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(54) Title: SWARM IMMUNIZATION WITH ENVELOPES FROM CH505

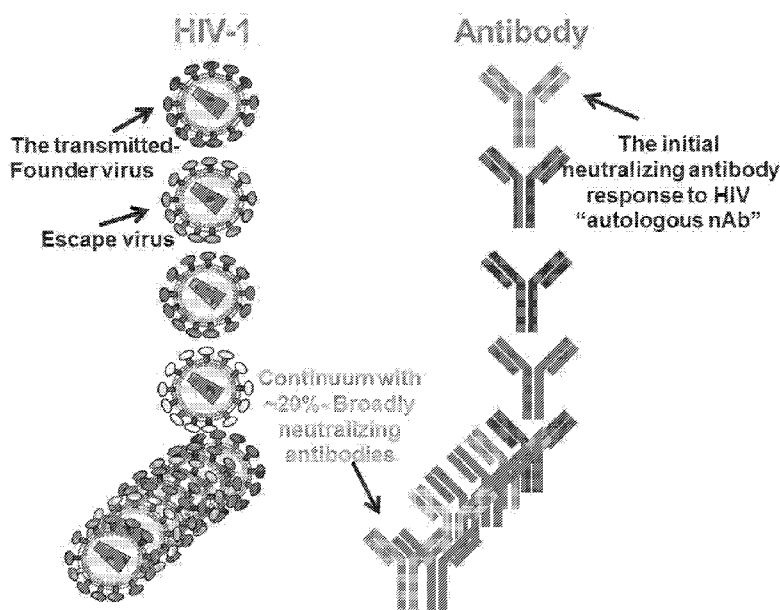


Fig. 15

(57) Abstract: In certain aspects the invention provides HIV-1 immunogens, including CH505 HIV-1 envelopes and selections therefrom, and methods for swarm immunizations using combinations of HIV-1 envelopes.



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## **SWARM IMMUNIZATION WITH ENVELOPES FROM CH505**

[0001] This application claims the benefit of and priority to U.S. Serial No. 62/303,248 filed March 3, 2016, the contents of which is hereby incorporated by reference in its entirety.

[0002] This invention was made with government support under Center for HIV/AIDS Vaccine Immunology-Immunogen Design grant UM1-AI100645 from the NIH, NIAID, Division of AIDS. The government has certain rights in the invention. The United States government also has rights in this invention pursuant to Contract No. DE-AC52-06NA25396 between the United States Department of Energy and Los Alamos National Security, LLC for the operation of Los Alamos National Laboratory.

## **SEQUENCE LISTING**

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 1, 2017, is named 1234300\_259WO1\_SL.TXT and is 2,795,937 bytes in size.

## **TECHNICAL FIELD**

[0004] The present invention relates in general, to a composition suitable for use in inducing anti-HIV-1 antibodies, and, in particular, to immunogenic compositions comprising envelope proteins and nucleic acids to induce cross-reactive neutralizing antibodies and increase their breadth of coverage. The invention also relates to methods of inducing such broadly neutralizing anti-HIV-1 antibodies using such compositions.

## **BACKGROUND**

[0005] The development of a safe and effective HIV-1 vaccine is one of the highest priorities of the scientific community working on the HIV-1 epidemic. While anti-retroviral treatment (ART) has dramatically prolonged the lives of HIV-1 infected patients, ART is not routinely available in developing countries.

## SUMMARY OF THE INVENTION

[0006] In certain embodiments, the invention provides compositions and method for induction of immune response, for example cross-reactive (broadly) neutralizing Ab induction. In certain embodiments, the methods use compositions comprising “swarms” of sequentially evolved envelope viruses that occur in the setting of bnAb generation in vivo in HIV-1 infection. The compositions and methods using swarms could include nucleic acids, proteins, or the combination thereof.

[0007] In certain aspects the invention provides compositions comprising a selection of HIV-1 envelopes or nucleic acids encoding these envelopes as described herein for example but not limited to Selections as described herein. In certain embodiments, these compositions are used in immunization methods as a prime and/or boosts as described in Selections as described herein.

[0008] In one aspect the invention provides selections of envelopes from individual CH505, which selections can be used in compositions for immunizations to induce lineages of broad neutralizing antibodies. In certain embodiments, there is some variance in the immunization regimen; in some embodiments, the selection of HIV-1 envelopes may be grouped in various combinations of primes and boosts, either as nucleic acids, proteins, or combinations thereof. In certain embodiments the compositions are pharmaceutical compositions which are immunogenic.

[0009] In one aspect the invention provides a composition comprising any one of the envelopes described herein, or any combination thereof (Tables 1 and 2, selection A). In some embodiments, CH505 transmitted/founder (T/F) Env is administered first as a prime, followed by a mixture of a next group of Envs, followed by a mixture of a next group of Envs, followed by a mixture of the final Envs. Envelopes and other immunogens could be administered as multiple primes and/or boosts.

[0010] In some embodiments, grouping of the envelopes is based on their binding affinity for the antibodies expected to be induced. In some embodiments, grouping of the envelopes is based on chronological evolution of envelope viruses that occurs in the setting of bnAb generation in vivo in HIV-1 infection. In some embodiments Loop D mutants could be included in either prime and/or boost. In some embodiments, the composition comprises an adjuvant. In some embodiments, the composition and methods comprise use of agents for transient modulation of the host immune response.

[0011] In one aspect the invention provides a composition comprising nucleic acids encoding HIV-1 envelope w000.T/F (or w004.03) and a loop D mutant, e.g. M11 or any other suitable D loop mutant or combination thereof. In some embodiments, the compositions and methods of the invention comprise use of any one of the mutants in Figure 23, e.g., M14 and/or M24. In one aspect the invention provides a composition comprising nucleic acids encoding HIV-1 envelope w000.T/F (or w004.03), M11, w014.32, and w014.12. In one aspect the invention provides a composition comprising nucleic acids encoding a HIV-1 envelope.

[0012] In one aspect the invention provides compositions comprising, consisting essentially of, consisting of nucleic acids encoding and/or the polypeptides of HIV-1 envelopes w000.TF, M5, and/or M11.

[0013] In one aspect the invention provides compositions comprising, consisting essentially of, consisting of nucleic acids encoding and/or the polypeptides of HIV-1 envelopes w004.03, w004.10, w004.26, w014.10, w014.2, w014.21, w014.3, w014.32, w014.8, w020.11, w020.13, w020.14, w020.15, w020.22, w020.23, w020.26, w020.3, w020.4, w020.7, w020.8, w020.9, w030.10, w030.11, w030.12, w030.13, w030.20, w030.25, w030.27, w030.28, and/or w030.36.

[0014] In one aspect the invention provides compositions comprising, consisting essentially of, consisting of nucleic acids encoding and/or the polypeptides of HIV-1 envelopes w030.15, w030.17, w030.18, w030.19, w030.21, w030.23, w030.5, w030.6, w030.9, w053.13, w053.16, w053.19, w053.25, w053.29, w053.3, w053.31, w053.6, w078.10, w078.15, w078.33, w078.38, w078.6, w078.9, w100.A10, w100.A13, w100.A4, w136.B18, w136.B2, w136.B3, and/or w160.T4.

[0015] In one aspect the invention provides compositions comprising, consisting essentially of, consisting of nucleic acids encoding and/or the polypeptides of HIV-1 envelopes w078.1, w078.17, w078.25, w078.7, w100.A12, w100.A3, w100.A6, w100.B2, w100.B4, w100.B6, w100.B7, w100.C7, w136.B10, w136.B12, w136.B20, w136.B27, w136.B29, w136.B36, w136.B4, w136.B5, w136.B8, w160.A1, w160.C11, w160.C12, w160.C14, w160.C2, w160.C4, w160.D1, w160.D5, and/or w160.T2.

[0016] In one aspect the invention provides a composition comprising nucleic acids encoding HIV-1 envelope w000.TF, w004.03, M10, M11, M19, M20, M21, M5, M6, M7, M8, and/or M9.

[0017] In another aspect the invention provides a method of inducing an immune response in a subject comprising administering a composition comprising HIV-1 envelope T/F (or w004.03),

M5 and M11 as a prime in an amount sufficient to induce an immune response, wherein the envelope is administered as a polypeptide or a nucleic acid encoding the same. A method of inducing an immune response in a subject comprising administering a composition comprising HIV-1 envelope T/F (or w004.03), M5, M11, as a prime in an amount sufficient to induce an immune response, wherein the envelope is administered as a polypeptide or a nucleic acid encoding the same.

[0018] In certain embodiments the methods further comprise administering a composition comprising of the selected combination of HIV-1 envelopes in Example 2 or 3, wherein the envelope is administered as a polypeptide or a nucleic acid encoding the same.

[0019] In certain embodiments, the compositions contemplate nucleic acid, as DNA and/or RNA, or proteins immunogens either alone or in any combination. In certain embodiments, the methods contemplate genetic, as DNA and/or RNA, immunization either alone or in combination with envelope protein(s).

[0020] In certain embodiments the nucleic acid encoding an envelope is operably linked to a promoter inserted an expression vector. In certain aspects the compositions comprise a suitable carrier. In certain aspects the compositions comprise a suitable adjuvant.

[0021] In certain embodiments the induced immune response includes induction of antibodies, including but not limited to autologous and/or cross-reactive (broadly) neutralizing antibodies against HIV-1 envelope. Various assays that analyze whether an immunogenic composition induces an immune response, and the type of antibodies induced are known in the art and are also described herein.

[0022] In certain aspects the invention provides an expression vector comprising any of the nucleic acid sequences of the invention, wherein the nucleic acid is operably linked to a promoter. In certain aspects the invention provides an expression vector comprising a nucleic acid sequence encoding any of the polypeptides of the invention, wherein the nucleic acid is operably linked to a promoter. In certain embodiments, the nucleic acids are codon optimized for expression in a mammalian cell, in vivo or in vitro. In certain aspects the invention provides nucleic acid comprising any one of the nucleic acid sequences of invention. A nucleic acid consisting essentially of any one of the nucleic acid sequences of invention. A nucleic acid consisting of any one of the nucleic acid sequences of invention. In certain embodiments the nucleic acid of invention, is operably linked to a promoter and is inserted in an expression vector.

In certain aspects the invention provides an immunogenic composition comprising the expression vector.

[0023] In certain aspects the invention provides a composition comprising at least one of the nucleic acid sequences of the invention. In certain aspects the invention provides a composition comprising any one of the nucleic acid sequences of invention. In certain aspects the invention provides a composition comprising at least one nucleic acid sequence encoding any one of the polypeptides of the invention.

[0024] In certain aspects the invention provides a composition comprising at least one nucleic acid encoding a HIV-1 envelope w000.TF, w004.03, w004.26, M10, M11, M19, M20, M21, M5, M6, M7, M8, M9, w014.10, w014.2, w014.21, w014.3, w014.32, w014.8, w020.3, w020.4, w020.7, w020.8, w020.9, w020.11, w020.13, w020.14, w020.15, w020.19, w020.22, w020.23, w020.24, w020.26, w030.5, w030.6, w030.9, w030.10, w030.11, w030.13, w030.15, w030.17, w030.18, w030.19, w030.20, w030.21, w030.23, w030.25, w030.27, w030.28, w030.36, w053.3, w053.6, w053.13, w053.16, w053.25, w053.29, w053.31, w078.1, w078.6, w078.7, w078.9, w078.10, w078.15, w078.17, w078.25, w078.33, w078.38, w100.A3, w100.A4, w100.A6, w100.A10, w100.A12, w100.A13, w100.B2, w100.B4, w100.B6, w100.B7, w100.C7, w136.B2, w136.B3, w136.B4, w136.B5, w136.B8, w136.B10, w136.B12, w136.B18, w136.B20, w136.B27, w136.B29, w136.B36, w160.A1, w160.C1, w160.C2, w160.C4, w160.C11, w160.C12, w160.C14, w160.D1, w160.D5, w160.T2, w160.T4, or any combination thereof.

[0025] In certain embodiments, the compositions and methods employ an HIV-1 envelope as polypeptide instead of a nucleic acid sequence encoding the HIV-1 envelope. In certain embodiments, the compositions and methods employ an HIV-1 envelope as polypeptide, a nucleic acid sequence encoding the HIV-1 envelope, or a combination thereof.

[0026] The envelope used in the compositions and methods of the invention can be a gp160, gp150, gp145, gp140, gp120, gp41, N-terminal deletion variants as described herein, cleavage resistant variants as described herein, or codon optimized sequences thereof.

[0027] The polypeptide contemplated by the invention can be a polypeptide comprising any one of the polypeptides described herein. The polypeptide contemplated by the invention can be a polypeptide consisting essentially of any one of the polypeptides described herein. The polypeptide contemplated by the invention can be a polypeptide consisting of any one of the polypeptides described herein. In certain embodiments, the polypeptide is recombinantly

produced. In certain embodiments, the polypeptides and nucleic acids of the invention are suitable for use as an immunogen, for example to be administered in a human subject.

[0028] In certain aspects, the invention provides a kit comprising a combination/selection of immunogens of Table 2. In some embodiments the selection of immunogens described in Example 3. In some embodiments the kit comprises instructions on how to carry out the immunization regimen. In some embodiments the kit comprises instructions on administration of the selection of immunogens as a prime or boost as part of a prime/boost immunization regimen. In certain aspects, the invention provides a kit comprising any one of the immunogens of Example 3, and instructions on how to carry out an immunization regimen with the immunogen of the kit, including which immunogen(s) are a prime immunization and which immunogen(s) comprise a boost immunization. In some embodiments the kit comprises instructions on administration of the immunogen as a prime or as a boost as part of a prime/boost immunization regimen. In some embodiments the immunogen could be administered sequentially or additively.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0029] The patent or application file contains at least one drawing executed in color. To conform to the requirements for PCT patent applications, many of the figures presented herein are black and white representations of images originally created in color.

[0030] Figs. 1A-C show CH505 Env polymorphisms, neutralization, vaccine regimes, and phylogeny.

[0031] Figs. 2A-C shows swarm vaccine variant frequencies in concatenated Env “hot-spot” sites, numbered as in Figs. 3A-C. These sites were used to identify immunogens because they include polymorphisms resulting from immune selection by neutralizing antibodies. Three criteria identified Env sites of outstanding interest (“hot spots”) for antibody evolution: (a) “selected” sites with T/F frequency below 20% in any time-point sampled, (b) single or PNG sites with  $q < 0.1$  for tree-corrected signatures of neutralization activity, and (c) CD4 binding-site and known CH103 contacts with any variation. We extracted these sites from aligned sequences and concatenated them to see how each candidate clone varies in Env “hot spots” (Figs. 3A-C). Rather than eliminate sites found by multiple methods, duplicate sites are included multiple times, for emphasis.

[0032] Figs. 3A-C show one embodiment of alignment columns in Env “hot-spot” concatamer summaries.

[0033] Figs. 4A-C show another embodiment of alignment columns in Env “hot-spot” concatamer summaries.

[0034] Fig. 5 shows one embodiment of ten selected Envs as concatenated sites. Columns follow rows of Figs. 1A-C. Potentially glycosylated asparagines are shown as “O”. Asterisks (\*) to the left indicate candidates required for inclusion. Pound signs (#) indicate candidates we recommend be excluded. Names of ten clones recommended for inclusion are bold underlined.

[0035] Fig. 6 shows another embodiment of ten selected Envs as concatenated sites. Columns follow rows of Figs. 1A-C. Potentially glycosylated asparagines are shown as “O”. Asterisks (\*) to the left indicate candidates required for inclusion. Pound signs (#) indicate candidates we recommend be excluded. Names of ten clones recommended for inclusion are bold underlined.

[0036] Fig. 7 shows one embodiment of a proposed swarm of CH505 envelopes.

[0037] Fig. 8 shows another embodiment of a proposed swarm of CH505 envelopes.

[0038] Fig. 9 shows temporal development of CH505 variant frequencies for 36 Env sites from time of infection (Y0) through three years of follow-up (Y3), resulting from development of neutralizing antibody responses with increasing heterologous neutralization breadth. An O indicates a potentially N- (asparagine) linked glycosylation site. For clarity, only variants that exceed 20% frequency in any given sample are shown.

