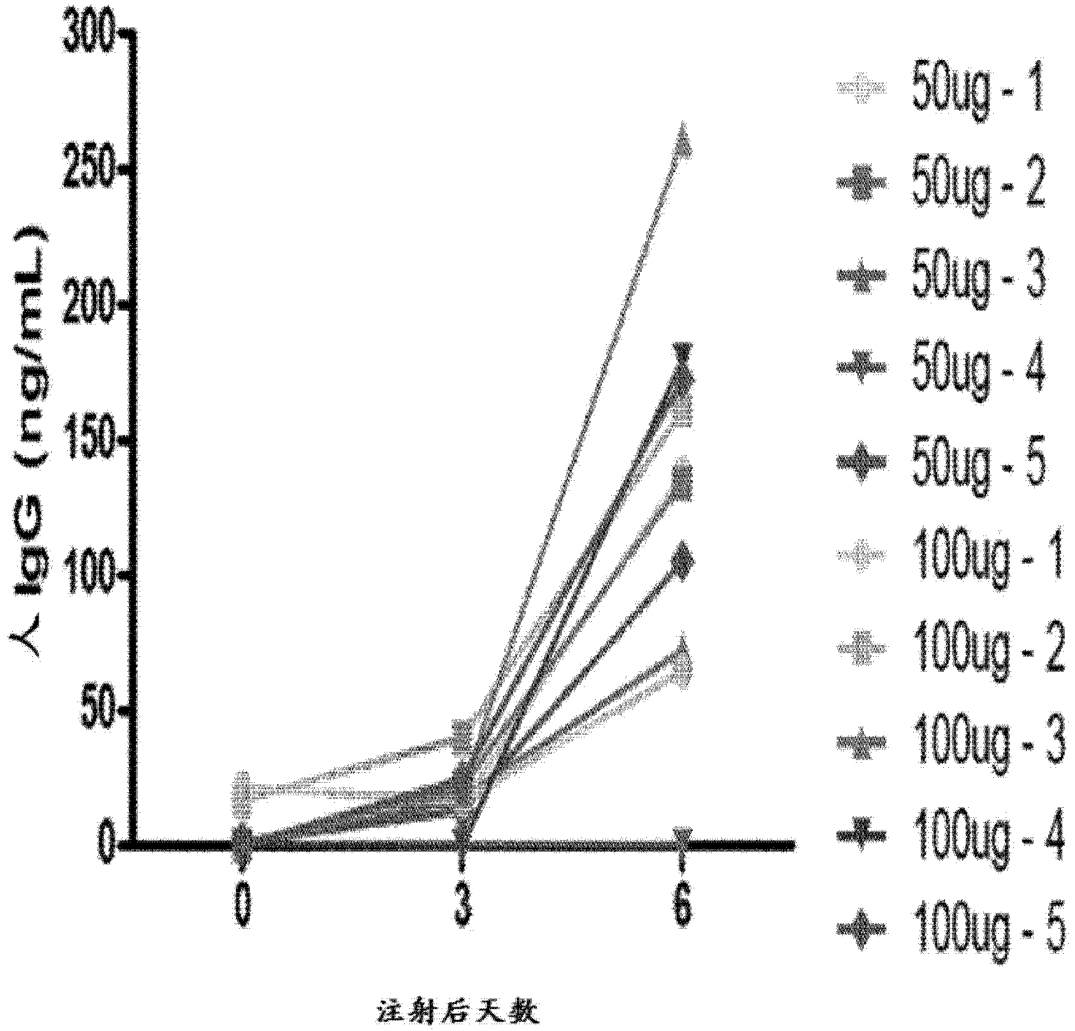

图 41

IgG 重链

METDTLLLWVLLLWVPGSTGDGAQVQLVQSGAVIKTPGSSVKISCRASGYNFRDYSIHWVRLIPDKGFEWIGWIKPLWGAV
SYARQLQGRVSMTRQLSQDPDDPDWGVAYMEFSGLTPADTAEYFCVRRGSCDYCGDFPWQYWCQGTVVVVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKKVEPKSCYPYDVPDYA (SEQ ID NO:46)

图 42

IgG 轻链

METDTLLWVLLLWVPGSTGDGAQVQIVLTQSPGILSLSPGETATLFCKASQGGNAMTWYQKRRGQVPRLLIYDTSRRASG
VPDRFVGSGSGTDFFLTINKLDREDFAVYYCQQFEFFGLGSELEVHRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECYPYDVPDYA
(SEQ ID NO:47)

图 43

HIV-1 Env Fab 的重链 (VH-CH1) 的氨基酸序列

METDTLLWVLLLWVPGSTGDGAQVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGG
AVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSPSTKGPSVFPLAPSSK
STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKA
EPKSCYPYDVPDYA (SEQ ID NO:48)

图 44

HIV-1 Env Fab 的轻链 (VL-CL) 的氨基酸序列

METDTLLWVLLLWVPGSTGDGAQVQIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPD
RFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGECYPYDVPDYA
(SEQ ID NO:49)