[0039] Fig. 10 shows temporal progression of CH505 variant frequencies for 40 Env sites from time of infection with the Transmitted/Founder virus (w000) through three years of follow-up (w160). Height of each character indicates its frequency per sample. In all except the top row, the Transmitted/Founder virus is not shown and constitutes the remaining portion of the sample. Insertions or deletions (indels) appear as grey blocks. Multiple sites with the same HXB2 numbering correspond to un-numbered insertions towards the C-terminal end of the position numbered.

[0040] Fig. 11 shows hierarchical clustering of CH505 variant frequencies per longitudinal sample (x-axis) for 26 selected CH505 Env mutations. Frequency of non-Transmitted/Founder mutations is proportional to the grey-scale value in each cell, and cells clustered together on the vertical axis indicate Env sites that vary in a concerted manner (i.e. in the same temporal window), rather than independently. Where a numbered site appears more than once (e.g., 359/

V281G and 359/ V281S), it depicts alternative non-Transmitted/Founder variant forms. Sites with indels and variant forms that fail to exceed 25% frequency of any given sample were excluded for clarity.

[0041] Fig. 12 shows hierarchical clustering of Shannon entropies per longitudinal sample (x-axis) for 40 selected CH505 Env sites. Low entropy means high prevalence of a single variant, whether Transmitted/Founder or an escape mutation, and high entropy indicates high variability. This uses the same information as Figs. 9-11 but shows when and where variation is most active, clustering together on the vertical axis sites that share variability (entropy) profiles.

[0042] Figs. 13A-13C is an enlarged version of Figs. 1A-C. Figs. 13A-13C shows the genotype variation (A, left panel), neutralization titers (B, center panel), and Envelope phylogenetic relations (C, right panel) among CH505 Envelope variants. The vertical position in each panel corresponds to the same CH505 Env clone named on the right side of the tree. Distance from the Transmitted/Founder form generally increases from top towards bottom of the figure. In the left panel (A), sites not colored correspond to the Transmitted/Founder virus, red sites show mutations, and black sites correspond to insertions or deletions relative to the Transmitted/Founder virus. Additional annotation indicates the known CD4 binding-site contacts (short, vertical black bars towards top), CH103 binding-site contacts for the resolved structure (short, vertical blue bars with a horizontal line to indicate the region resolved by X-Ray Crystallography), gp120 landmarks (vertical grey rectangular regions, V1-V5 hypervariable loops, Loop D, and CD4 Loops), a dashed vertical line delineating the gp120/gp41 boundary, and results from testing for CTL epitopes with ELISpot assays (magenta bands at top and bottom show where peptides were tested and negative, and a magenta rectangle for the tested positive region outside the C-terminal end of V4). The center panel (B) depicts IC<sub>50</sub> (50% inhibitory concentrations, in µg/ml) values from autologous neutralization assays against 13 monoclonal antibodies (MAbs) of the CH103 lineage and each of 134 CH505 Env-pseudotyped viruses. Color-scale values indicate neutralization potency and range from grey (no neutralization detected) through dark red (potent neutralization, i.e. <0.2 µg/ml; empty cells correspond to absence of information). The cumulative progression of neutralization potency from left to right, corresponding to developmental stages in the CH103 lineage, indicates accumulation of neutralization potency. Similarly, increased presence neutralization signal from top to bottom corresponds to increasing neutralization breadth per MAb in the CH103 lineage. In the right-

most panel (C) is the phylogeny of CH505 Envs, with the x-axis indicating distance from the Transmitted-Founder virus per the scale bar (units are mutations per site). The tree is ordered vertically such that lineages with the most descendants appear towards the bottom. Each leaf on the tree corresponds to a CH505 autologous Env, with the name of the sequence depicted ('w' and symbol color indicate the sample time-point; 'M' indicates a synthetic mutant Env). The color of text in each leaf name indicates its inclusion in a possible embodiment, or grey for exclusion from any embodiments described herein. Three long, vertical lines to the left of the tree depict the phylogenetic distribution of envelopes in three distinct alternative embodiments (identified as "Vaccination Regimes 1-3"), with diamonds used to identify each.

[0043] Fig. 14A shows one embodiment of a swarm of CH505 envelopes (SEQ ID NOS 216, 310, 217, 254, 262, 261, 267, 270, 283, 425, 426, 276 and 273, respectively, in order of appearance). Fig. 14B shows another embodiment of a swarm of CH505 envelopes (SEQ ID NOs 217, 310, 221, 427, 254, 261, 262, 267, 270, 273, 276 and 283, respectively, in order of appearance).

[0044] Fig. 15 shows "The HIV-1 Arms Race" as a graphical representation of mapping the Virus and Antibody from the Time of Transmission.

[0045] Fig. 16 shows isolation of broad neutralizing antibodies from chronically Infected Individual CH505 Followed From Time of Transmission

[0046] Fig. 17 shows tempo and site of accumulation of mutations at the contact sites between virus and CH103 mAb.

[0047] Fig. 18 shows an assay for identification of CD4 Binding Site broad neutralizing lineage antibodies. VRC01 and CH103 CD4Binding Site BnAbs do not bind RSCdelta371(D371). For plasma, a greater than 2.5 fold loss of binding when the titer is over 200 suggests the presence of CD4bs BnAb (Lynch, JVI, 2012).

[0048] Figs. 19A-19B show FACS analysis identifying CH505 TF gp120 Reactive Memory B Cells that Demonstrate RSC3 Binding Reactivity (Gr. 1, animal 5346 in NHP study #79). FACS analysis is carried out essentially as described in Example 1. Fig. 26A shows CH505 TF gp120 DP = 109; RSC3-positive (black DP) = 10 (9%). Fig. 26B shows CH505 TF gp120 DP = 110; RSC3-positive (black DP) = 8 (7%).

[0049] Fig. 20 shows RSC3+, RSC3D371- Memory B Cells in CH505 T/F Env-Immunized #79 NHPs. FACS analysis is carried out essentially as described in Example 1.

[0050] Fig. 21 shows induction of autologous neutralization of both the transmitted/founder CH505 Env and neutralization sensitive CH505 Env variant w004.3 in NHPs. Shown is week 14 neutralization data from TZMbl assay after three immunizations.

[0051] Fig. 22 is a heatmap showing neutralization potency of antibodies in the CH103 lineage against early CH505 mutations. M11 shows enhanced sensitivity relative to the TF, so might serve as a good trigger of the CH103 like lineage.

[0052] Fig. 23 shows a heatmap showing neutralization potency of antibodies in the CH103 lineage against population signature mutations. M14 confers partial resistance on its own, while the others need to be given in combination to confer resistance. In certain embodiments, adding M14 and M24 after affinity maturation is initiated may expand breadth.

[0053] Fig. 24 shows Env diversity accompanies heterologous neutralization breadth. (Left) Frequency of mutations among sites with at least 80% TF loss in any timepoint sampled through week 160; these are the sites we consider candidates for being under the greatest selective pressure from the immune response. (Right) Breadth develops over longitudinal plasma neutralization ID50s against Tier 1 (autologous CH0505.TF, then B|SF162 through B|BG1168) and Tier 2 (A|Q842.d12 through B|AC10.0.29) Env-pseudotyped viruses. Heatmaps summarize neutralization ID50 values.

[0054] Fig. 25. Binding phenotypes. Heatmap of ELISA binding log-AUCs from 40 Abs (columns) and 93 Env gp120s (rows). To illustrate the progression of binding sensitivity over longitudinally sampled Envs, rows are sorted by the number of Abs with log AUCs over 0.1, then by mean log AUC. Sorting by other criteria gives similar results but shows less clearly the progression of binding sensitivity.

[0055] Figs. 26A-B show Env mutations and phenotype. In the heatmaps, row order roughly follows binding susceptibility, modified to group related sequences together. Among mutations, 32 of 35 sites in Fig. 24 occur in gp120; three gp41 sites are not shown. Column order among mutations differs from Fig. 24 to show when TF loss first reached 50% in any sample. The site labeled 144h is a V1 insertion flanked by two non-HBX2 sites (i.e. 144g on its left and 144f right). In the pixel representation (left panel), blanks match the TF, red and blue depict negative and positive charge change, black shows indels, cyan a gain of PNG asparagine, and grey shows other mutations.

[0056] Figs. 27A-B show Env mutations, phenotypes, and phylogeny. Row order follows the leaves in the tree, which was made by maximum likelihood (in phyML with HIVw+G4+I) from gp120s. Column order among mutations follows Figs. 26A-26B. (See Figs. 26A-26B for missing column names.)

[0057] Fig. 28. Sequence logos summarize variant frequency in selected sites. (a) Prime, (b) Boost 1, (c) Boost 2, and (d) Boost 3. Below the top-most row, variants that match the TF sequence are left blank, to emphasize variation. Other colors follow representations in pixel plots (Figs. 26A & 27A). The letter O represents an asparagine in a potential N-linked glycosylation motif.

[0058] Fig. 29A-D. Entropy per site on the Env trimer structure. Shannon entropy values were computed among sequences in (a) Prime, (b) Boost 1, (c) Boost 2, and (d) Boost 3. Sequence entropy varies from 0 where the site is invariant to a maximum value of 1.407 bits, which occurs at HXB2 position 464 (located in the V5 hypervariable loop) in Boost 2. The structure is PDB ID 4TVP. Renderings labeled “side” are oriented with the virus membrane at the bottom and renderings labeled “top” show trimer viewed from the host cell membrane as it is approached by the virion. Entropy quantifies amino-acid variation in a site, independently of TF loss. Boost 3 has lower entropy than Boost 2 in most sites, but more TF loss (compare with Fig. 28).

## DETAILED DESCRIPTION OF THE INVENTION

[0059] The development of a safe, highly efficacious prophylactic HIV-1 vaccine is of paramount importance for the control and prevention of HIV-1 infection. A major goal of HIV-1 vaccine development is the induction of broadly neutralizing antibodies (bnAbs) (Immunol. Rev. 254: 225-244, 2013). BnAbs are protective in rhesus macaques against SHIV challenge, but as yet, are not induced by current vaccines.

[0060] For the past 25 years, the HIV vaccine development field has used single or prime boost heterologous Envs as immunogens, but to date has not found a regimen to induce high levels of bnAbs.

[0061] Recently, a new paradigm for design of strategies for induction of broadly neutralizing antibodies was introduced, that of B cell lineage immunogen design (Nature Biotech. 30: 423, 2012) in which the induction of bnAb lineages is recreated. It was recently demonstrated the power of mapping the co-evolution of bnAbs and founder virus for elucidating the Env evolution

pathways that lead to bnAb induction (Nature 496: 469, 2013). From this type of work has come the hypothesis that bnAb induction will require a selection of antigens to recreate the “swarms” of sequentially evolved viruses that occur in the setting of bnAb generation in vivo in HIV infection (Nature 496: 469, 2013).

[0062] A critical question is why the CH505 immunogens are better than other immunogens. This rationale comes from three recent observations. First, a series of immunizations of single putatively “optimized” or “native” trimers when used as an immunogen have not induced bnAbs as single immunogens. Second, in all the chronically infected individuals who do develop bnAbs, they develop them in plasma after ~2 years. When these individuals have been studied at the time soon after transmission, they do not make bnAbs immediately. Third, now that individual’s virus and bnAb co-evolution has been mapped from the time of transmission to the development of bnAbs, the identification of the specific Envs that lead to bnAb development have been identified-thus taking the guess work out of env choice.

[0063] Two other considerations are important. The first is that for the CH103 bnAb CD4 binding site lineage, the VH4-59 and Vλ3-1 genes are common as are the VDJ, VJ recombinations of the lineage (Liao, Nature 496: 469, 2013). In addition, the bnAb sites are so unusual, we are finding that the same VH and VL usage is recurring in multiple individuals. Thus, we can expect the CH505 Envs to induce CD4 binding site antibodies in many different individuals.

[0064] Finally, regarding the choice of gp120 vs. gp160, for the genetic immunization we would normally not even consider not using gp160. However, in acute infection, gp41 non-neutralizing antibodies are dominant and overwhelm gp120 responses (Tomaras, G et al. J. Virol. 82: 12449, 2008; Liao, HX et al. JEM 208: 2237, 2011). Recently we have found that the HVTN 505 DNA prime, rAd5 vaccine trial that utilized gp140 as an immunogen, also had the dominant response of non-neutralizing gp41 antibodies. Thus, we will evaluate early on the use of gp160 vs gp120 for gp41 dominance.

[0065] In certain aspects the invention provides a strategy for induction of bnAbs is to select and develop immunogens designed to recreate the antigenic evolution of Envs that occur when bnAbs do develop in the context of infection. Therefore, we believe that the groups of CH505 Envs proposed in this study is the “best in class” of current Env immunogens.

[0066] That broadly neutralizing antibodies (bnAbs) occur in nearly all sera from chronically infected HIV-1 subjects suggests anyone can develop some bnAb response if exposed to immunogens via vaccination. Working back from mature bnAbs through intermediates enabled understanding their development from the unmutated ancestor, and showed that antigenic diversity preceded the development of population breadth. See Liao et al. (2013) Nature 496, 469–476. In this study, an individual “CH505” was followed from HIV-1 transmission to development of broadly neutralizing antibodies. This individual developed antibodies targeted to CD4 binding site on gp120. In this individual the virus was sequenced over time, and broadly neutralizing antibody clonal lineage (“CH103”) was isolated by antigen-specific B cell sorts, memory B cell culture, and amplified by VH/VL next generation pyrosequencing. The CH103 lineage began by binding the T/F virus, autologous neutralization evolved through somatic mutation and affinity maturation, escape from neutralization drove rapid (clearly by 20 weeks) accumulation of variation in the epitope, antibody breadth followed this viral diversification (Fig. 15-16).

[0067] Further analysis of envelopes and antibodies from the CH505 individual indicated that a non-CH103 Lineage participates in driving CH103-BnAb induction. For example V1 loop, V5 loop and CD4 binding site loop mutations escape from CH103 and are driven by CH103 lineage. Loop D mutations *enhanced* neutralization by CH103 lineage and are driven by another lineage. Transmitted/founder Env, or another early envelope for example W004.26, triggers naïve B cell with CH103 Unmutated Common Ancestor (UCA) which develop in to intermediate antibodies. Transmitted/founder Env, or another early envelope for example W004.26, also triggers non-CH103 autologous neutralizing Abs that drive loop D mutations in Env that have enhanced binding to intermediate and mature CH103 antibodies and drive remainder of the lineage. In certain embodiments, the inventive composition and methods also comprise loop D mutant envelopes (e.g. but not limited to M10, M11, M19, M20, M21, M5, M6, M7, M8, M9) as immunogens. In certain embodiments, the D-loop mutants are included in a composition used as a prime.

[0068] The invention provides various methods to choose a subset of viral variants, including but not limited to envelopes, to investigate the role of antigenic diversity in serial samples. In other aspects, the invention provides compositions comprising viral variants, for example but not limited to envelopes, selected based on various criteria as described herein to be used as

immunogens. In some embodiments, the immunogens are selected based on the envelope binding to the UCA, and/or intermediate antibodies. In other embodiments the immunogens are selected based on their chronological appearance during infection.

[0069] In other aspects, the invention provides immunization strategies using the selections of immunogens to induce cross-reactive neutralizing antibodies. In certain aspects, the immunization strategies as described herein are referred to as “swarm” immunizations to reflect that multiple envelopes are used to induce immune responses. The multiple envelopes in a swarm could be combined in various immunization protocols of priming and boosting.

[0070] In certain embodiments the invention provides that sites losing the ancestral, transmitted-founder (T/F) state are most likely under positive selection. From acute, homogenous infections with 3-5 years of follow-up, identified herein are sites of interest among plasma single genome analysis (SGA) Envs by comparing the proportion of sequences per time-point in the T/F state with a threshold, typically 5%. Sites with T/F frequencies below threshold are putative escapes. We then selected clones with representative escape mutations. Where more information was available, such as tree-corrected neutralization signatures and antibody contacts from co-crystal structure, additional sites of interest were considered.

[0071] Co-evolution of a broadly neutralizing HIV-1 antibody (CH103) and founder virus was previously reported in African donor (CH505). See Liao et al. (2013) Nature 496, 469–476. In CH505, which had an early antibody that bound autologous T/F virus, we studied 398 envs from 14 time-points over three years (median per sample: 25, range: 18-53). We found 36 sites with T/F frequencies under 20% in any sample. Neutralization and structure data identified 28 and 22 interesting sites, respectively. Together, six gp41 and 53 gp120 sites were identified, plus six V1 or V5 insertions not in HXB2.

[0072] The invention provides an approach to select reagents for neutralization assays and subsequently investigate affinity maturation, autologous neutralization, and the transition to heterologous neutralization and breadth. Given the sustained coevolution of immunity and escape this antigen selection based on antibody and antigen coevolution has specific implications for selection of immunogens for vaccine design.

[0073] In one embodiment, 100 clones were selected that represent the selected sites. In another embodiment, 101 clones were selected that represent the selected sites. In another embodiment, 103 clones were selected that represent the selected sites. In another embodiment, 104 clones

were selected that represent the selected sites. one embodiment, 10 clones were selected that represent the selected sites. In one embodiment, 12 clones were selected that represent the selected sites. In one embodiment, 4 clones were selected that represent the selected sites. These sets of clones represent antigenic diversity by deliberate inclusion of polymorphisms that result from immune selection by neutralizing antibodies, and had a lower clustering coefficient and greater diversity in selected sites than sets sampled randomly. These selections of clones represent various levels of antigenic diversity in the HIV-1 envelope and are based on the genetic diversity of longitudinally sampled SGA envelopes, and correlated with other factors such as antigenic/neutralization diversity, and antibody coevolution.

[0074] Sequences/Clones

[0075] Described herein are nucleic and amino acids sequences of HIV-1 envelopes. The sequences for use as immunogens are in any suitable form. In certain embodiments, the described HIV-1 envelope sequences are gp160s. In certain embodiments, the described HIV-1 envelope sequences are gp120s. Other sequences, for example but not limited to stable SOSIP trimer designs, gp145s, gp140s, both cleaved and uncleaved, gp140 Envs with the deletion of the cleavage (C) site, fusion (F) and immunodominant (I) region in gp41--named as gp140 $\Delta$ CFI (gp140CFI), gp140 Envs with the deletion of only the cleavage (C) site and fusion (F) domain -- named as gp140 $\Delta$ CF (gp140CF), gp140 Envs with the deletion of only the cleavage (C)--named gp140 $\Delta$ C (gp140C) (See e.g. Liao et al. Virology 2006, 353, 268-282), gp150s, gp41s, which are readily derived from the nucleic acid and amino acid gp160 sequences. In certain embodiments the nucleic acid sequences are codon optimized for optimal expression in a host cell, for example a mammalian cell, a rBCG cell or any other suitable expression system.

[0076] An HIV-1 envelope has various structurally defined fragments/forms: gp160; gp140--- including cleaved gp140 and uncleaved gp140 (gp140C), gp140CF, or gp140CFI; gp120 and gp41. A skilled artisan appreciates that these fragments/forms are defined not necessarily by their crystal structure, but by their design and bounds within the full length of the gp160 envelope. While the specific consecutive amino acid sequences of envelopes from different strains are different, the bounds and design of these forms are well known and characterized in the art.

[0077] For example, it is well known in the art that during its transport to the cell surface, the gp160 polypeptide is processed and proteolytically cleaved to gp120 and gp41 proteins.

Cleavages of gp160 to gp120 and gp41 occurs at a conserved cleavage site “REKR.” *See* Chakrabarti et al. Journal of Virology vol. 76, pp. 5357-5368 (2002) see for example Figure 1, and Second paragraph in the Introduction on p. 5357; Binley et al. Journal of Virology vol. 76, pp. 2606-2616 (2002) for example at Abstract; Gao et al. Journal of Virology vol. 79, pp. 1154-1163 (2005); Liao et al. Virology vol. 353(2): 268–282 (2006).

[0078] The role of the furin cleavage site was well understood both in terms of improving cleavage efficiency, *see* Binley et al. *supra*, and eliminating cleavage, *see* Bosch and Pawlita, Virology 64 (5):2337-2344 (1990); Guo et al. Virology 174: 217-224 (1990); McCune et al. Cell 53:55-67 (1988); Liao et al. J Virol. Apr;87(8):4185-201 (2013).

[0079] Likewise, the design of gp140 envelope forms is also well known in the art, along with the various specific changes which give rise to the gp140C (uncleaved envelope), gp140CF and gp140CFI forms. Envelope gp140 forms are designed by introducing a stop codon within the gp41 sequence. *See* Chakrabarti et al. at Figure 1.

[0080] Envelope gp140C refers to a gp140 HIV-1 envelope design with a functional deletion of the cleavage (C) site, so that the gp140 envelope is not cleaved at the furin cleavage site. The specification describes cleaved and uncleaved forms, and various furin cleavage site modifications that prevent envelope cleavage are known in the art. In some embodiments of the gp140C form, two of the R residues in and near the furin cleavage site are changed to E, e.g., **RRVVEREKR** is changed to **ERVVEREKE**, and is one example of an uncleaved gp140 form. Another example is the gp140C form which has the REKR site changed to SEKS. *See supra* for references.

[0081] Envelope gp140CF refers to a gp140 HIV-1 envelope design with a deletion of the cleavage (C) site and fusion (F) region. Envelope gp140CFI refers to a gp140 HIV-1 envelope design with a deletion of the cleavage (C) site, fusion (F) and immunodominant (I) region in gp41. *See* Chakrabarti et al. Journal of Virology vol. 76, pp. 5357-5368 (2002) see for example Figure 1, and Second paragraph in the Introduction on p. 5357; Binley et al. Journal of Virology vol. 76, pp. 2606-2616 (2002) for example at Abstract; Gao et al. Journal of Virology vol. 79, pp. 1154-1163 (2005); Liao et al. Virology vol. 353(2): 268–282 (2006). In certain embodiments, the envelope design in accordance with the present invention involves deletion of residues (e.g., 5-11, 5, 6, 7, 8, 9, 10, or 11 amino acids) at the N-terminus. For delta N-terminal design, amino acid residues ranging from 4 residues or even fewer to 14 residues or even more are deleted.

These residues are between the maturation (signal peptide, usually ending with CX, X can be any amino acid) and "VPVXXXX...". In case of CH505 T/F Env as an example, 8 amino acids (italicized and underlined in the below sequence) were deleted:

MRVMGIQRNYPQWWIWSMLGFWMLMICNG*MWVTYYYG*VPVWKEAKTTLFCASDAKA  
YEKEVHNVWATHACVPTDPNPQE... (rest of envelope sequence is indicated as "...").

In other embodiments, the delta N-design described for CH505 T/F envelope can be used to make delta N-designs of other CH505 envelopes. In certain embodiments, the invention relates generally to an immunogen, gp160, gp120 or gp140, without an N-terminal Herpes Simplex gD tag substituted for amino acids of the N-terminus of gp120, with an HIV leader sequence (or other leader sequence), and without the original about 4 to about 25, for example 11, amino acids of the N-terminus of the envelope (e.g. gp120). See WO2013/006688, e.g. at pages 10-12, the contents of which publication is hereby incorporated by reference in its entirety.

[0082] The general strategy of deletion of N-terminal amino acids of envelopes results in proteins, for example gp120s, expressed in mammalian cells that are primarily monomeric, as opposed to dimeric, and, therefore, solves the production and scalability problem of commercial gp120 Env vaccine production. In other embodiments, the amino acid deletions at the N-terminus result in increased immunogenicity of the envelopes.

[0083] In certain embodiments, the invention provides envelope sequences, amino acid sequences and the corresponding nucleic acids, and in which the V3 loop is substituted with the following V3 loop sequence TRPNNNTRKSIRIGPGQTFY ATGDIIGNIRQAH. This substitution of the V3 loop reduced product cleavage and improves protein yield during recombinant protein production in CHO cells.

[0084] In certain embodiments, the CH505 envelopes will have added certain amino acids to enhance binding of various broad neutralizing antibodies. Such modifications could include but not limited to, mutations at W680G or modification of glycan sites for enhanced neutralization.

[0085] In certain aspects, the invention provides composition and methods which use a selection of sequential CH505 Envs, as gp120s, gp 140s cleaved and uncleaved, gp145s, gp150s and gp160s, as proteins, DNAs, RNAs, or any combination thereof, administered as primes and boosts to elicit immune response. Sequential CH505 Envs as proteins would be co-administered with nucleic acid vectors containing Envs to amplify antibody induction. In a non-limiting embodiment the CH505 Envs include transmitted/founder, week 53, week 58, week 100

envelopes. In certain embodiments, the compositions and methods include any immunogenic HIV-1 sequences to give the best coverage for T cell help and cytotoxic T cell induction. In certain embodiments, the compositions and methods include mosaic and/or consensus HIV-1 genes to give the best coverage for T cell help and cytotoxic T cell induction. In certain embodiments, the compositions and methods include mosaic group M and/or consensus genes to give the best coverage for T cell help and cytotoxic T cell induction. In some embodiments, the mosaic genes are any suitable gene from the HIV-1 genome. In some embodiments, the mosaic genes are Env genes, Gag genes, Pol genes, Nef genes, or any combination thereof. See e.g. US Patent No. 7951377. In some embodiments the mosaic genes are bivalent mosaics. In some embodiments the mosaic genes are trivalent. In some embodiments, the mosaic genes are administered in a suitable vector with each immunization with Env gene inserts in a suitable vector and/or as a protein. In some embodiments, the mosaic genes, for example as bivalent mosaic Gag group M consensus genes, are administered in a suitable vector, for example but not limited to HSV2, would be administered with each immunization with Env gene inserts in a suitable vector, for example but not limited to HSV-2.

[0086] In certain aspects the invention provides compositions and methods of Env genetic immunization either alone or with Env proteins to recreate the swarms of evolved viruses that have led to bnAb induction. Nucleotide-based vaccines offer a flexible vector format to immunize against virtually any protein antigen. Currently, two types of genetic vaccination are available for testing—DNAs and mRNAs.

[0087] In certain aspects the invention contemplates using immunogenic compositions wherein immunogens are delivered as DNA. See Graham BS, Enama ME, Nason MC, Gordon IJ, Peel SA, et al. (2013) DNA Vaccine Delivered by a Needle-Free Injection Device Improves Potency of Priming for Antibody and CD8+ T-Cell Responses after rAd5 Boost in a Randomized Clinical Trial. PLoS ONE 8(4): e59340, page 9. Various technologies for delivery of nucleic acids, as DNA and/or RNA, so as to elicit immune response, both T-cell and humoral responses, are known in the art and are under developments. In certain embodiments, DNA can be delivered as naked DNA. In certain embodiments, DNA is formulated for delivery by a gene gun. In certain embodiments, DNA is administered by electroporation, or by a needle-free injection technologies, for example but not limited to Biojector® device. In certain embodiments, the DNA is inserted in vectors. The DNA is delivered using a suitable vector for expression in

mammalian cells. In certain embodiments the nucleic acids encoding the envelopes are optimized for expression. In certain embodiments DNA is optimized, e.g. codon optimized, for expression. In certain embodiments the nucleic acids are optimized for expression in vectors and/or in mammalian cells. In non-limiting embodiments these are bacterially derived vectors, adenovirus based vectors, rAdenovirus (e.g. Barouch DH, et al. *Nature Med.* 16: 319-23, 2010), recombinant mycobacteria (e.g. rBCG or *M smegmatis*) (Yu, JS et al. *Clinical Vaccine Immunol.* 14: 886-093, 2007; *ibid* 13: 1204-11, 2006), and recombinant vaccinia type of vectors (Santra S. *Nature Med.* 16: 324-8, 2010), for example but not limited to ALVAC, replicating (Kibler KV et al., *PLoS One* 6: e25674, 2011 nov 9.) and non-replicating (Perreau M et al. *J. virology* 85: 9854-62, 2011) NYVAC, modified vaccinia Ankara (MVA)), adeno-associated virus, Venezuelan equine encephalitis (VEE) replicons, Herpes Simplex Virus vectors, and other suitable vectors.

[0088] In certain aspects the invention contemplates using immunogenic compositions wherein immunogens are delivered as DNA or RNA in suitable formulations. Various technologies which contemplate using DNA or RNA, or may use complexes of nucleic acid molecules and other entities to be used in immunization. In certain embodiments, DNA or RNA is administered as nanoparticles consisting of low dose antigen-encoding DNA formulated with a block copolymer (amphiphilic block copolymer 704). See Cany et al., *Journal of Hepatology* 2011 vol. 54 j 115–121; Arnaoty et al., Chapter 17 in Yves Bigot (ed.), *Mobile Genetic Elements: Protocols and Genomic Applications, Methods in Molecular Biology*, vol. 859, pp293-305 (2012); Arnaoty et al. (2013) *Mol Genet Genomics*. 2013 Aug;288(7-8):347-63. Nanocarrier technologies called Nanotaxi® for immunogenic macromolecules (DNA, RNA, Protein) delivery are under development. See for example technologies developed by Incellart.

[0089] Nucleic acids, e.g. but not limited to mRNA immunogens, could be delivered by a lipid nanoparticle (LNP) technology. The LNPs could comprise various different lipids that could self assemble to 80-100nm size particles.

[0090] In certain aspects the invention contemplates using immunogenic compositions wherein immunogens are delivered as recombinant proteins. Various methods for production and purification of recombinant proteins suitable for use in immunization are known in the art.

[0091] The immunogenic envelopes can also be administered as a protein boost in combination with a variety of nucleic acid envelope primes (e.g., HIV -1 Envs delivered as DNA expressed in viral or bacterial vectors).

[0092] Dosing of proteins and nucleic acids can be readily determined by a skilled artisan. A single dose of nucleic acid can range from a few nanograms (ng) to a few micrograms ( $\mu$ g) or milligram of a single immunogenic nucleic acid. Recombinant protein dose can range from a few  $\mu$ g micrograms to a few hundred micrograms, or milligrams of a single immunogenic polypeptide.

[0093] Administration: The compositions can be formulated with appropriate carriers using known techniques to yield compositions suitable for various routes of administration. In certain embodiments the compositions are delivered via intramuscular (IM), via subcutaneous, via intravenous, via nasal, via mucosal routes, or any other suitable route of immunization.

[0094] The compositions can be formulated with appropriate carriers and adjuvants using techniques to yield compositions suitable for immunization. The compositions can include an adjuvant, such as, for example but not limited to, GLA-SE, alum, poly IC, MF-59 or other squalene-based adjuvant, ASOIB, or other liposomal based adjuvant suitable for protein or nucleic acid immunization. In certain embodiments, TLR agonists are used as adjuvants. In other embodiment, adjuvants which break immune tolerance are included in the immunogenic compositions.

[0095] In certain embodiments, the methods and compositions comprise any suitable agent or immune modulation which could modulate mechanisms of host immune tolerance and release of the induced antibodies. In non-limiting embodiments modulation includes PD-1 blockade; T regulatory cell depletion; CD40L hyperstimulation; soluble antigen administration, wherein the soluble antigen is designed such that the soluble agent eliminates B cells targeting dominant epitopes, or a combination thereof. In certain embodiments, an immunomodulatory agent is administered in at time and in an amount sufficient for transient modulation of the subject's immune response so as to induce an immune response which comprises broad neutralizing antibodies against HIV-1 envelope. Non-limiting examples of such agents is any one of the agents described herein: e.g. chloroquine (CQ), PTP1B Inhibitor - CAS 765317-72-4 - Calbiochem or MSI 1436 clodronate or any other bisphosphonate; a Foxo1 inhibitor, e.g. 344355 | Foxo1 Inhibitor, AS1842856 - Calbiochem; Gleevec, anti-CD25 antibody, anti-CTLA4

antibody, anti-CCR4 Ab, an agent which binds to a B cell receptor for a dominant HIV-1 envelope epitope, or any combination thereof. In certain embodiments, the methods comprise administering a second immunomodulatory agent, wherein the second and first immunomodulatory agents are different.