图 45

编码 HIV-1 PG9 Fab 的核酸序列

GGATCCGCCACCATGGCAAGACCCCTGTGCACCCTGCTGCTGCTGATGGCAACCCTGGCCGGAGCCCTGGCACAGAGC
GCCCTGACCCAGCCCGCAAGCGTCTCCGGCTCACCAGGCCAGAGCATCACTATTAGTTGCAACGGGACTAGCAACGAC
GTGGGAGGCTATGAGAGTGTCAGCTGGTACCAGCAGCATCCCGGAAAAGCACCAAAAGTGGTCATCTACGATGTCAGT
AAAAGGCCAAGTGGGGTCTCAAATAGGTTCTCAGGGAGTAAATCTGGGAATACAGCATCTCTGACCATCTCCGGACTG
GGCGCAGAAGATGAAGGCGACTACTATTGCAAAAGCCTGACCTCAACCAGACGGCGAGTCTTTGGGACAGGCACCAA
GCTGACAGTCCTGACAGTCGCTGCCCCCTCCGTCTTCATTTTTCCACCTTCAGATGAGCAGCTGAAATCTGGCACTGCAT
CTGTGGTCTGCCTGCTGAACAACTTCTATCCACGAGAGGCCAAGGTGCAGTGGAAAGTGGATAACGCACTGCAGTCCG
GCAATAGTCAGGAAAGCGTGACTGAGCAGGATTCCAAGGACAGTACCTATAGCCTGTCCAGTACACTGACCCTGTCCA
AGGCTGACTACGAAAAACATAAGGTGTATGCATGTGAAGTGACTCACCAGGGACTGAGGTCACCAGTCACTAAGTCTT
TTAACAGGGGAGAGTGCGGCGGGGGAGGATCTGGAGGCGGCGGCTCTGGAGGGGGAGGCTCAGGGGGCGGAGGAAG
CGGCGGAGGAGGGTCCGGAGGAGGAGGCAGTCAGAGACTGGTCGAAAGCGGGGGAGGAGTGGTGCAGCCTGGGTCCT
CACTGAGACTGTCATGCGCTGCCAGTGGCTTTGATTTTTCACGACAGGGAATGCATTGGGTCAGGCAGGCACCCGGACA
GGGCCTGGAATGGGTCGCCTTCATTAAGTACGACGGAAGCGAGAAGTACCATGCCGACTCAGTGTGGGGAAGGCTGAG
CATCTCAAGGGACAACTCAAAGGACACCCTGTACCTGCAGATGAATAGCCTGAGAGTGGAAGATACCGCTACTTATTT
CTGCGTGCGAGAGGCCGGAGGGCCAGATTACCGGAACGGGTACAATTACTATGATTTCTACGACGGCTACTACAATTA
CCATTATATGGATGTCTGGGGCAAAGGAACTACAGTCACCGTGAGCTCCGCAAGTACTAAGGGACCTTCCGTGTTTCCT
CTGGCTCCCAGTTCCAAAAGTACATCCGGAGGAACAGCCGCTCTGGGATGTCTGGTCAAGGACTATTTTCCCGAGCCCG
TGACTGTCTCCTGGAACAGCGGGGCTCTGACAAGCGGGGTGCACACCTTTCCTGCCGTGCTGCAGTCCAGTGGGCTGTA
CAGTCTGTCTAGTGTCGTCACTGTGCCAAGCTCAAGTCTGGGGACCCAGACATACATTTGTAATGTGAACCATAAACCC
TCAAACACCAAAGTGGACAAGAAAGTGGAACCTAAAAGCTGATAACTCGAG (SEQ ID NO:50)

图 46

HIV-1 PG9 Fab的氨基酸序列

MARPLCTLLLLMATLAGALAQSALTQPASVSGSPGQSITISCNGTSNDVGGYESVSWYQQHPGKAPKVVIYDVSKRPSGVSN
RFSGSKSGNTASLTISGLGAEDEGDYYCKSLTSTRRRVFGTGTKLTVLTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGECGGGGSGGGGS
GGGGSGGGGSGGGGSGGGGSQRLVESGGGVVQPGSSLRLSCAASGFDFSRQGMHWVRQAPGQGLEWVAFIKYDGSEKYH
ADSVWGRLSISRDNSKDTLYLQMNSLRVEDTATYFCVREAGGPDYRNGYNYYDFYDGYYNYHYMDVWGKGTTVTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV
NHKPSNTKVDKKVEPKS (SEQ ID NO:51)

图 47

编码 HIV-1 4E10 Fab 的核酸序列

GGATCCGCCACCATGGCAAGACCTCTGTGCACTCTGCTGCTGCTGATGGCTACTCTGGCCGGGGCTCTGGCTGAGATTG
TCCTGACCCAGTCCCCTGGCACTCAGTCACTGTCCCCCGGCGAGCGCGCAACTCTGTCCTGCAGAGCAAGCCAGTCCGT
CGGGAACAACAAGCTGGCATGGTACCAGCAGCGCCCAGGACAGGCACCCAGGCTGCTGATCTACGGAGCAAGCTCCC
GGCCTAGCGGAGTCGCTGATAGATTCTCCGGAAGCGGCTCCGGGACCGATTTCACTCTGACCATCTCCAGGCTGGAACC
TGAGGATTTTGCCGTGTATTACTGTCAGCAGTACGGGCAGAGCCTGTCAACTTTCGGCCAGGGAACTAAAGTCGAAAA
GAGAACCGTGGCCGCACCAAGCGTCTTTATTTTTCCCCCTAGCGATGAACAGCTGAAATCCGGGACTGCTTCCGTGGTC
TGCCTGCTGAATAACTTCTATCCAAGAGAGGCAAAGGTGCAGTGGAAAGTGGACAACGCCCTGCAGAGCGGAAACTCA
CAGGAATCTGTGACAGAGCAGGACTCCAAGGATAGCACATACAGTCTGTCCTCAACTCTGACCCTGTCCAAAGCTGAC
TATGAGAAGCATAAAGTCTACGCATGTGAGGTGACCCACCAGGGACTGAGGTCCCCCGTCACTAAGTCCTTCAATAGA
GGCGAGTGCGGGGGCGGGGGCAGTGGCGGAGGGGGAAGTGGGGGCGGAGGGAGTGGCGGCGGCGGGAGTGGCGGCG
GCGGCTCAGGGGGCGGCGGCTCCCAGGTCCAGCTGGTCCAGAGCGGAGCCGAGGTCAAGAGACCAGGCTCTTCAGTCA
CCGTGAGCTGCAAAGCCAGCGGAGGCTCCTTTAGCACTTACGCCCTGTCATGGGTGCGGCAGGCCCCAGGCCGAGGCC
TGGAGTGGATGGGCGGCGTGATCCCCCTGCTGACCATTACTAACTATGCCCCTAGATTTGGAGGCCGGATCACCATCAC
AGCTGACAGATCCACATCCACAGCTTACCTGGAGCTGAACAGTCTGAGGCCCGAGGACACTGCAGTCTACTACTGTGC
ACGAGAAGGCACCACTGGATGGGGGTGGCTGGGGAAGCCCATCGGGGCTTTTGCACATTGGGGCGGAGGGACACTGGT
GACTGTGAGCTCTGCCAGCACTAAAGGGCCCAGTGTCTTCCCTCTGGCCCCAAGTTCCAAGAGTACATCAGGGGGCACC
GCCGCACTGGGGTGTCTGGTGAAGGATTACTTCCCAGAGCCCGTGACAGTCAGTTGGAACAGCGGCGCTCTGACCAGT
GGGGTGCACACTTTCCCAGCCGTGCTGCAGAGTTCAGGGCTGTACTCCCTGTCCTCAGTGGTGACTGTGCCCTCAAGCA
GTCTGGGGACTCAGACTTACATTTGTAATGTGAACCATAAACCCTCAAATACTAAAGTGGACAAAAAAGTGGAACCAA
AGAGCTGATAACTCGAG (SEQ ID NO:52)

图 48

HIV-1 4E10 Fab 的氨基酸序列

MARPLCTLLLLMATLAGALAEIVLTQSPGTQSLSPGERATLSCRASQSVGNNKLAWYQQRPGQAPRLLIYGASSRPSGVADR
FSGSGSGTDFTLTISRLEPEDFAVYYCQQYGQSLSTFGQGTKVEKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGECGGGGSGGGGSGG
GGSGGGGSGGGGSGGGGSQVQLVQSGAEVKRPGSSVTVSCKASGGSFSTYALSWVRQAPGRGLEWMGGVIPLLTITNYAP
RFGGRITITADRSTSTAYLELNSLRPEDTAVYYCAREGTTGWGWLGKPIGAFAHWGGGTLVTVSSASTKGPSVFPLAPSSKST
SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KS (SEQ ID NO:53)