[0096] There are various host mechanisms that control bNAbs. For example highly somatically mutated antibodies become autoreactive and/or less fit (Immunity 8: 751, 1998; PloS Comp. Biol. 6 e1000800, 2010; J. Thoret. Biol. 164:37, 1993); Polyreactive/autoreactive naïve B cell receptors (unmutated common ancestors of clonal lineages) can lead to deletion of Ab precursors (Nature 373: 252, 1995; PNAS 107: 181, 2010; J. Immunol. 187: 3785, 2011); Abs with long HCDR3 can be limited by tolerance deletion (JI 162: 6060, 1999; JCI 108: 879, 2001). BnAb knock-in mouse models are providing insights into the various mechanisms of tolerance control of MPER BnAb induction (deletion, anergy, receptor editing). Other variations of tolerance control likely will be operative in limiting BnAbs with long HCDR3s, high levels of somatic hypermutations. 2F5 and 4E10 BnAbs were induced in mature antibody knock-in mouse models with MPER peptide-liposome-TLR immunogens. Next step is immunization of germline mouse models and humans with the same immunogens.

[0097] The invention is described in the following non-limiting examples.

Table 1. Summary of nomenclature used to identify sequences

|    | envelope  | gp120            | Plasmid ID   | gp145          | Plasmid ID |
|----|-----------|------------------|--------------|----------------|------------|
| 1. | CH505.M5  | CH505.M5D8gp120  | HV1300531_v2 | CH505.M5gp145  | HV1300656  |
| 2. | CH505.M6  | CH505.M6D8gp120  | HV1300532_v2 | CH505.M6gp145  | HV1300657  |
| 3. | CH505.M7  | CH505.M7D8gp120  | HV1300533_v2 | CH505.M7gp145  | HV1300658  |
| 4. | CH505.M8  | CH505.M8D8gp120  | HV1300534_v2 | CH505.M8gp145  | HV1300659  |
| 5. | CH505.M9  | CH505.M9D8gp120  | HV1300535_v2 | CH505.M9gp145  | HV1300660  |
| 6. | CH505.M10 | CH505.M10D8gp120 | HV1300536_v2 | CH505.M10gp145 | HV1300661  |
| 7. | CH505.M11 | CH505.M11D8gp120 | HV1300537_v2 | CH505.M11gp145 | HV1300662  |
| 8. | CH505.M19 | CH505.M19D8gp120 | HV1300538_v2 | CH505.M19gp145 | HV1300663  |
| 9. | CH505.M20 | CH505.M20D8gp120 | HV1300539_v2 | CH505.M20gp145 | HV1300664  |

|     |              |                     |              |                   |           |
|-----|--------------|---------------------|--------------|-------------------|-----------|
| 10. | CH505.M21    | CH505.M21D8gp120    | HV1300540_v2 | CH505.M21gp145    | HV1300665 |
| 11. | CH505TF      | CH505TFD8gp120      | HV1300541_v2 | CH505TFgp145      | HV1300631 |
| 12. | CH505w004.03 | CH505w004.03D8gp120 |              | CH505w004.03gp145 | HV1300632 |
| 13. | CH505w004.10 | CH505w004.10D8gp120 | HV1300542_v2 | CH505w04.10gp145  | HV1300666 |
| 14. | CH505w014.2  | CH505w014.2D8gp120  | HV1300543_v2 | CH505w14.2gp145   | HV1300667 |
| 15. | CH505w014.3  | CH505w014.3D8gp120  | HV1300544_v2 | CH505w014.3gp145  | HV1300641 |
| 16. | CH505w014.8  | CH505w014.8D8gp120  | HV1300545_v2 | CH505w014.8gp145  | HV1300642 |
| 17. | CH505w014.10 | CH505w014.10D8gp120 | HV1300546_v2 | CH505w14.10gp145  | HV1300668 |
| 18. | CH505w014.21 | CH505w014.21D8gp120 | HV1300547_v2 | CH505w014.21gp145 | HV1300634 |
| 19. | CH505w014.32 | CH505w014.32D8gp120 | HV1300548_v2 | CH505w14.32gp145  | HV1300669 |
| 20. | CH505w020.3  | CH505w020.3D8gp120  | HV1300549_v2 | CH505w20.3gp145   | HV1300670 |
| 21. | CH505w020.4  | CH505w020.4D8gp120  | HV1300550_v2 | CH505w20.4gp145   | HV1300671 |
| 22. | CH505w020.7  | CH505w020.7D8gp120  | HV1300551_v2 | CH505w020.7gp145  | HV1300643 |
| 23. | CH505w020.8  | CH505w020.8D8gp120  | HV1300552_v2 | CH505w20.8gp145   | HV1300672 |
| 24. | CH505w020.9  | CH505w020.9D8gp120  | HV1300553_v2 | CH505w020.9gp145  | HV1300645 |
| 25. | CH505w020.11 | CH505w020.11D8gp120 | HV1300554_v2 | CH505w20.11gp145  | HV1300673 |
| 26. | CH505w020.13 | CH505w020.13D8gp120 | HV1300555_v2 | CH505w20.13gp145* | HV1300684 |
| 27. | CH505w020.14 | CH505w020.14D8gp120 | HV1300556_v2 | CH505w020.14gp145 | HV1300635 |
| 28. | CH505w020.15 | CH505w020.15D8gp120 | HV1300557_v2 | CH505w20.15gp145  | HV1300674 |
| 29. | CH505w020.19 | CH505w020.19D8gp120 | HV1300558_v2 | CH505w20.19gp145  | HV1300675 |
| 30. | CH505w020.22 | CH505w020.22D8gp120 | HV1300559_v2 | CH505w20.22gp145  | HV1300676 |
| 31. | CH505w020.23 | CH505w020.23D8gp120 | HV1300560_v2 | CH505w20.23gp145  | HV1300677 |
| 32. | CH505w020.24 | CH505w020.24D8gp120 | HV1300561_v2 | CH505w20.24gp145  | HV1300678 |
| 33. | CH505w020.26 | CH505w020.26D8gp120 | HV1300562_v2 | CH505w020.26gp145 | HV1300644 |
| 34. | CH505w030.5  | CH505w030.5D8gp120  | HV1300563_v2 | CH505w30.5gp145   | HV1300679 |
| 35. | CH505w030.6  | CH505w030.6D8gp120  | HV1300564_v2 | CH505w30.6gp145   | HV1300680 |
| 36. | CH505w030.9  | CH505w030.9D8gp120  | HV1300565_v2 | CH505w30.9gp145   | HV1300681 |

|     |              |                     |              |                   |           |
|-----|--------------|---------------------|--------------|-------------------|-----------|
| 37. | CH505w030.10 | CH505w030.10D8gp120 | HV1300566_v2 | CH505w30.10gp145  | HV1300682 |
| 38. | CH505w030.11 | CH505w030.11D8gp120 | HV1300567_v2 | CH505w30.11gp145  | HV1300683 |
| 39. | CH505w030.13 | CH505w030.13D8gp120 | HV1300568_v2 | CH505w030.13gp145 | HV1300637 |
| 40. | CH505w030.15 | CH505w030.15D8gp120 | HV1300569_v2 | CH505w30.15gp145  | HV1300685 |
| 41. | CH505w030.17 | CH505w030.17D8gp120 | HV1300570_v2 | CH505w30.17gp145  | HV1300686 |
| 42. | CH505w030.18 | CH505w030.18D8gp120 | HV1300571_v2 | CH505w30.18gp145  | HV1300687 |
| 43. | CH505w030.19 | CH505w030.19D8gp120 | HV1300572_v2 | CH505w030.19gp145 | HV1300756 |
| 44. | CH505w030.20 | CH505w030.20D8gp120 | HV1300573_v2 | CH505w30.20gp145  | HV1300688 |
| 45. | CH505w030.21 | CH505w030.21D8gp120 | HV1300574_v2 | CH505w30.21gp145  | HV1300689 |
| 46. | CH505w030.23 | CH505w030.23D8gp120 | HV1300575_v2 | CH505w30.23gp145  | HV1300690 |
| 47. | CH505w030.25 | CH505w030.25D8gp120 | HV1300576_v2 | CH505w30.25gp145  | HV1300691 |
| 48. | CH505w030.27 | CH505w030.27D8gp120 | HV1300577_v2 | CH505w30.27gp145  | HV1300692 |
| 49. | CH505w030.28 | CH505w030.28D8gp120 | HV1300578_v2 | CH505w030.28gp145 | HV1300636 |
| 50. | CH505w030.36 | CH505w030.36D8gp120 | HV1300579_v2 | CH505w30.36gp145  | HV1300693 |
| 51. | CH505w053.3  | CH505w053.3D8gp120  | HV1300580_v2 | CH505w53.3gp145   | HV1300694 |
| 52. | CH505w053.6  | CH505w053.6D8gp120  | HV1300581    | CH505w53.6gp145   | HV1300695 |
| 53. | CH505w053.13 | CH505w053.13D8gp120 | HV1300582    | CH505w053.13gp145 | HV1300649 |
| 54. | CH505w053.16 | CH505w053.16D8gp120 | HV1300583    | CH505w53.16gp145  | HV1300696 |
| 55. | CH505w053.25 | CH505w053.25D8gp120 | HV1300584    | CH505w53.25gp145  | HV1300697 |
| 56. | CH505w053.29 | CH505w053.29D8gp120 | HV1300585    | CH505w53.29gp145  | HV1300698 |
| 57. | CH505w053.31 | CH505w053.31D8gp120 | HV1300586    | CH505w053.31gp145 | HV1300638 |
| 58. | CH505w078.1  | CH505w078.1D8gp120  | HV1300587    | CH505w078.1gp145  | HV1300650 |
| 59. | CH505w078.6  | CH505w078.6D8gp120  | HV1300588    | CH505w78.6gp145   | HV1300699 |
| 60. | CH505w078.7  | CH505w078.7D8gp120  | HV1300589    | CH505w78.7gp145   | HV1300700 |
| 61. | CH505w078.9  | CH505w078.9D8gp120  | HV1300590    | CH505w78.9gp145   | HV1300701 |
| 62. | CH505w078.10 | CH505w078.10D8gp120 | HV1300591    | CH505w78.10gp145  | HV1300702 |
| 63. | CH505w078.15 | CH505w078.15D8gp120 | HV1300592    | CH505w078.15gp145 | HV1300639 |

|     |               |                      |           |                    |           |
|-----|---------------|----------------------|-----------|--------------------|-----------|
| 64. | CH505w078.17  | CH505w078.17D8gp120  | HV1300593 | CH505w78.17gp145   | HV1300703 |
| 65. | CH505w078.25  | CH505w078.25D8gp120  | HV1300594 | CH505w78.25gp145   | HV1300704 |
| 66. | CH505w078.33  | CH505w078.33D8gp120  | HV1300595 | CH505w78.33gp145   | HV1300705 |
| 67. | CH505w078.38  | CH505w078.38D8gp120  | HV1300596 | CH505w78.38gp145   | HV1300706 |
| 68. | CH505w100.A3  | CH505w100.A3D8gp120  | HV1300597 | CH505w100.A3gp145  | HV1300707 |
| 69. | CH505w100.A4  | CH505w100.A4D8gp120  | HV1300598 | CH505w100.A4gp145  | HV1300708 |
| 70. | CH505w100.A6  | CH505w100.A6D8gp120  | HV1300599 | CH505w100.A6gp145  | HV1300709 |
| 71. | CH505w100.A10 | CH505w100.A10D8gp120 | HV1300600 | CH505w100.A10gp145 | HV1300710 |
| 72. | CH505w100.A12 | CH505w100.A12D8gp120 | HV1300601 | CH505w100.A12gp145 | HV1300711 |
| 73. | CH505w100.A13 | CH505w100.A13D8gp120 | HV1300602 | CH505w100.A13gp145 | HV1300712 |
| 74. | CH505w100.B2  | CH505w100.B2D8gp120  | HV1300603 | CH505w100.B2gp145  | HV1300713 |
| 75. | CH505w100.B4  | CH505w100.B4D8gp120  | HV1300604 | CH505w100.B4gp145  | HV1300640 |
| 76. | CH505w100.B6  | CH505w100.B6D8gp120  | HV1300605 | CH505w100.B6gp145  | HV1300714 |
| 77. | CH505w100.B7  | CH505w100.B7D8gp120  | HV1300606 | CH505w100.B7gp145  | HV1300715 |
| 78. | CH505w100.C7  | CH505w100.C7D8gp120  | HV1300607 | CH505w100.C7gp145  | HV1300716 |
| 79. | CH505w136.B2  | CH505w136.B2D8gp120  | HV1300608 | CH505w136.B2gp145  | HV1300717 |
| 80. | CH505w136.B3  | CH505w136.B3D8gp120  | HV1300609 | CH505w136.B3gp145  | HV1300718 |
| 81. | CH505w136.B4  | CH505w136.B4D8gp120  | HV1300610 | CH505w136.B4gp145  | HV1300719 |
| 82. | CH505w136.B5  | CH505w136.B5D8gp120  | HV1300611 | CH505w136.B5gp145  | HV1300720 |
| 83. | CH505w136.B8  | CH505w136.B8D8gp120  | HV1300612 | CH505w136.B8gp145  | HV1300721 |
| 84. | CH505w136.B10 | CH505w136.B10D8gp120 | HV1300613 | CH505w136.B10gp145 | HV1300722 |
| 85. | CH505w136.B12 | CH505w136.B12D8gp120 | HV1300614 | CH505w136.B12gp145 | HV1300723 |
| 86. | CH505w136.B18 | CH505w136.B18D8gp120 | HV1300615 | CH505w136.B18gp145 | HV1300724 |
| 87. | CH505w136.B20 | CH505w136.B20D8gp120 | HV1300616 | CH505w136.B20gp145 | HV1300725 |
| 88. | CH505w136.B27 | CH505w136.B27D8gp120 | HV1300617 | CH505w136.B27gp145 | HV1300726 |
| 89. | CH505w136.B29 | CH505w136.B29D8gp120 | HV1300618 | CH505w136.B29gp145 | HV1300727 |
| 90. | CH505w136.B36 | CH505w136.B36D8gp120 | HV1300619 | CH505w136.B36gp145 | HV1300728 |

|      |               |                      |           |                    |           |
|------|---------------|----------------------|-----------|--------------------|-----------|
| 91.  | CH505w160.A1  | CH505w160.A1D8gp120  | HV1300620 | CH505w160.A1gp145  | HV1300729 |
| 92.  | CH505w160.C2  | CH505w160.C2D8gp120  | HV1300621 | CH505w160.C2gp145  | HV1300730 |
| 93.  | CH505w160.C4  | CH505w160.C4D8gp120  | HV1300622 | CH505w160.C4gp145  | HV1300731 |
| 94.  | CH505w160.C11 | CH505w160.C11D8gp120 | HV1300623 | CH505w160.C11gp145 | HV1300732 |
| 95.  | CH505w160.C12 | CH505w160.C12D8gp120 | HV1300624 | CH505w160.C12gp145 | HV1300733 |
| 96.  | CH505w160.C14 | CH505w160.C14D8gp120 | HV1300625 | CH505w160.C14gp145 | HV1300734 |
| 97.  | CH505w160.D1  | CH505w160.D1D8gp120  | HV1300626 | CH505w160.D1gp145  | HV1300735 |
| 98.  | CH505w160.D5  | CH505w160.D5D8gp120  | HV1300627 | CH505w160.D5gp145  | HV1300736 |
| 99.  | CH505w160.T2  | CH505w160.T2D8gp120  | HV1300628 | CH505w160.T2gp145  | HV1300737 |
| 100. | CH505w160.T4  | CH505w160.T4D8gp120  | HV1300629 | CH505w160.T4gp145  | HV1300738 |
| 101. | CH505.w4.26   | CH505.w4.26D8gp120   | HV1300777 | CH505.w4.26gp145   | HV1300633 |
| 102. | CH505.w30.12  | CH505.w30.12D8gp120  | HV1300778 | CH505.w30.12gp145  | HV1300646 |
| 103. | CH505.w53.19  | CH505.w53.19D8gp120  | HV1300779 | CH505.w53.19gp145  | HV1300648 |
| 104. | CH05w020.2    | CH05w020.2.D8gp120   | HV1300749 | CH505w020.2.gp145  | HV1300748 |