图 49

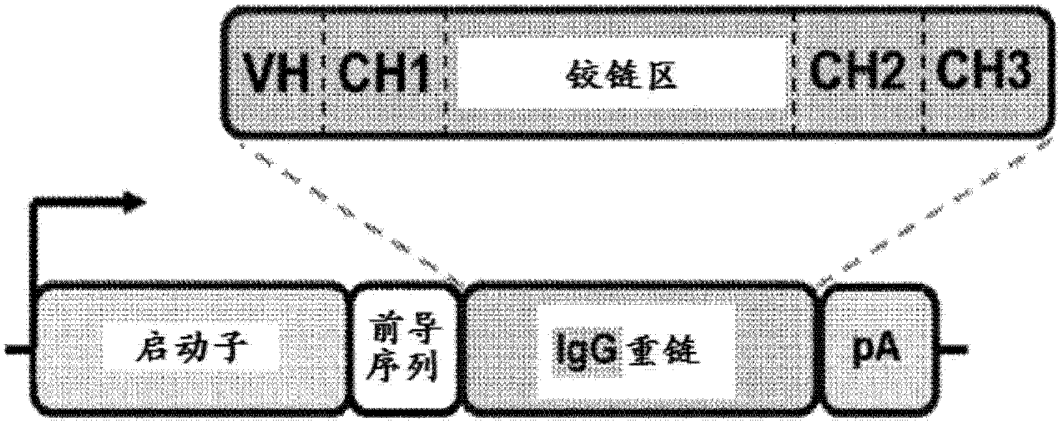

图 50

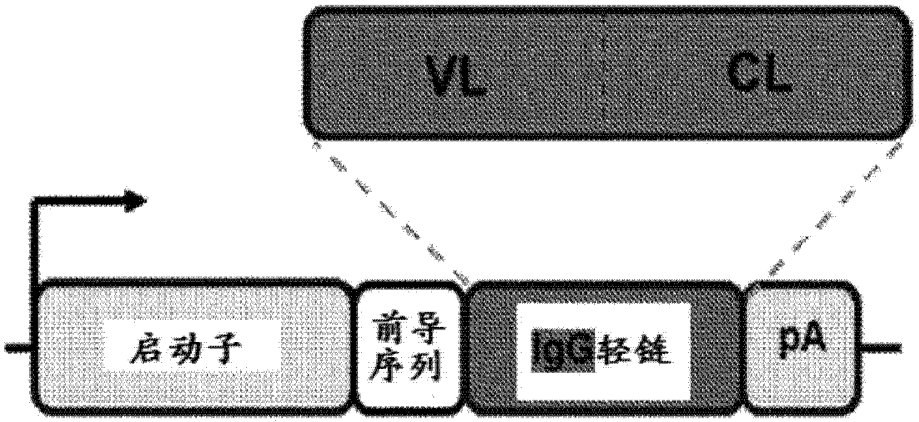

图 51

编码HIV-1 VRC01 IgG1重链(VH/CH1/铰链/CH2/CH3)的核酸序列

GGATCCGCCACCATGGATTGGACATGGATTCTGTTCCTGGTCGCCGCCGCAACTAGAGTGCATTCACAGGTGCAGCTGG
TGCAGTCAGGCGGGCAGATGAAGAAACCCGGCGAGAGTATGCGAATCTCATGCCGGGCTAGCGGGTACGAATTCATCG
ACTGTACCCTGAACTGGATTAGACTGGCACCTGGGAAGAGGCCAGAGTGGATGGGATGGCTGAAACCTAGAGGCGGG
GCAGTGAATTACGCCAGACCACTGCAGGGCAGGGTCACTATGACCCGCGACGTGTATTCTGATACCGCATTCCTGGAG
CTGCGAAGTCTGACAGTCGACGATACTGCCGTGTACTTCTGCACACGGGGCAAGAACTGTGACTATAATTGGGATTTTG
AACACTGGGGCAGGGGGACACCTGTCATTGTGAGCTCCCCAAGTACTAAGGGACCCTCAGTGTTTCCCCTGGCCCCTTC
TAGTAAAAGTACCTCAGGAGGCACAGCCGCTCTGGGATGCCTGGTGAAGGATTACTTCCCTGAGCCAGTCACCGTGAG
TTGGAACTCAGGCGCCCTGACAAGCGGGGTCCATACTTTTCCAGCTGTGCTGCAGTCAAGCGGGCTGTACTCCCTGTCC
TCTGTGGTCACAGTGCCCAGTTCAAGCCTGGGAACACAGACTTATATCTGTAACGTCAATCACAAGCCTAGCAATACTA
AAGTGGACAAGAAAGCCGAGCCTAAGAGCTGCGAACCAAAGTCCTGTGATAAAACCCATACATGCCCTCCCTGTCCAG
CTCCTGAACTGCTGGGCGGCCCATCCGTGTTCCTGTTTCCACCCAAGCCCAAAGACACCCTGATGATTAGCAGGACTCC
TGAGGTCACCTGCGTGGTCGTGGACGTGTCCCACGAGGACCCCGAAGTCAAGTTTAACTGGTACGTGGATGGCGTCGA
AGTGCATAATGCCAAGACAAAACCCCGGGAGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTCCTGACAGTGCT
GCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTGTCTAATAAGGCCCTGCCAGCTCCCATCGAGAAAAC
AATTTCCAAGGCAAAAGGCCAGCCAAGGGAACCCCAGGTGTACACTCTGCCTCCATCCCGCGACGAGCTGACTAAGAA
CCAGGTCTCTCTGACCTGTCTGGTGAAAGGATTCTATCCAAGCGATATCGCCGTGGAGTGGGAATCCAATGGCCAGCCC
GAGAACAATTACAAGACCACACCCCCTGTGCTGGACAGCGATGGCTCCTTCTTTCTGTATTCAAAGCTGACCGTGGATA
AAAGCCGCTGGCAGCAGGGGAACGTCTTTAGCTGCTCCGTGATGCACGAAGCTCTGCACAATCATTACACCCAGAAGT
CTCTGAGTCTGTCACCTGGCAAGTGATAACTCGAG (SEQ ID NO:54)

图 52

HIV-1 VRC01 IgG1重链(VH/CH1/CH2/CH3)的氨基酸序列

MDWTWILFLVAAATRVHSQVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYA
RPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSPSTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCE
PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:55)

图 53

编码HIV-1 VRC01 IgG轻链(VL/CL)的核酸序列

GGATCCGCCACCATGGATTGGACTTGGATTCTGTTCCTGGTGGCAGCCGCTACCAGAGTCCATTCCGAAATTGTGCTGA
CCCAGTCTCCCGGAACACTGTCTCTGAGTCCTGGCGAGACAGCCATCATTTCCTGTAGGACTTCTCAGTACGGGAGTCT
GGCATGGTATCAGCAGCGACCAGGACAGGCTCCTCGACTGGTCATCTACTCAGGAAGCACTCGGGCAGCCGGCATTCC
CGACCGATTCTCCGGGTCTCGGTGGGGACCTGATTACAACCTGACCATCTCAAATCTGGAAAGCGGAGACTTTGGCGTG
TACTATTGCCAGCAGTATGAGTTCTTTGGGCAGGGAACCAAGGTCCAGGTGGACATCAAACGCACAGTCGCTGCACCA
AGCGTGTTCATCTTTCCACCCTCAGATGAACAGCTGAAGTCCGGCACCGCCTCTGTGGTGTGCCTGCTGAACAATTTCTA
CCCCCGGGAGGCAAAGGTCCAGTGGAAAGTGGACAACGCCCTGCAGTCTGGCAATAGTCAGGAGTCAGTGACTGAAC
AGGACAGCAAGGATTCCACCTATTCTCTGTCCTCTACTCTGACCCTGAGCAAAGCTGATTACGAGAAGCACAAAGTGTA
TGCATGTGAGGTCACCCACCAGGGACTGCGGTCACCCGTCACCAAGAGCTTCAATCGCGGAGAGTGTTGATAACTCGA
G (SEQ ID NO:56)

图 54

HIV-1 VRC01 IgG轻链(VL/CL)的氨基酸序列

MDWTWILFLVAAATRVHSEIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRW
GPDYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLRSPVTKSFNRGEC (SEQ ID NO:57)

图 55

编码CHIKV-Env-Fab的重链(VH-CH1)的核酸序列

GGATCCGCCACCATGGATTGGACATGGAGGATTCTGTTTCTGGTCGCCGCCGCTACTGGAACTCACGCTCAGGTGCAGC
TGGTGCAGTCAGGGTCCGAACTGAAGAAACCAGGGGCATCTGTGAAGGTCAGTTGCAAAGCCTCAGGCTACACCCTGA
CACGGTATGCCATGACTTGGGTGCGCCAGGCTCCTGGACAGGGACTGGAGTGGATGGGCTGGATCAACACTTACACCG
GAAATCCAACTTATGTGCAGGGGTTCACCGGCCGATTCGTGTTTTCTCTGGACACTTCCGTCTCTACCGCCTTTCTGCAC
ATTACAAGTCTGAAGGCAGAGGACACTGCCGTGTACTTCTGCGCTAGGGAAGGCGGAGCAAGAGGCTTTGATTATTGG
GGCCAGGGAACCCTGGTGACAGTCAGCTCCGCCAGCACAAAGGGACCCTCCGTGTTCCCACTGGCTCCCTCTAGTAAA
AGTACATCAGGGGGCACTGCCGCTCTGGGATGTCTGGTCAAAGATTACTTCCCCGAACCTGTGACCGTCAGCTGGAACT
CCGGAGCTCTGACCAGCGGGGTGCATACATTTCCCGCAGTCCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTGGT
CACAGTGCCTAGTTCAAGCCTGGGGACACAGACTTATATCTGTAATGTGAACCATAAGCCAAGCAACACCAAAGTGGA
CAAAAAAGTGGAACCTAAGAGCTGCTGATAACTCGAG (SEQ ID NO:58)

图 56

CHIKV-Env-Fab的重链(VH-CH1)的氨基酸序列

MDWTWRILFLVAAATGTHAQVQLVQSGSELKKPGASVKVSCKASGYTLTRYAMTWVRQAPGQGLEWMGWINTYTGNPT
YVQGFTGRFVFSLDTSVSTAFLHITSLKAEDTAVYFCAREGGARGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
(SEQ ID NO:59)

图 57

编码CHIKV-Env-Fab的轻链(VL-CL)的核酸序列

GGATCCGCCACCATGGCATGGACCCCACTGTTCCTGTTCCTGCTGACTTGTTGTCCTGGCGGGAGCAATTCACAGAGCG
TCCTGACCCAGCCCCCTTCTGTGTCCGGAGCACCAGGACAGCGAGTCACAATCTCTTGCACTGGAAGCTCCTCTAACAT
TGGGGCCAGCCACGACGTGCATTGGTACCAGCAGCTGCCAGGGACCGCTCCCACACTGCTGATCTATGTGAACTCTAAT
AGGCCTAGTGGCGTCCCAGATAGATTTTCAGGGAGCAAGTCCGGCACCTCTGCTAGTCTGGCAATTACAGGACTGCAG
GCTGAGGACGAAGCAGATTACTATTGCCAGAGTTACGACTCAAACCTGTCAGGCAGCGCAGTGTTCGGAGGAGGAACT
AAGCTGACCGTCCTGGGACAGCCCAAAGCCGCTCCTTCTGTGACCCTGTTTCCCCCTAGTTCAGAGGAACTGCAGGCCA
ACAAGGCTACTCTGGTGTGTCTGATCTCCGACTTCTACCCTGGAGCAGTGACCGTCGCATGGAAGGCCGATAGCTCCCC
AGTGAAAGCTGGGGTCGAGACCACAACTCCCAGCAAGCAGTCCAACAACAAGTACGCAGCCTCTAGTTATCTGTCACT
GACACCTGAACAGTGGAAGAGCCACAAATCCTATTCTTGCCAAGTGACTCATGAGGGCAGTACCGTGGAAAAGACAGT
CGCCCCAACTGAGTGTTCCTGATAACTCGAG (SEQ ID NO:60)