[0098] Table 2 shows a summary of sequence names and sequence identifiers.

|     | Name         | Gp160 aa<br>SEQ ID NO | Gp160 nt<br>SEQ ID NO | Gp145 aa<br>SEQ ID NO | Gp145 nt<br>SEQ ID NO | Gp120 aa<br>D8<br>SEQ ID NO | Gp120D nt<br>SEQ ID NO |
|-----|--------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------------|------------------------|
| 1.  | CH505.M5     | 307                   | 102                   | 447                   | 551                   | 322                         | 113                    |
| 2.  | CH505.M6     | 316                   | 111                   | 448                   | 552                   | 323                         | 114                    |
| 3.  | CH505.M7     | 312                   | 107                   | 449                   | 553                   | 324                         | 115                    |
| 4.  | CH505.M8     | 314                   | 109                   | 450                   | 554                   | 325                         | 116                    |
| 5.  | CH505.M9     | 311                   | 106                   | 451                   | 555                   | 326                         | 117                    |
| 6.  | CH505.M10    | 309                   | 104                   | 452                   | 556                   | 327                         | 118                    |
| 7.  | CH505.M11    | 310                   | 105                   | 453                   | 557                   | 328                         | 119                    |
| 8.  | CH505.M19    | 308                   | 103                   | 454                   | 558                   | 329                         | 120                    |
| 9.  | CH505.M20    | 313                   | 108                   | 455                   | 559                   | 330                         | 121                    |
| 10. | CH505.M21    | 315                   | 110                   | 456                   | 560                   | 331                         | 122                    |
| 11. | CH505TF      | 216                   | 11                    | 428                   | 532                   | 332*                        | 123*                   |
| 12. | CH505w004.03 | 217                   | 12                    | 429                   | 533                   | 332*                        | 123*                   |
| 13. | CH505w004.10 | 219                   | 14                    | 457                   | 561                   | 333                         | 124                    |
| 14. | CH505w014.2  | 222                   | 17                    | 458                   | 562                   | 334                         | 125                    |
| 15. | CH505w014.3  | 220                   | 15                    | 438                   | 542                   | 335                         | 126                    |
| 16. | CH505w014.8  | 225                   | 20                    | 439                   | 543                   | 336                         | 127                    |

|     |              |     |    |     |     |     |     |
|-----|--------------|-----|----|-----|-----|-----|-----|
| 17. | CH505w014.10 | 224 | 19 | 459 | 563 | 337 | 128 |
| 18. | CH505w014.21 | 223 | 18 | 431 | 535 | 338 | 129 |
| 19. | CH505w014.32 | 221 | 16 | 460 | 564 | 339 | 130 |
| 20. | CH505w020.3  | 239 | 34 | 461 | 565 | 340 | 131 |
| 21. | CH505w020.4  | 229 | 24 | 462 | 566 | 341 | 132 |
| 22. | CH505w020.7  | 227 | 22 | 440 | 544 | 342 | 133 |
| 23. | CH505w020.8  | 230 | 25 | 463 | 567 | 343 | 134 |
| 24. | CH505w020.9  | 236 | 31 | 442 | 546 | 344 | 135 |
| 25. | CH505w020.11 | 233 | 28 | 464 | 568 | 345 | 136 |
| 26. | CH505w020.13 | 238 | 33 | 475 | 578 | 346 | 137 |
| 27. | CH505w020.14 | 231 | 26 | 432 | 536 | 347 | 138 |
| 28. | CH505w020.15 | 232 | 27 | 465 | 569 | 348 | 139 |
| 29. | CH505w020.19 | 237 | 32 | 466 | 570 | 349 | 140 |
| 30. | CH505w020.22 | 226 | 21 | 467 | 571 | 350 | 141 |
| 31. | CH505w020.23 | 234 | 29 | 468 | 572 | 351 | 142 |
| 32. | CH505w020.24 | 235 | 30 | 469 | 573 | 352 | 143 |
| 33. | CH505w020.26 | 228 | 23 | 441 | 545 | 353 | 144 |
| 34. | CH505w030.5  | 249 | 44 | 470 | 644 | 354 | 145 |
| 35. | CH505w030.6  | 243 | 38 | 471 | 574 | 355 | 146 |
| 36. | CH505w030.9  | 247 | 42 | 472 | 575 | 356 | 147 |
| 37. | CH505w030.10 | 253 | 48 | 473 | 576 | 357 | 148 |
| 38. | CH505w030.11 | 240 | 35 | 474 | 577 | 358 | 149 |
| 39. | CH505w030.13 | 255 | 50 | 434 | 538 | 359 | 150 |
| 40. | CH505w030.15 | 252 | 47 | 476 | 579 | 360 | 151 |
| 41. | CH505w030.17 | 242 | 37 | 477 | 580 | 361 | 152 |
| 42. | CH505w030.18 | 246 | 41 | 478 | 581 | 362 | 153 |
| 43. | CH505w030.19 | 256 | 51 | 531 | 634 | 363 | 154 |
| 44. | CH505w030.20 | 241 | 36 | 479 | 582 | 364 | 155 |
| 45. | CH505w030.21 | 245 | 40 | 480 | 583 | 365 | 156 |
| 46. | CH505w030.23 | 251 | 46 | 481 | 584 | 366 | 157 |
| 47. | CH505w030.25 | 244 | 39 | 482 | 585 | 367 | 158 |
| 48. | CH505w030.27 | 250 | 45 | 483 | 586 | 368 | 159 |
| 49. | CH505w030.28 | 254 | 49 | 433 | 537 | 369 | 160 |
| 50. | CH505w030.36 | 248 | 43 | 484 | 587 | 370 | 161 |
| 51. | CH505w053.3  | 257 | 52 | 485 | 588 | 371 | 162 |
| 52. | CH505w053.6  | 259 | 54 | 486 | 589 | 372 | 163 |
| 53. | CH505w053.13 | 263 | 58 | 445 | 549 | 373 | 164 |
| 54. | CH505w053.16 | 262 | 57 | 487 | 590 | 374 | 165 |
| 55. | CH505w053.25 | 260 | 55 | 488 | 591 | 375 | 166 |
| 56. | CH505w053.29 | 258 | 53 | 489 | 592 | 376 | 167 |
| 57. | CH505w053.31 | 261 | 56 | 435 | 539 | 377 | 168 |
| 58. | CH505w078.1  | 269 | 64 | 446 | 550 | 378 | 169 |
| 59. | CH505w078.6  | 264 | 59 | 490 | 593 | 379 | 170 |
| 60. | CH505w078.7  | 273 | 68 | 491 | 594 | 380 | 171 |
| 61. | CH505w078.9  | 266 | 61 | 492 | 595 | 381 | 172 |
| 62. | CH505w078.10 | 271 | 66 | 493 | 596 | 382 | 173 |
| 63. | CH505w078.15 | 270 | 65 | 436 | 540 | 383 | 174 |
| 64. | CH505w078.17 | 268 | 63 | 494 | 597 | 384 | 175 |
| 65. | CH505w078.25 | 265 | 60 | 495 | 598 | 385 | 176 |
| 66. | CH505w078.33 | 267 | 62 | 496 | 599 | 386 | 177 |
| 67. | CH505w078.38 | 272 | 67 | 497 | 600 | 387 | 178 |

|      |                                                                              |                                                    |     |     |     |     |     |
|------|------------------------------------------------------------------------------|----------------------------------------------------|-----|-----|-----|-----|-----|
| 68.  | CH505w100.A3                                                                 | 278                                                | 73  | 498 | 601 | 388 | 179 |
| 69.  | CH505w100.A4                                                                 | 276                                                | 71  | 499 | 602 | 389 | 180 |
| 70.  | CH505w100.A6                                                                 | 274                                                | 69  | 500 | 603 | 390 | 181 |
| 71.  | CH505w100.A10                                                                | 277                                                | 72  | 501 | 604 | 391 | 182 |
| 72.  | CH505w100.A12                                                                | 275                                                | 70  | 502 | 605 | 392 | 183 |
| 73.  | CH505w100.A13                                                                | 284                                                | 79  | 503 | 606 | 393 | 184 |
| 74.  | CH505w100.B2                                                                 | 279                                                | 74  | 504 | 607 | 394 | 185 |
| 75.  | CH505w100.B4                                                                 | 280                                                | 75  | 437 | 541 | 395 | 186 |
| 76.  | CH505w100.B6                                                                 | 283                                                | 78  | 505 | 608 | 396 | 187 |
| 77.  | CH505w100.B7                                                                 | 282                                                | 77  | 506 | 609 | 397 | 188 |
| 78.  | CH505w100.C7                                                                 | 281                                                | 76  | 507 | 610 | 398 | 189 |
| 79.  | CH505w136.B2                                                                 | 294                                                | 89  | 508 | 611 | 399 | 190 |
| 80.  | CH505w136.B3                                                                 | 295                                                | 90  | 509 | 612 | 400 | 191 |
| 81.  | CH505w136.B4                                                                 | 288                                                | 83  | 510 | 613 | 401 | 192 |
| 82.  | CH505w136.B5                                                                 | 293                                                | 88  | 511 | 614 | 402 | 193 |
| 83.  | CH505w136.B8                                                                 | 290                                                | 85  | 512 | 615 | 403 | 194 |
| 84.  | CH505w136.B10                                                                | 285                                                | 80  | 513 | 616 | 404 | 195 |
| 85.  | CH505w136.B12                                                                | 287                                                | 82  | 514 | 617 | 405 | 196 |
| 86.  | CH505w136.B18                                                                | 296                                                | 91  | 515 | 618 | 406 | 197 |
| 87.  | CH505w136.B20                                                                | 292                                                | 87  | 516 | 619 | 407 | 198 |
| 88.  | CH505w136.B27                                                                | 286                                                | 81  | 517 | 620 | 408 | 199 |
| 89.  | CH505w136.B29                                                                | 289                                                | 84  | 518 | 621 | 409 | 200 |
| 90.  | CH505w136.B36                                                                | 291                                                | 86  | 519 | 622 | 410 | 201 |
| 91.  | CH505w160.A1                                                                 | 297                                                | 92  | 520 | 623 | 411 | 202 |
| 92.  | CH505w160.C2                                                                 | 301                                                | 96  | 521 | 624 | 412 | 203 |
| 93.  | CH505w160.C4                                                                 | 302                                                | 97  | 522 | 625 | 413 | 204 |
| 94.  | CH505w160.C11                                                                | 298                                                | 93  | 523 | 626 | 414 | 205 |
| 95.  | CH505w160.C12                                                                | 299                                                | 94  | 524 | 627 | 415 | 206 |
| 96.  | CH505w160.C14                                                                | 300                                                | 95  | 525 | 628 | 416 | 207 |
| 97.  | CH505w160.D1                                                                 | 303                                                | 98  | 526 | 629 | 417 | 208 |
| 98.  | CH505w160.D5                                                                 | 304                                                | 99  | 527 | 630 | 418 | 209 |
| 99.  | CH505w160.T2                                                                 | 305                                                | 100 | 528 | 631 | 419 | 210 |
| 100. | CH505w160.T4                                                                 | 306                                                | 101 | 529 | 632 | 420 | 211 |
| 101. | CH505.w4.26                                                                  | 318                                                | 13  | 430 | 534 | 422 | 213 |
| 102. | CH505.w30.12                                                                 | 319                                                |     |     |     | 423 | 214 |
| 103. | CH505.w53.19                                                                 | 320                                                |     |     |     | 424 | 215 |
| 104. | CH05w020.2                                                                   | 321                                                |     |     | 633 | 421 | 212 |
|      | Other sequences                                                              |                                                    |     |     |     |     |     |
|      | CH505 virus                                                                  | 1                                                  |     |     |     |     |     |
|      | CH505 viral genes: Gag, Pol, Vif, Vpr, Tat, Rev, VPU, Env, Nef, respectively | 2;<br>3;<br>4;<br>5;<br>6;<br>7;<br>8;<br>9;<br>10 |     |     |     |     |     |

\*The gp120 aa and nt sequence for TF and w004.3 envelope is the same.

[0099] SEQ ID NOs. 113-215 and 322-424 comprise non-coding nucleotide sequences flanking the coding sequence. It will be readily understood that the nucleotide sequence encoding the protein may be derived from SEQ ID NOs. 113-215 and 322-424 by reading each sequence from the first ATG in the sequence, which is a start codon, to the first in-frame stop codon following the start codon.

[0100] It is readily understood that the envelope glycoproteins referenced in various examples and figures comprise a signal/leader sequence. It is well known in the art that HIV-1 envelope glycoprotein is a secretory protein with a signal or leader peptide sequence that is removed during processing and recombinant expression (without removal of the signal peptide, the protein is not secreted). *See* for example Li et al. Control of expression, glycosylation, and secretion of HIV-1 gp120 by homologous and heterologous signal sequences. *Virology* 204(1):266-78 (1994) (“Li et al. 1994”), at first paragraph, and Li et al. Effects of inefficient cleavage of the signal sequence of HIV-1 gp120 on its association with calnexin, folding, and intracellular transport. *PNAS* 93:9606-9611 (1996) (“Li et al. 1996”), at 9609. Any suitable signal sequence could be used. In some embodiments the leader sequence is the endogenous leader sequence. Most of the gp120 and gp160 amino acid sequences include the endogenous leader sequence. In other non-limiting examples the leader sequence is human Tissue Plasminogen Activator (TPA) sequence, human CD5 leader sequence (e.g. MPMGSLQPLATLYLLGMLVASVLA). Most of the chimeric designs include CD5 leader sequence. A skilled artisan appreciates that when used as immunogens, and for example when recombinantly produced, the amino acid sequences of these proteins do not comprise the leader peptide sequences.

[0101] Immunogenic compositions and immunization protocols contemplated by the invention include envelope sequences as described herein including but not limited to nucleic acids and/or amino acid sequences of gp160s, gp150s, gp145, cleaved and uncleaved gp140s, stabilized trimers, e.g. but not limited to SOSIP trimers, gp120s, gp41s, N-terminal deletion variants as described herein, cleavage resistant variants as described herein, or codon optimized sequences thereof. A skilled artisan can readily modify the gp160 and gp120 sequences described herein to obtain these envelope variants. The swarm immunization selections can be administered in any subject, for example monkeys, mice, guinea pigs, or human subjects.

[0102] In certain aspects the invention provides a selection of nucleic acids encoding HIV-1 envelopes for immunization wherein the nucleic acid encodes a gp120 envelope, gp120D8 envelope, a gp140 envelope (gp140C, gp140CF, gp140CFI) as soluble or stabilized protomer of a SOSIP trimer, a gp145 envelope, a gp150 envelope, or a transmembrane bound envelope.

## EXAMPLES

### Example 1

[0103] HIV-1 sequences, including envelopes, and antibodies from HIV-1 infected individual CH505 were isolated as described in Liao et al. (2013) Nature 496, 469–476 including supplementary materials.

[0104] Recombinant HIV-1 proteins

[0105] HIV-1 Env genes for subtype B, 63521, subtype C, 1086, and subtype CRF\_01, 427299, as well as subtype C, CH505 autologous transmitted/founder Env were obtained from acutely infected HIV-1 subjects by single genome amplification, codon-optimized by using the codon usage of highly expressed human housekeeping genes, de novo synthesized (GeneScript) as gp140 or gp120 (AE.427299) and cloned into a mammalian expression plasmid pcDNA3.1/hygromycin (Invitrogen). Recombinant Env glycoproteins were produced in 293F cells cultured in serum-free medium and transfected with the HIV-1 gp140- or gp120-expressing pcDNA3.1 plasmids, purified from the supernatants of transfected 293F cells by using Galanthus nivalis lectin-agarose (Vector Labs) column chromatography, and stored at –80 °C. Select Env proteins made as CH505 transmitted/founder Env were further purified by superose 6 column chromatography to trimeric forms, and used in binding assays that showed similar results as with the lectin-purified oligomers.