图 58

CHIKV-Env-Fab的轻链(VL-CL)的氨基酸序列

MAWTPLFLFLLTCCPGGSNSQSVLTQPPSVSGAPGQRVTISCTGSSSNIGASHDVHWYQQLPGTAPTLLIYVNSNRPSGVPDR
FSGSKSGTSASLAITGLQAEDEADYYCQSYDSNLSGSAVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFY
PGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS (SEQ ID
NO:61)

图 59

编码HIV-1 Env-4E10 Ig的核酸序列

GGATCCGCCACCATGGATTGGACATGGAGGATTCTGTTTCTGGTCGCCGCCGCTACAGGAACTCACGCCCAGGTGCAG
CTGGTGCAGTCAGGAGCCGAAGTGAAGCGACCAGGCAGCTCCGTCACTGTGTCCTGCAAAGCATCTGGCGGATCATTC
AGCACCTACGCCCTGAGCTGGGTGAGACAGGCTCCTGGACGAGGACTGGAATGGATGGGAGGCGTCATCCCACTGCTG
ACAATTACTAACTACGCCCCCCGATTTCAGGGCAGGATCACCATTACAGCAGACCGCTCCACTTCTACCGCCTATCTGG
AGCTGAATAGCCTGAGACCAGAAGATACCGCAGTGTACTATTGCGCCCGGGAGGGAACCACAGGATGGGGATGGCTG
GGAAAGCCCATCGGGGCTTTCGCACACTGGGGCCAGGGAACCCTGGTCACAGTGTCTAGTGCCAGCACAAAGGGCCCC
TCCGTGTTTCCCCTGGCTCCTTCAAGCAAAAGTACTTCAGGAGGGACCGCCGCTCTGGGATGTCTGGTGAAGGACTACT
TCCCTGAGCCAGTCACCGTGTCCTGGAACTCTGGCGCTCTGACCTCCGGAGTGCATACATTTCCCGCAGTCCTGCAGTC
CTCTGGGCTGTACTCTCTGAGTTCAGTGGTCACTGTGCCTAGCTCCTCTCTGGGCACACAGACTTATATCTGCAACGTGA
ATCACAAGCCCTCCAATACCAAAGTCGACAAGAAAGTGGAACCTAAGTCTTGTGATAAAACCCATACATGCCCACCTT
GTCCAGCACCTGAGCTGCTGGGCGGACCTTCCGTGTTCCTGTTTCCACCCAAGCCAAAAGACACACTGATGATTAGCCG
GACACCTGAAGTGACTTGTGTGGTCGTGGACGTCAGCCACGAGGACCCCGAAGTGAAGTTCAACTGGTACGTGGATGG
CGTCGAGGTGCATAATGCCAAGACCAAACCCAGGGAGGAACAGTACAACTCTACTTATAGGGTCGTGAGTGTCCTGAC
CGTGCTGCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTGTCCAATAAGGCCCTGCCAGCTCCCATCGA
GAAAACAATTTCTAAGGCTAAAGGCCAGCCACGCGAACCCCAGGTGTACACTCTGCCTCCCAGCAGGGACGAGCTGAC
CAAGAACCAGGTGAGTCTGACATGTCTGGTCAAAGGCTTCTATCCAAGCGATATCGCCGTGGAGTGGGAATCCAATGG
ACAGCCCGAAAACAATTACAAGACTACCCCCCCTGTGCTGGACAGTGATGGATCATTCTTTCTGTATTCCAAGCTGACC
GTGGACAAATCTCGCTGGCAGCAGGGGAACGTCTTTAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCATTACACA
CAGAAGTCTCTGAGTCTGTCACCAGGCAAGCGGGGACGCAAAAGGAGAAGCGGGTCCGGCGCTACTAACTTCAGCCTG
CTGAAACAGGCAGGGGATGTGGAGGAAAATCCTGGCCCAATGGTCCTGCAGACCCAGGTGTTTATCTCACTGCTGCTGT
GGATTAGCGGGGCTTATGGCGAAATCGTGCTGACTCAGAGCCCCGGAACCCAGTCTCTGAGTCCTGGGGAGCGCGCTA
CACTGAGCTGTCGAGCATCACAGAGCGTGGGGAACAATAAGCTGGCATGGTACCAGCAGAGGCCTGGCCAGGCTCCAA
GACTGCTGATCTATGGCGCAAGTTCACGGCCTAGCGGAGTGGCAGACCGCTTCTCCGGATCTGGGAGTGGCACCGATTT
TACTCTGACCATTAGCAGGCTGGAGCCAGAAGACTTCGCTGTGTACTATTGCCAGCAGTACGGCCAGTCACTGAGCACA
TTTGGACAGGGGACTAAGGTCGAAAAAAGAACCGTGGCAGCCCCAAGTGTCTTCATTTTTCCACCCTCAGACGAGCAG
CTGAAGAGTGGAACAGCCTCAGTCGTGTGTCTGCTGAACAATTTCTACCCCAGGGAGGCCAAGGTCCAGTGGAAAGTG
GATAACGCTCTGCAGAGCGGCAATTCCCAGGAGTCTGTGACAGAACAGGACAGTAAGGATTCAACTTATAGCCTGAGC
TCCACACTGACTCTGTCCAAAGCAGATTACGAGAAGCACAAAGTGTATGCCTGCGAAGTCACCCATCAGGGACTGTCT
AGTCCTGTGACAAAGTCTTTTAACAGAGGGGAGTGATAACTCGAG (SEQ ID NO:62)

图 60

编码HIV-1 Env-PG9 Ig的核酸序列

GGATCCGCCACCATGGACTGGACTTGGAGGATTCTGTTTCTGGTCGCCGCCGCAACTGGAACTCACGCTGAATTTGGAC
TGTCATGGGTCTTTCTGGTGGCCTTTCTGCGAGGGGTCCAGTGCCAGAGGCTGGTGGAGTCCGGAGGAGGAGTGGTCCA
GCCAGGCAGCTCCCTGCGACTGAGTTGTGCCGCTTCAGGGTTCGACTTTTCTAGACAGGGCATGCACTGGGTGCGGCAG
GCACCAGGACAGGGACTGGAGTGGGTGGCTTTCATCAAGTACGACGGAAGTGAAAAATATCATGCCGATTCAGTGTGG
GGGCGGCTGTCAATTAGCCGCGACAACTCCAAGGATACCCTGTACCTGCAGATGAATTCTCTGAGGGTCGAGGACACA
GCTACTTATTTCTGCGTGAGGGAAGCAGGCGGACCTGATTACAGAAACGGGTATAATTACTATGACTTTTACGATGGCT
ACTATAACTACCACTATATGGACGTGTGGGGCAAGGGAACCACAGTCACAGTGTCTAGTGCATCAACTAAAGGCCCAA
GCGTGTTTCCCCTGGCCCCTTCAAGCAAGTCCACTTCTGGAGGAACCGCAGCACTGGGATGTCTGGTGAAGGATTACTT
CCCTGAGCCAGTCACCGTGAGTTGGAACTCAGGCGCCCTGACTAGCGGAGTCCATACCTTTCCTGCTGTGCTGCAGTCC
TCTGGGCTGTACAGCCTGAGTTCAGTGGTCACAGTGCCAAGCTCCTCTCTGGGCACCCAGACATATATCTGCAACGTGA
ATCACAAGCCTAGCAATACTAAGGTCGACAAAAGAGTGGAACCAAAGAGCTGTGATAAAACTCATACCTGCCCACCTT
GTCCAGCACCTGAGCTGCTGGGAGGGCCTTCCGTGTTCCTGTTTCCACCCAAGCCAAAAGACACCCTGATGATTAGCCG
GACACCAGAAGTCACTTGCGTGGTCGTGGACGTGAGCCACGAGGACCCCGAAGTCAAGTTTAACTGGTACGTGGATGG
CGTCGAGGTGCATAATGCTAAGACAAAACCACGGGAGGAACAGTACAACTCCACATATCGCGTCGTGTCTGTCCTGAC
TGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTATAAGTGCAAAGTGTCCAATAAGGCACTGCCAGCCCCCATCGA
GAAAACCATTTCTAAGGCCAAAGGCCAGCCACGAGAACCCCAGGTGTACACACTGCCTCCAAGTAGGGACGAGCTGAC
TAAGAACCAGGTCTCTCTGACCTGTCTGGTGAAAGGCTTCTATCCCTCTGATATCGCTGTGGAGTGGGAAAGTAATGGA
CAGCCTGAAAACAATTACAAGACTACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTATAGCAAGCTGACCG
TGGACAAATCCAGATGGCAGCAGGGGAACGTCTTTAGTTGCTCAGTGATGCACGAGGCACTGCACAATCATTACACCC
AGAAAAGCCTGTCCCTGTCTCCTGGCAAGAGGGGAAGAAAAAGGAGAAGTGGGTCAGGCGCAACAAACTTCAGCCTG
CTGAAGCAGGCCGGAGATGTGGAGGAAAATCCTGGGCCAATGGCTTGGACCCCCCTGTTCCTGTTTCTGCTGACATGCT
GTCCTGGCGGAAGCAACTCCCAGTCTGCACTGACACAGCCAGCAAGTGTGTCAGGGAGCCCAGGACAGAGCATCACCA
TTTCCTGTAACGGCACAAGCAATGACGTCGGGGGCTACGAGTCCGTGTCTTGGTATCAGCAGCATCCTGGAAAGGCCCC
AAAAGTCGTGATCTACGATGTCAGCAAACGCCCCTCTGGGGTGAGTAACCGATTCAGTGGATCAAAGAGCGGGAATAC
CGCTTCTCTGACAATTAGTGGCCTGCAGGCAGAGGACGAAGGAGATTACTATTGCAAATCACTGACAAGCACTCGGCG
CCGAGTCTTCGGAACCGGGACAAAGCTGACTGTGCTGGGCCAGCCCAAAGCTGCACCTAGCGTGACCCTGTTTCCACCC
AGTTCAGAGGAACTGCAGGCTAATAAGGCAACACTGGTGTGTCTGATCTCCGACTTCTACCCTGGCGCTGTCACTGTGG
CCTGGAAGGCTGATAGCTCCCCAGTCAAAGCAGGAGTGGAAACAACTACCCCCTCCAAGCAGTCTAACAACAAGTACG
CCGCTTCTAGTTATCTGTCACTGACTCCCGAGCAGTGGAAGAGCCACAAATCCTATTCTTGCCAGGTGACCCATGAGGG
CTCCACTGTCGAAAAGACCGTGGCCCCTACAGAGTGTTCTTGATAACTCGAG (SEQ ID NO:63)