[0106] ELISA

[0107] Binding of patient plasma antibodies and CH103 clonal lineage antibodies to autologous and heterologous HIV-1 Env proteins was measured by ELISA as described previously. Plasma samples in serial threefold dilutions starting at 1:30 to 1:521,4470 or purified monoclonal antibodies in serial threefold dilutions starting at 100 µg ml<sup>-1</sup> to 0.000 µg ml<sup>-1</sup> diluted in PBS were assayed for binding to autologous and heterologous HIV-1 Env proteins. Binding of biotin-

labelled CH103 at the subsaturating concentration was assayed for cross-competition by unlabelled HIV-1 antibodies and soluble CD4-Ig in serial fourfold dilutions starting at  $10 \mu\text{g ml}^{-1}$ . The half-maximal effective concentration ( $\text{EC}_{50}$ ) of plasma samples and monoclonal antibodies to HIV-1 Env proteins were determined and expressed as either the reciprocal dilution of the plasma samples or concentration of monoclonal antibodies.

[0108] **Surface plasmon resonance affinity and kinetics measurements**

[0109] Binding  $K_d$  and rate constant (association rate ( $K_a$ )) measurements of monoclonal antibodies and all candidate UCAs to the autologous Env C. CH05 gp140 and/or the heterologous Env B.63521 gp120 were carried out on BIAcore 3000 instruments as described previously. Anti-human IgG Fc antibody (Sigma Chemicals) was immobilized on a CM5 sensor chip to about 15,000 response units and each antibody was captured to about 50–200 response units on three individual flow cells for replicate analysis, in addition to having one flow cell captured with the control Synagis (anti-RSV) monoclonal antibody on the same sensor chip. Double referencing for each monoclonal antibody–HIV-1 Env binding interactions was used to subtract nonspecific binding and signal drift of the Env proteins to the control surface and blank buffer flow, respectively. Antibody capture level on the sensor surface was optimized for each monoclonal antibody to minimize rebinding and any associated avidity effects. C.CH505 Env gp140 protein was injected at concentrations ranging from 2 to  $25 \mu\text{g ml}^{-1}$ , and B.63521 gp120 was injected at 50–400  $\mu\text{g ml}^{-1}$  for UCAs and early intermediates IA8 and IA4, 10–100  $\mu\text{g ml}^{-1}$  for intermediate IA3, and 1–25  $\mu\text{g ml}^{-1}$  for the distal and mature monoclonal antibodies. All curve-fitting analyses were performed using global fit of to the 1:1 Langmuir model and are representative of at least three measurements. All data analysis was performed using the BIAevaluation 4.1 analysis software (GE Healthcare).

[0110] Neutralization assays

[0111] Neutralizing antibody assays in TZM-bl cells were performed as described previously. Neutralizing activity of plasma samples in eight serial threefold dilutions starting at 1:20 dilution and for recombinant monoclonal antibodies in eight serial threefold dilutions starting at  $50 \mu\text{g ml}^{-1}$  were tested against autologous and herologous HIV-1 Env-pseudotyped viruses in TZM-bl-based neutralization assays using the methods known in the art. Neutralization breadth of CH103 was determined using a panel of 196 of geographically and genetically diverse Env-pseudoviruses representing the major circulated genetic subtypes and circulating recombinant

forms. HIV-1 subtype robustness is derived from the analysis of HIV-1 clades over time. The data were calculated as a reduction in luminescence units compared with control wells, and reported as IC<sub>50</sub> in either reciprocal dilution for plasma samples or in micrograms per microlitre for monoclonal antibodies.

[0112] The GenBank accession numbers for 292 CH505 Env proteins are KC247375–KC247667, and accessions for 459 V<sub>H</sub>DJ<sub>H</sub> and 174 V<sub>L</sub>J<sub>L</sub> sequences of antibody members in the CH103 clonal lineage are KC575845–KC576303 and KC576304–KC576477, respectively..

## Example 2

[0113] Combinations of antigens derived from CH505 envelope sequences for swarm immunizations

[0114] Provided herein are non-limiting examples of combinations of antigens derived from CH505 envelope sequences for a swarm immunization. The selection includes priming with a virus which binds to the UCA, for example a T/F virus or another early (e.g. but not limited to week 004.3, or 004.26) virus envelope. In certain embodiments the prime could include D-loop variants. In certain embodiments the boost could include D-loop variants. In certain embodiments, these D-loop variants are envelope escape mutants not recognized by the UCA. Non-limiting examples of such D-loop variants are envelopes designated as M10, M11, M19, M20, M21, M5, M6, M7, M8, M9, M14 (TF\_M14), M24 (TF\_24), M15, M16, M17, M18, M22, M23, M24, M25, M26.

[0115] Non-limiting embodiments of envelopes selected for swarm vaccination are shown as the selections described below. A skilled artisan would appreciate that a vaccination protocol can include a sequential immunization starting with the “prime” envelope(s) and followed by sequential boosts, which include individual envelopes or combination of envelopes. In another vaccination protocol, the sequential immunization starts with the “prime” envelope(s) and is followed with boosts of cumulative prime and/or boost envelopes. In certain embodiments, the prime does not include T/F sequence (W000.TF). In certain embodiments, the prime includes w004.03 envelope. In certain embodiments, the prime includes w004.26 envelope. In certain embodiments, the immunization methods do not include immunization with HIV-1 envelope T/F. In other embodiments for example the T/F envelope may not be included when w004.03 or w004.26 envelope is included. In certain embodiments, the immunization methods do not

include a schedule of four valent immunization with HIV-1 envelopes T/F, w053.16, w078.33, and w100.B6.

[0116] In certain embodiments, there is some variance in the immunization regimen; in some embodiments, the selection of HIV-1 envelopes may be grouped in various combinations of primes and boosts, either as nucleic acids, proteins, or combinations thereof.

[0117] In certain embodiments the immunization includes a prime administered as DNA, and MVA boosts. See Goepfert, et al. 2014; “Specificity and 6-Month Durability of Immune Responses Induced by DNA and Recombinant Modified Vaccinia Ankara Vaccines Expressing HIV-1 Virus-Like Particles” J Infect Dis. 2014 Feb 9. [Epub ahead of print].

[0118] **HIV-1 Envelope selection A:** w000.TF, M5, M11; w004.03, w004.10, w004.26, w014.10, w014.2, w014.21, w014.3, w014.32, w014.8, w020.11, w020.13, w020.14, w020.15, w020.22, w020.23, w020.26, w020.3, w020.4, w020.7, w020.8, w020.9, w030.10, w030.11, w030.12, w030.13, w030.20, w030.25, w030.27, w030.28, w030.36; w030.15, w030.17, w030.18, w030.19, w030.21, w030.23, w030.5, w030.6, w030.9, w053.13, w053.16, w053.19, w053.25, w053.29, w053.3, w053.31, w053.6, w078.10, w078.15, w078.33, w078.38, w078.6, w078.9, w100.A10, w100.A13, w100.A4, w136.B18, w136.B2, w136.B3, w160.T4; w078.1, w078.17, w078.25, w078.7, w100.A12, w100.A3, w100.A6, w100.B2, w100.B4, w100.B6, w100.B7, w100.C7, w136.B10, w136.B12, w136.B20, w136.B27, w136.B29, w136.B36, w136.B4, w136.B5, w136.B8, w160.A1, w160.C11, w160.C12, w160.C14, w160.C2, w160.C4, w160.D1, w160.D5, w160.T2.

[0119] These envelopes are listed among the envelopes in Tables 1 and 2 as gp160s, gp145s, gp140s; 103 envelopes as gp120D8s.

[0120] The selections of CH505-Envs were down-selected from a series of 400 CH505 Envs isolated by single-genome amplification followed for 3 years after acute infection, based on experimental data. The enhanced neutralization breadth that developed in the CD4-binding site (bs) CH103 antibody lineage that arose in subject CH505 developed in conjunction with epitope diversification in the CH505's viral quasispecies. It was observed that at 6 months post-infection in there was more diversification in the CD4bs epitope region in this donor than sixteen other acutely infected donors. Population breadth did not arise in the CH103 antibody lineage until the epitope began to diversify. A hypothesis is that the CH103 lineage drove viral escape, but then the antibody adapted to the relatively resistant viral variants. As this series of events was

repeated, the emerging antibodies evolved to tolerate greater levels of diversity in relevant sites, and began to be able to recognize and neutralize diverse heterologous forms for the virus and manifest population breadth. In certain embodiments, the envs are selected from CH505 sequences to reflect diverse variants for making Env pseudoviruses, with the goal of recapitulating CH505 HIV-1 antigenic diversity over time, making sure selected site (i.e. those sites reflecting major antigenic shifts) diversity was represented.

[0121] Specifically, for CH505 the virus and envelope evolution were mapped, and the CH103 CD4 binding-site bnAb evolution. In addition, 135 CH505 varied envelope pseudotyped viruses were made and tested them for neutralization sensitivity by members of the CH103 bnAb lineage (e.g, Figures 13, 22-23). From this large dataset, in one embodiment, Env variants were chosen for immunization based on three major criteria: Env mutants with sites under diversifying selection, in which the transmitted/founder (T/F) Env form vanished below 20% in any sample, i.e. escape variants; signature sites based on autologous neutralization data, i.e. Envs with statistically supported signatures for escape from members of the CH103 bnAb lineage; and sites with mutations at the contact sites of the CH103 antibody and HIV Env. From a set of candidate envs, Envs with mutations in these characteristic sites and representative of Envs with these criteria were chosen. In this manner, a sequential swarm of Envs was selected for immunization to represent the progression of virus escape mutants that evolved during bnAb induction and increasing neutralization breadth in the CH505 donor.

[0122] In certain embodiments, additional two sequences are selected to contain five additional specific amino acid signatures of resistance that were identified at the global population level. These sequences contain statistically defined resistance signatures, which are common at the population level and enriched among heterologous viruses that CH103 fails to neutralize. When they were introduced into the TF sequence, they were experimentally shown to confer partial resistance to antibodies in the CH103 lineage. Following the reasoning that serial viral escape and antibody adaptation to escape is what ultimately selects for neutralizing antibodies that exhibit breadth and potency against diverse variants, in certain embodiments, inclusion of these variants in a vaccine may extend the breadth of vaccine-elicited antibodies even beyond that of the CH103 lineage. Thus the overarching goal will be to trigger a CH103-like lineage first using the CH505TF modified M11, that is well recognized by early CH103 ancestral states, then

vaccinating with antigenic variants, to allow the antibody lineage to adapt through somatic mutation to accommodate the natural variants that arose in CH505.

[0123] The above selection of 93 CH505 sequences is one representation of the accumulation of viral sequence and antigenic diversity in the CD4bs epitope of CH103 in subject CH505

[0124] M5 is a mutant generated to include one mutation in the loop D (N279K). M11 is a mutant generated to include two mutations in the loop D (N279D + V281G relative to the TF sequence) that enhanced binding to the CH103 lineage (see Figure 22). These were early escape mutations for another CD4bs autologous neutralizing antibody lineage, but might have served to promote early expansion of the CH103 lineage.

[0125] In certain embodiments, the two CH103 resistance signature-mutation sequences added to the antigenic swarm are: M14 (TF with S364P), and M24 (TF with S375H + T202K + L520F + G459E) (See Fig. 23). They confer partial resistance to the TF with respect to the CH103 lineage. In certain embodiments, these D-loop mutants are administered in the boost.

### Example 3

[0126] **Env mixtures of the CH505 virus induce CD4 binding site BnAb lineages:**

#### **Immunogen Diversity for Sequential Immunogens Administration**

[0127] Background. CH505 is an HIV-1 infected individual who eventually developed CD4bs antibodies with desirable breadth and potency. Some 398 viruses from CH505 were sequenced by SGA from longitudinal samples, and we selected about 100 to represent diversity that developed in this individual, based on the phylogeny and visualizing the accrual of mutations. (A strategy to select Envs was later formalized and optimized computationally in the LASSIE program, but these Envs were selected prior to LASSIE, with selection aided by the figures.) Of these, roughly 90 sequences were cloned and assayed for binding and/or neutralization sensitivity.

[0128] We (Liao et al. 2013, Gao et al. Cell. 2014 Jul 31; 158(3): 481–491, Bonsignori et al. 2016/ E-Publication March 3, 2016, the contents of each publications including supplemental materials and sequence by accession numbers are incorporated by reference) have previously reported unmutated germ-line ancestors of two B-cell lineages that target the CD4bs in HIV-1 Env, and development of neutralization breadth in response to Env diversification from immune escape. In particular, plasmas from CH505 begin to show increasing neutralization of Tier 1

(easily neutralized) viruses through the first year of infection, followed by gradual development of breadth against Tier 2 viruses that peaks by week 136, within the first three years of infection (Fig. 32).

[0129] Objective. To evaluate the hypothesis that ongoing processes of immune escape and antibody coevolution eventually result in cross-reactive antibody breadth, we will evaluate use of a sequential immunization strategy, which mimics in vivo diversity, to determine whether it induces broadly neutralizing antibodies against HIV-1. A low-diversity prime of three viruses will be followed by three boosts, which to roughly equal numbers ( $n=30$ ) from about 90 different Envs.

[0130] Our hypothesis is two-fold, first that antibody lineages with potential to develop breadth must be triggered, and once triggered, exposure to viral diversity is necessary for breadth to evolve. In this case, CH505 Env diversity sampled through at least week 136 must have contained mutations required to increase breadth. Week 160 is the latest sample we are using for immunogen design, which covers the interval of expanding plasma neutralization breadth (Fig. 32). Antibodies with 78% and 90% breadth were isolated from week 152 and 323 plasmas, respectively.

[0131] We further hypothesize that gradual exposure to accumulating escape variants may be necessary for bnAb induction, so antibody lineages can adapt sequentially to recognize small changes due to mutations in or near an epitope, and increase recognition to tolerate the range of virus diversity ultimately seen among these sites. Jumping too abruptly from the TF to later forms may yield complete escape from the developing antibody lineages or trigger independent lineages.

[0132] Priming will include the transmitted founder (TF) virus and two constructs that carry mutations with enhanced binding to unmutated ancestors of the B-cell lineages of interest. With progressive envelope diversification that reflects diversity in vivo, we hypothesize that sequentially administering immunogens will induce de novo broadly neutralizing antibody development.

[0133] The Env variants (including the TF virus) are complemented by two mutants synthesized by site-directed mutagenesis. These mutated Envs were made to study effects on antibody specificity to alternate forms in Loop D of the CD4 binding site. The 91 Envs from CH505 represent phylogenetic diversity among samples sequenced.

[0134] ELISA binding assays of the Env gp120s against Abs from the CH103 and CH235 lineages give a measure of the interaction phenotype as log-transformed area under the dilution curve, such that high AUCs indicate strong binding affinities and values of 0 represent no binding (Fig. 33).

[0135] Recommendations. Priming will consist of low diversity and contain three Envs, the CH505 TF and two with mutations in Loop D of the CD4 binding site, M5 (N279K) and M11 (N279D + V281A). Three subsequent booster vaccinations will follow, each to contain roughly the same number of immunogens (n=30), in different booster compositions. Sequence diversity in each boost will approximately follow genetic diversity found in vivo by longitudinal sampling. We use binding data from each Env against desirable antibody lineages to help choose the groupings.

[0136] Grouping the binding data first by breadth of sensitivity to the CH103 and CH235 lineages and then by mean binding per Env suggests a partitioning of 93 Env gp120s into 3 groups (Fig. 34). After priming, sequential boosts of immunogens in each group are initially broadly susceptible to binding by both lineages (Boost 1). Mutations among these gp120s occur primarily in Loop D of the CD4bs, a V1 insertion, and the V3 glycan shift from 334 to 332, though mutations in other sites are also present at lower frequencies. Boost 2 increases mutations in V5 and V3 while gradually increasing resistance to the CH235 lineage and the early ancestral intermediates of CH103. Boost 3 is almost entirely resistant to CH235 Abs and immature CH103 precursors, while maintaining diversity among sites associated with Env evolution in CH505. The groups used in each boost loosely reflect temporal Env development, though the timing of sequences in Boost 2 overlaps somewhat with the other Boosts 1 and 3. Boost 1 includes Envs from weeks 4 through 30, Boost 2 from weeks 30 through 136, and Boost 3 includes only Envs sampled on or after week 78 (Fig. 3). The listing below summarizes the Envs in each group. Envelopes from boost 1, 2, and/or 3 could be used as further boosts.

[0137] The grouping Envs by related binding responses does not strictly follow the gp120 phylogeny (Fig. 35). In particular, ordering Envs by their evolutionary distance from the TF Env yields a more scattered distribution of mutations in selected sites and uneven distribution of binding phenotypes than is obtained by the ordering used in Figs. 26A-B. This uneven distribution is most clear among sequences sampled from weeks 30 through week 78, and shown

in the middle half of the tree (i.e., excluding the top and bottom 25% of leaves, which roughly correspond to week 0 through week 20 and week 136 through week 160, respectively).

[0138] These groupings give Env compositions with progressive sequence diversification in selected sites (Fig. 36). A rendering of the trimer structure shows entropy per site for each set (Fig. 37).

[0139] Prime and boost compositions.

[0140] Prime w000.TF, M5, M11.

[0141] Boost 1: w004.03, w004.10, w004.26, w014.10, w014.2, w014.21, w014.3, w014.32, w014.8, w020.11, w020.13, w020.14, w020.15, w020.22, w020.23, w020.26, w020.3, w020.4, w020.7, w020.8, w020.9, w030.10, w030.11, w030.12, w030.13, w030.20, w030.25, w030.27, w030.28, w030.36.

[0142] Boost 2: w030.15, w030.17, w030.18, w030.19, w030.21, w030.23, w030.5, w030.6, w030.9, w053.13, w053.16, w053.19, w053.25, w053.29, w053.3, w053.31, w053.6, w078.10, w078.15, w078.33, w078.38, w078.6, w078.9, w100.A10, w100.A13, w100.A4, w136.B18, w136.B2, w136.B3, w160.T4.

[0143] Boost 3: w078.1, w078.17, w078.25, w078.7, w100.A12, w100.A3, w100.A6, w100.B2, w100.B4, w100.B6, w100.B7, w100.C7, w136.B10, w136.B12, w136.B20, w136.B27, w136.B29, w136.B36, w136.B4, w136.B5, w136.B8, w160.A1, w160.C11, w160.C12, w160.C14, w160.C2, w160.C4, w160.D1, w160.D5, w160.T2.

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## Example 4

- [0153] Immunization protocols in subjects with swarms of HIV-1 envelopes.

[0154] Immunization protocols contemplated by the invention include envelopes sequences as described herein including but not limited to nucleic acids and/or amino acid sequences of gp160s, gp150s, gp145, cleaved and uncleaved gp140s, gp120s, gp41s, N-terminal deletion variants as described herein, cleavage resistant variants as described herein, or codon optimized sequences thereof. A skilled artisan can readily modify the gp160 and gp120 sequences described herein to obtain these envelope variants. The swarm immunization protocols can be administered in any subject, for example monkeys, mice, guinea pigs, or human subjects.

[0155] In non-limiting embodiments, the immunization includes a nucleic acid is administered as DNA, for example in a modified vaccinia vector (MVA). In non-limiting embodiments, the nucleic acids encode gp160 envelopes. In other embodiments, the nucleic acids encode gp120 envelopes. In other embodiments, the boost comprises a recombinant gp120 envelope. The vaccination protocols include envelopes formulated in a suitable carrier and/or adjuvant, for example but not limited to alum. In certain embodiments the immunizations include a prime, as a nucleic acid or a recombinant protein, followed by a boost, as a nucleic acid or a recombinant protein. A skilled artisan can readily determine the number of boosts and intervals between boosts.

[0156] Table 3 A non-limiting example of an immunization protocol with HIV-1 envelopes.

| Envelope                                                                                                                                                                                                                                   | Prime                            | Boost(s)                         | Boost(s) | Boost(s) | Boost(s) |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|----------------------------------|----------|----------|----------|
| w000.TF, M11, M5,                                                                                                                                                                                                                          | As nucleic acids and/or proteins |                                  |          |          |          |
| w004.03, w004.10, w004.26, w014.10, w014.2, w014.21, w014.3, w014.32, w014.8, w020.11, w020.13, w020.14, w020.15, w020.22, w020.23, w020.26, w020.3, w020.4, w020.7, w020.8, w020.9, w030.10, w030.11, w030.12, w030.13, w030.20, w030.25, |                                  | As nucleic acids and/or proteins |          |          |          |

|                                                                                                                                                                                                                                                                                                                                    |  |  |                                           |                                           |  |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|-------------------------------------------|-------------------------------------------|--|
| w030.27, w030.28,<br>w030.36                                                                                                                                                                                                                                                                                                       |  |  |                                           |                                           |  |
| w030.15, w030.17,<br>w030.18, w030.19,<br>w030.21, w030.23,<br>w030.5, w030.6, w030.9,<br>w053.13, w053.16,<br>w053.19, w053.25,<br>w053.29, w053.3,<br>w053.31, w053.6,<br>w078.10,<br>w078.15, w078.33,<br>w078.38, w078.6,<br>w078.9, w100.A10,<br>w100.A13, w100.A4,<br>w136.B18, w136.B2,<br>w136.B3, w160.T4.                |  |  | As nucleic<br>acids<br>and/or<br>proteins |                                           |  |
| w078.1, w078.17,<br>w078.25, w078.7,<br>w100.A12, w100.A3,<br>w100.A6, w100.B2,<br>w100.B4, w100.B6,<br>w100.B7, w100.C7,<br>w136.B10, w136.B12,<br>w136.B20,<br>w136.B27, w136.B29,<br>w136.B36, w136.B4,<br>w136.B5, w136.B8,<br>w160.A1,<br>w160.C11, w160.C12,<br>w160.C14, w160.C2,<br>w160.C4, w160.D1,<br>w160.D5, w160.T2. |  |  |                                           | As nucleic<br>acids<br>and/or<br>proteins |  |

[0157] In certain embodiments an immunization protocol could include the gag protein. This could be for example, a bivalent or trivalent Gag mosaic (Gag1 and Gag 2, Gag 1, Gag 2 and Gag3) in a suitable vector.

### Example 5

[0158] DNA and mRNA vaccination for mimicking HIV envelope evolution during broad neutralizing antibody induction

[0159] In certain aspects the invention provides compositions and methods for HIV-1 vaccine development: DNA and RNA delivery system (for example but not limited by the Nanotaxi® nanoparticle delivery technology), as well as the B Cell Lineage Vaccine Design concept. This example will study the hypothesis that the critical factor for generation of broadly neutralizing antibodies (bnAbs) is exposure of the B cell repertoire to swarms of Env mutants that have developed over time such that the B cells induced both retain the ability to neutralize swarms of autologous viruses, while acquiring the ability to neutralize heterologous viruses.

[0160] B Cell lineage vaccine design concepts envision multiple immunogens to target the unmutated common ancestors (UAs) and intermediate antibodies (IAs) of clonal lineages of potentially protective antibodies to induce these UAs to begin maturation to generate protective antibody responses. Translational studies aimed at testing such concepts are required; however, the key would be to select appropriate immunogens that can be easily delivered either as a mix or in sequential manner and to determine the appropriate frequency of administrations. Nanotaxi®-based immunogens allows for easy handling and manipulations for such a complex set of vaccine immunogens.

[0161] The example will use the new CH505 set of T/F and sequential evolved Env envelopes that gave rise to the CH103 and CH235 bNAb lineages to generated broadly neutralizing CD4 binding site (bs) bNAb responses. A series of evolved viruses were chosen which will be tested as either mRNAs or DNAs, for example but not limited administered by the Nanotaxi® technology.

[0162] Once synthesized, the Nanotaxi® immunogens will be fully characterized as chemical entities using existing analytical approaches. Physico-chemical analyses will be performed by Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS) and High-Performance Liquid Chromatography (HPLC) to ensure both the identity and the purity of the compounds. Once the Nanotaxi® are prepared, they will be formulated with DNA and mRNA, following a self-assembling process. The formulation will in turn be characterized, in terms of size and zeta potential of the complexed Nanotaxi®.

[0163] **CH103 germline knock-in mice:** The selected immunogens could be used to immunize

[0164] **Group 1** Immunization with DNA or mRNA formulated with Nanotaxi® with CH505 transmitted/founder (T/F) Env M5, M11 first, followed by a mixture of Boost 1, followed by a

mixture of the next 30 Envs, followed by a mixture of the final 30 Envs Example 3. Loop D mutants are included in the prime.

[0165] **Group 2** Immunization with DNA or mRNA formulated with Nanotaxi® with CH505 transmitted/founder (T/F) Env M5, M11 first, followed by a mixture of Boost 1, followed by a mixture of the next 30 Envs, followed by a mixture of the final 30 Envs Example 3. Loop D mutants are included in the prime. Here the genetic immunization will be the same as in group 1 except each immunization will be accompanied by selected CH505 Env proteins as gp120s. These envelope proteins could be autologous, i.e. corresponding to prime and/or boost DNAs, or heterologous, i.e. encoding proteins which are not used in the prime/boost DNA.

[0166] Immunization studies could be conducted in **Rhesus Macaques**.

[0167] All immunizations in monkeys will be performed IM. Immunizations could continue for 2.5 years in the rhesus macaques. NHP immunizations will be analyzed for various immune response, including but not limited to induction of titers of CH505 Env antibodies, and the repertoire of clonal lineages of antibodies induced will be determined by a) memory B cell sorts using the CH505 gp120 as a fluorophor-labeled “hook”, b) clonal memory B cell cultures with screening for single cells producing bnAbs, c) Atreca Inc. (Immune Repertoire Capture™ technology) screens of extent of clonal diversity using either plasma cells or memory B cells sorts with maintenance of VH and VL natural pairs, and d) Illumina MiSeq analysis of clonal expansions in NHPs with the vaccinations.

[0168] Table 4 Below is one embodiment of an immunization study. The gp120 proteins could be gp120 deltaN variants as described herein.

| Bleed Date | Receive @ BIDMC | Instructions                                                                                                                                                                         | Samples Qty/Volume Needed @ Bioqual | Assay @ BIDMC                                  |
|------------|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|------------------------------------------------|
| Wk 0       | Wk 0            | <b>Immunization #1</b><br><br><b>Group 1-</b> w.000.TF + CH505 M5 + M11 gp145<br>DNA alone (IM) + w.000.TF gp 120 M5 + M11<br>gp 120 in GLA-SE (IM)<br><br><b>Monkey ID (n = 4):</b> | 8 ml EDTA + 3 ml SST                | Freeze<br>Plasma,<br>serum,<br>PBMC<br><br>CBC |

|      |      |                                                                                                                                                                                                                                                                                          |                      |                                       |
|------|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|---------------------------------------|
|      |      | <p><b>Group 2- w.000.TF + CH505 MS + M11 gp145 DNA (IM) with NanoTaxi + w.000.TF gp 120 MS + M11 gp 120 in GLA-SE (IM)</b></p> <p><b>Monkey ID (n = 4):</b></p> <p><b>1.0 ml IM (2 sites x 500 ul)</b></p> <p><b>Total DNA dose = 4 mg</b></p> <p><b>Total protein dose = 150 ug</b></p> |                      |                                       |
| Wk 2 | Wk 2 |                                                                                                                                                                                                                                                                                          | 8 ml EDTA + 3 ml SST | Freeze Plasma, serum, PBMC<br><br>CBC |
| Wk 6 | Wk 6 | <p><b>Immunization #2</b></p> <p><b>Group 1- 30 gp145 DNA alone + 30 gp 120's in GLA-SE</b></p> <p><b>Monkey ID (n = 4):</b></p> <p><b>Group 2- 30 gp145 DNA with NanoTaxi + 30 gp 120's in GLA-SE</b></p> <p><b>Monkey ID (n = 4):</b></p>                                              | 8 ml EDTA + 3 ml SST | Freeze Plasma, serum, PBMC<br><br>CBC |

|       |       |                                                                                                                                                                                                                                                                                 |                         |                                                |
|-------|-------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------------------------------|
|       |       | <b>1.0 ml IM (2 sites x 500 ul)</b>                                                                                                                                                                                                                                             |                         |                                                |
| Wk 8  | Wk 8  |                                                                                                                                                                                                                                                                                 | 8 ml EDTA + 3 ml<br>SST | Freeze<br>Plasma,<br>serum,<br>PBMC<br><br>CBC |
| Wk 12 | Wk 12 | <b>Immunization #3</b><br><br><b>Group 1-</b> 30 gp145 DNA alone + 30 gp 120's in GLA-SE<br><br><b>Monkey ID (n = 4):</b><br><br><b>Group 2-</b> 30 gp145 DNA with NanoTaxi + 30 gp 120's in GLA-SE<br><br><b>Monkey ID (n = 4):</b><br><br><b>1.0 ml IM (2 sites x 500 ul)</b> | 8 ml EDTA + 3 ml<br>SST | Freeze<br>Plasma,<br>serum,<br>PBMC<br><br>CBC |
| Wk 14 | Wk 14 |                                                                                                                                                                                                                                                                                 | 8 ml EDTA + 3 ml<br>SST | Freeze<br>Plasma,<br>serum,<br>PBMC<br><br>CBC |
| Wk 18 | Wk 18 | <b>Immunization #4</b><br><br><b>Group 1-</b> 30 gp145 DNA alone + 30 gp 120's in                                                                                                                                                                                               | 8 ml EDTA + 3 ml<br>SST | Freeze<br>Plasma,<br>serum,                    |

|       |       |                                                                                                                                                                                                                                                                               |                      |                                                                   |
|-------|-------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|-------------------------------------------------------------------|
|       |       | <p>GLA-SE</p> <p><b>Monkey ID (n = 4):</b></p> <p><b>Group 2- 30 gp145 DNA with NanoTaxi + 30 gp 120's in GLA-SE</b></p> <p><b>Monkey ID (n = 4):</b></p> <p><b>1.0 ml IM (2 sites x 500 ul)</b></p>                                                                          |                      | <p>PBMC</p> <p>CBC</p>                                            |
| Wk 20 | Wk 20 |                                                                                                                                                                                                                                                                               | 8 ml EDTA + 3 ml SST | <p>Freeze</p> <p>Plasma,</p> <p>serum,</p> <p>PBMC</p> <p>CBC</p> |
|       |       | 3 month resting period                                                                                                                                                                                                                                                        |                      |                                                                   |
| Wk 30 | Wk 30 | <p><b>Immunization #5 with same immunogens as immune #4</b></p> <p><b>Group 1- 30 gp145 DNA alone + 30 gp 120's in GLA-SE</b></p> <p><b>Monkey ID (n = 4):</b></p> <p><b>Group 2- 30 gp145 DNA with NanoTaxi + 30 gp 120's in GLA-SE</b></p> <p><b>Monkey ID (n = 4):</b></p> | 8 ml EDTA + 3 ml SST | <p>Freeze</p> <p>Plasma,</p> <p>serum,</p> <p>PBMC</p> <p>CBC</p> |

|       |       |                              |                         |                                                |
|-------|-------|------------------------------|-------------------------|------------------------------------------------|
|       |       | 1.0 ml IM (2 sites x 500 ul) |                         |                                                |
| Wk 32 | Wk 32 |                              | 8 ml EDTA + 3 ml<br>SST | Freeze<br>Plasma,<br>serum,<br>PBMC<br><br>CBC |