图 61

编码VRC01 IgG的核酸序列

GGATCCGCCACCATGGATTGGACATGGATTCTGTTCCTGGTCGCCGCCGCAACTAGAGTGCATTCACAGGTGCAGCTGG
TGCAGTCAGGCGGGCAGATGAAGAAACCCGGCGAGAGTATGCGAATCTCATGCCGGGCTAGCGGGTACGAATTCATCG
ACTGTACCCTGAACTGGATTAGACTGGCACCTGGGAAGAGGCCAGAGTGGATGGGATGGCTGAAACCTAGAGGCGGG
GCAGTGAATTACGCCAGACCACTGCAGGGCAGGGTCACTATGACCCGCGACGTGTATTCTGATACCGCATTCCTGGAG
CTGCGAAGTCTGACAGTCGACGATACTGCCGTGTACTTCTGCACACGGGGCAAGAACTGTGACTATAATTGGGATTTTG
AACACTGGGGCAGGGGGACACCTGTCATTGTGAGCTCCCCAAGTACTAAGGGACCCTCAGTGTTTCCCCTGGCCCCTTC
TAGTAAAAGTACCTCAGGAGGCACAGCCGCTCTGGGATGCCTGGTGAAGGATTACTTCCCTGAGCCAGTCACCGTGAG
TTGGAACTCAGGCGCCCTGACAAGCGGGGTCCATACTTTTCCAGCTGTGCTGCAGTCAAGCGGGCTGTACTCCCTGTCC
TCTGTGGTCACAGTGCCCAGTTCAAGCCTGGGAACACAGACTTATATCTGTAACGTCAATCACAAGCCTAGCAATACTA
AAGTGGACAAGAAAGCCGAGCCTAAGAGCTGCGAACCAAAGTCCTGTGATAAAACCCATACATGCCCTCCCTGTCCAG
CTCCTGAACTGCTGGGCGGCCCATCCGTGTTCCTGTTTCCACCCAAGCCCAAAGACACCCTGATGATTAGCAGGACTCC
TGAGGTCACCTGCGTGGTCGTGGACGTGTCCCACGAGGACCCCGAAGTCAAGTTTAACTGGTACGTGGATGGCGTCGA
AGTGCATAATGCCAAGACAAAACCCCGGGAGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTCCTGACAGTGCT
GCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTGTCTAATAAGGCCCTGCCAGCTCCCATCGAGAAAAC
AATTTCCAAGGCAAAAGGCCAGCCAAGGGAACCCCAGGTGTACACTCTGCCTCCATCCCGCGACGAGCTGACTAAGAA
CCAGGTCTCTCTGACCTGTCTGGTGAAAGGATTCTATCCAAGCGATATCGCCGTGGAGTGGGAATCCAATGGCCAGCCC
GAGAACAATTACAAGACCACACCCCCTGTGCTGGACAGCGATGGCTCCTTCTTTCTGTATTCAAAGCTGACCGTGGATA
AAAGCCGCTGGCAGCAGGGGAACGTCTTTAGCTGCTCCGTGATGCACGAAGCTCTGCACAATCATTACACCCAGAAGT
CTCTGAGTCTGTCACCTGGCAAGAGGGGACGAAAACGGAGAAGCGGCAGCGGAGCTACAAACTTCAGCCTGCTGAAA
CAGGCAGGCGACGTGGAGGAAAATCCTGGGCCAATGGATTGGACTTGGATTCTGTTCCTGGTGGCAGCCGCTACCAGA
GTCCATTCCGAAATTGTGCTGACCCAGTCTCCCGGAACACTGTCTCTGAGTCCTGGCGAGACAGCCATCATTTCCTGTA
GGACTTCTCAGTACGGGAGTCTGGCATGGTATCAGCAGCGACCAGGACAGGCTCCTCGACTGGTCATCTACTCAGGAA
GCACTCGGGCAGCCGGCATTCCCGACCGATTCTCCGGGTCTCGGTGGGGACCTGATTACAACCTGACCATCTCAAATCT
GGAAAGCGGAGACTTTGGCGTGTACTATTGCCAGCAGTATGAGTTCTTTGGGCAGGGAACCAAGGTCCAGGTGGACAT
CAAACGCACAGTCGCTGCACCAAGCGTGTTCATCTTTCCACCCTCAGATGAACAGCTGAAGTCCGGCACCGCCTCTGTG
GTGTGCCTGCTGAACAATTTCTACCCCCGGGAGGCAAAGGTCCAGTGGAAAGTGGACAACGCCCTGCAGTCTGGCAAT
AGTCAGGAGTCAGTGACTGAACAGGACAGCAAGGATTCCACCTATTCTCTGTCCTCTACTCTGACCCTGAGCAAAGCTG
ATTACGAGAAGCACAAAGTGTATGCATGTGAGGTCACCCACCAGGGACTGCGGTCACCCGTCACCAAGAGCTTCAATC
GCGGAGAGTGTTGATAACTCGAG (SEQ ID NO:64)

图 62