**What is claimed is:**

1. A composition comprising:
  - (a) a nucleic acid encoding a HIV-1 envelope from Table 2 or any combination thereof;  
and/or
  - (b) an HIV-1 envelope polypeptide from Table 2 or any combination thereof.
2. A composition comprising an HIV-1 envelope polypeptide from Table 2 or any combination thereof.
3. The composition of claim 1 or 2 wherein the HIV-1 envelope is T/F and a loop D mutant envelope.
4. The composition of claim 1 wherein the nucleic acid encodes a gp145 envelope.
5. The composition of claim 1 or 2 wherein the HIV-1 envelope is gp120D8 variant.
6. The composition of claim 1 or 2 further comprising an adjuvant.
7. The composition of any one of claim 1 or 4 wherein the nucleic acid is operably linked to a promoter inserted an expression vector.
8. The composition of claim 1 wherein the composition comprises nucleic acids encoding and/or the polypeptides of HIV-1 envelopes w000.TF, M5, and/or M11.
9. The composition of claim 1 wherein the composition comprises nucleic acids encoding and/or the polypeptides of HIV-1 envelopes w004.03, w004.10, w004.26, w014.10, w014.2, w014.21, w014.3, w014.32, w014.8, w020.11, w020.13, w020.14, w020.15, w020.22, w020.23, w020.26, w020.3, w020.4, w020.7, w020.8, w020.9, w030.10, w030.11, w030.12, w030.13, w030.20, w030.25, w030.27, w030.28, and/or w030.36.
10. The composition of claim 1 wherein the composition comprises nucleic acids encoding and/or the polypeptides of HIV-1 envelopes w030.15, w030.17, w030.18, w030.19, w030.21, w030.23, w030.5, w030.6, w030.9, w053.13, w053.16, w053.19, w053.25, w053.29, w053.3, w053.31, w053.6, w078.10, w078.15, w078.33, w078.38, w078.6, w078.9, w100.A10, w100.A13, w100.A4, w136.B18, w136.B2, w136.B3, and/or w160.T4.
11. The composition of claim 1 wherein the composition comprises nucleic acids encoding and/or the polypeptides of HIV-1 envelopes w078.1, w078.17, w078.25, w078.7, w100.A12, w100.A3, w100.A6, w100.B2, w100.B4, w100.B6, w100.B7, w100.C7, w136.B10, w136.B12, w136.B20, w136.B27, w136.B29, w136.B36, w136.B4, w136.B5, w136.B8,

- w160.A1, w160.C11, w160.C12, w160.C14, w160.C2, w160.C4, w160.D1, w160.D5, and/or w160.T2.
12. The composition of claim 2 wherein the composition comprises the polypeptides of HIV-1 envelopes w000.TF, M5, and/or M11.
  13. The composition of claim 2 wherein the composition comprises the polypeptides of HIV-1 envelopes w004.03, w004.10, w004.26, w014.10, w014.2, w014.21, w014.3, w014.32, w014.8, w020.11, w020.13, w020.14, w020.15, w020.22, w020.23, w020.26, w020.3, w020.4, w020.7, w020.8, w020.9, w030.10, w030.11, w030.12, w030.13, w030.20, w030.25, w030.27, w030.28, and/or w030.36.
  14. The composition of claim 2 wherein the composition the polypeptides of HIV-1 envelopes w030.15, w030.17, w030.18, w030.19, w030.21, w030.23, w030.5, w030.6, w030.9, w053.13, w053.16, w053.19, w053.25, w053.29, w053.3, w053.31, w053.6, w078.10, w078.15, w078.33, w078.38, w078.6, w078.9, w100.A10, w100.A13, w100.A4, w136.B18, w136.B2, w136.B3, and/or w160.T4.
  15. The composition of claim 2 wherein the composition comprises the polypeptides of HIV-1 envelopes w078.1, w078.17, w078.25, w078.7, w100.A12, w100.A3, w100.A6, w100.B2, w100.B4, w100.B6, w100.B7, w100.C7, w136.B10, w136.B12, w136.B20, w136.B27, w136.B29, w136.B36, w136.B4, w136.B5, w136.B8, w160.A1, w160.C11, w160.C12, w160.C14, w160.C2, w160.C4, w160.D1, w160.D5, and/or w160.T2.
  16. A method of inducing an immune response in a subject comprising administering a composition comprising a nucleic acid encoding an HIV-1 envelope(s) from Table 2 or an HIV-1 polypeptide(s) of Table 2, or any combination thereof, in an amount sufficient to induce an immune response.
  17. The method of claim 12, wherein the composition comprises nucleic acid(s) encoding and/or polypeptide(s) of HIV-1 envelopes w000.TF, M5, or M11 administered as a prime.
  18. The method of claim 12, further comprising administering an adjuvant.
  19. The method of claim 8, further comprising administering an agent which modulates host immune tolerance.
  20. The method of claim 12, further comprising administering an immunogenic HIV-1 sequences to give the best coverage for T cell help and cytotoxic T cell induction.

A. Envelope Genotype Variants

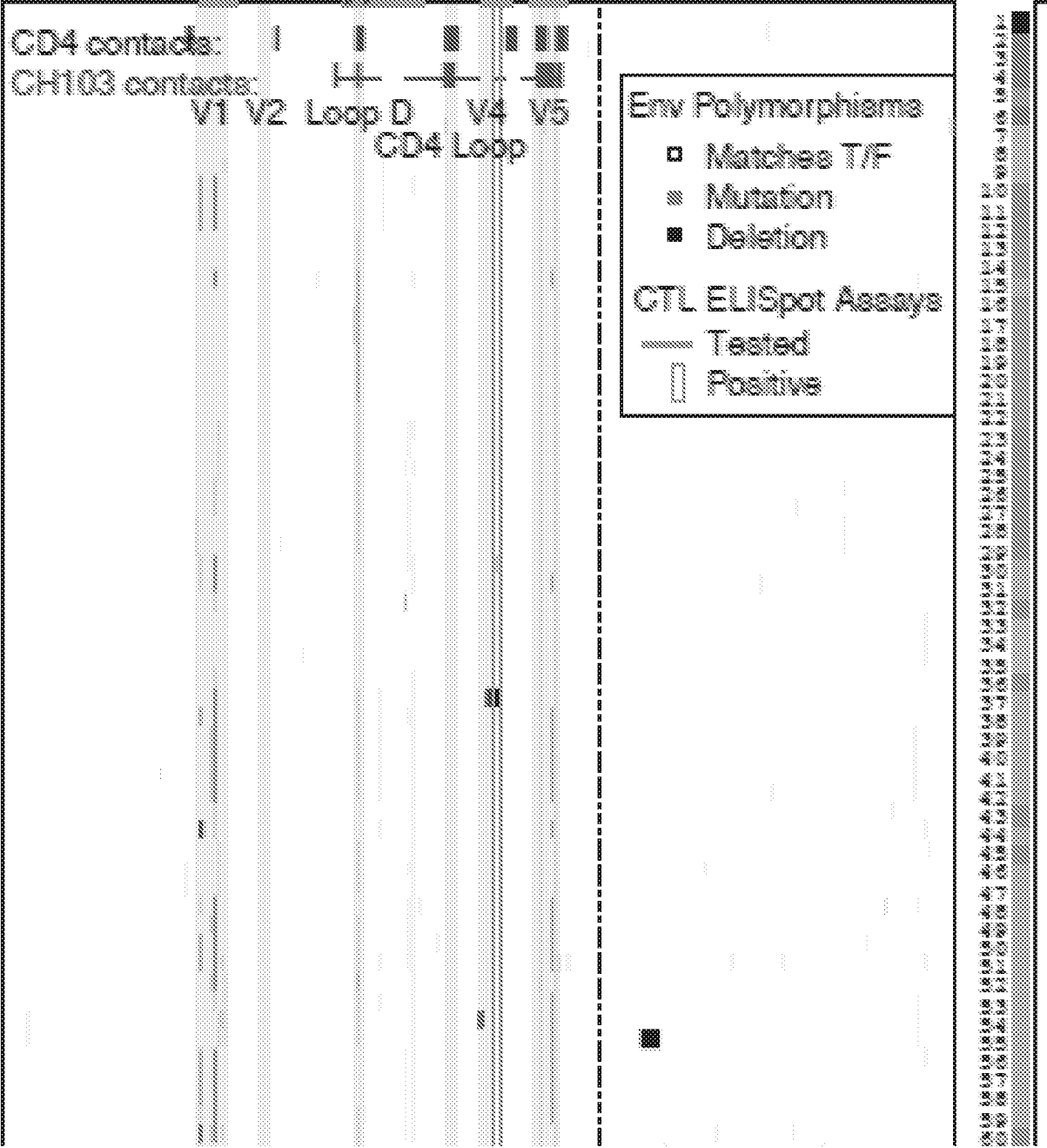


Fig. 1A

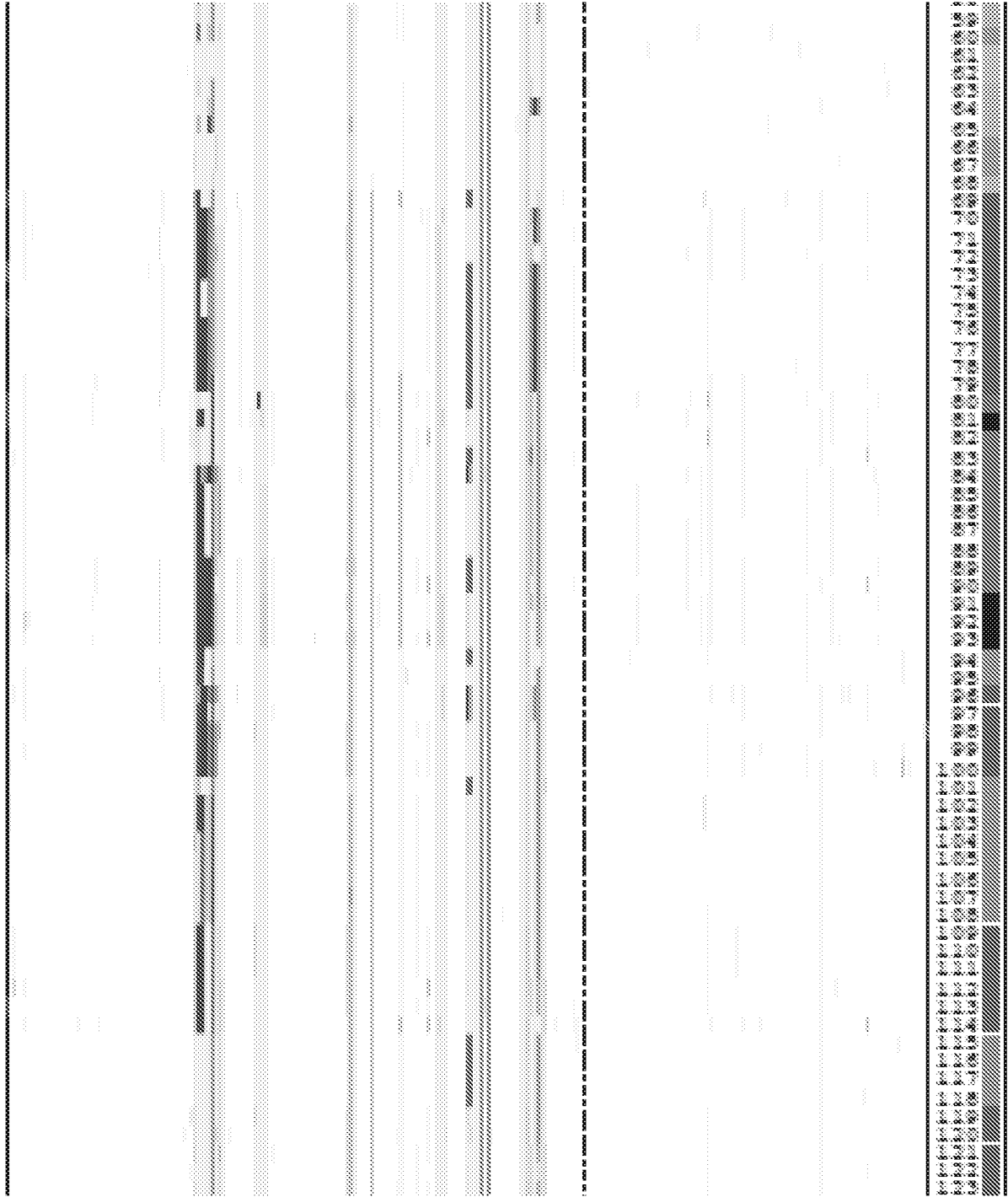


Fig. 1A (cont.)

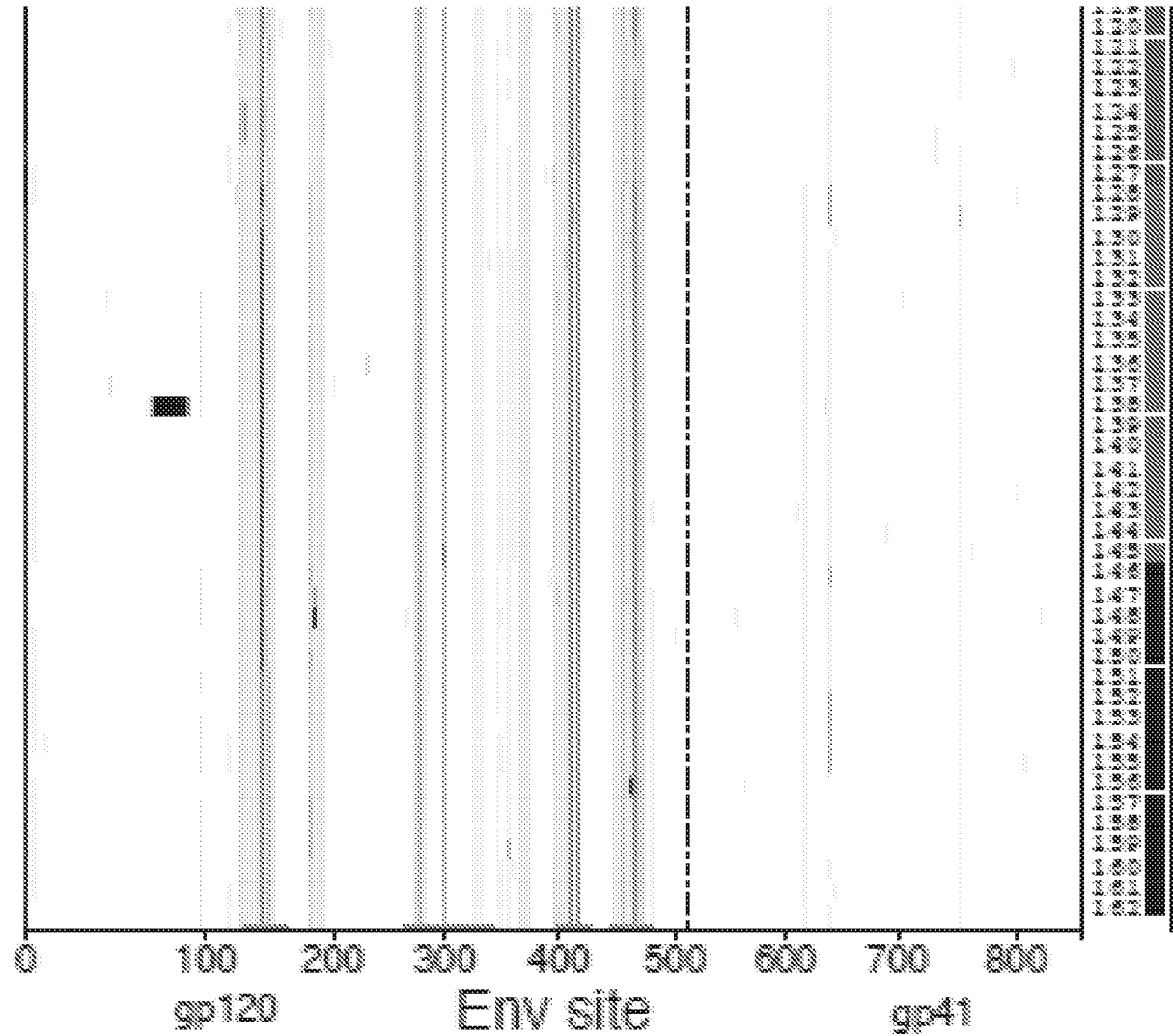
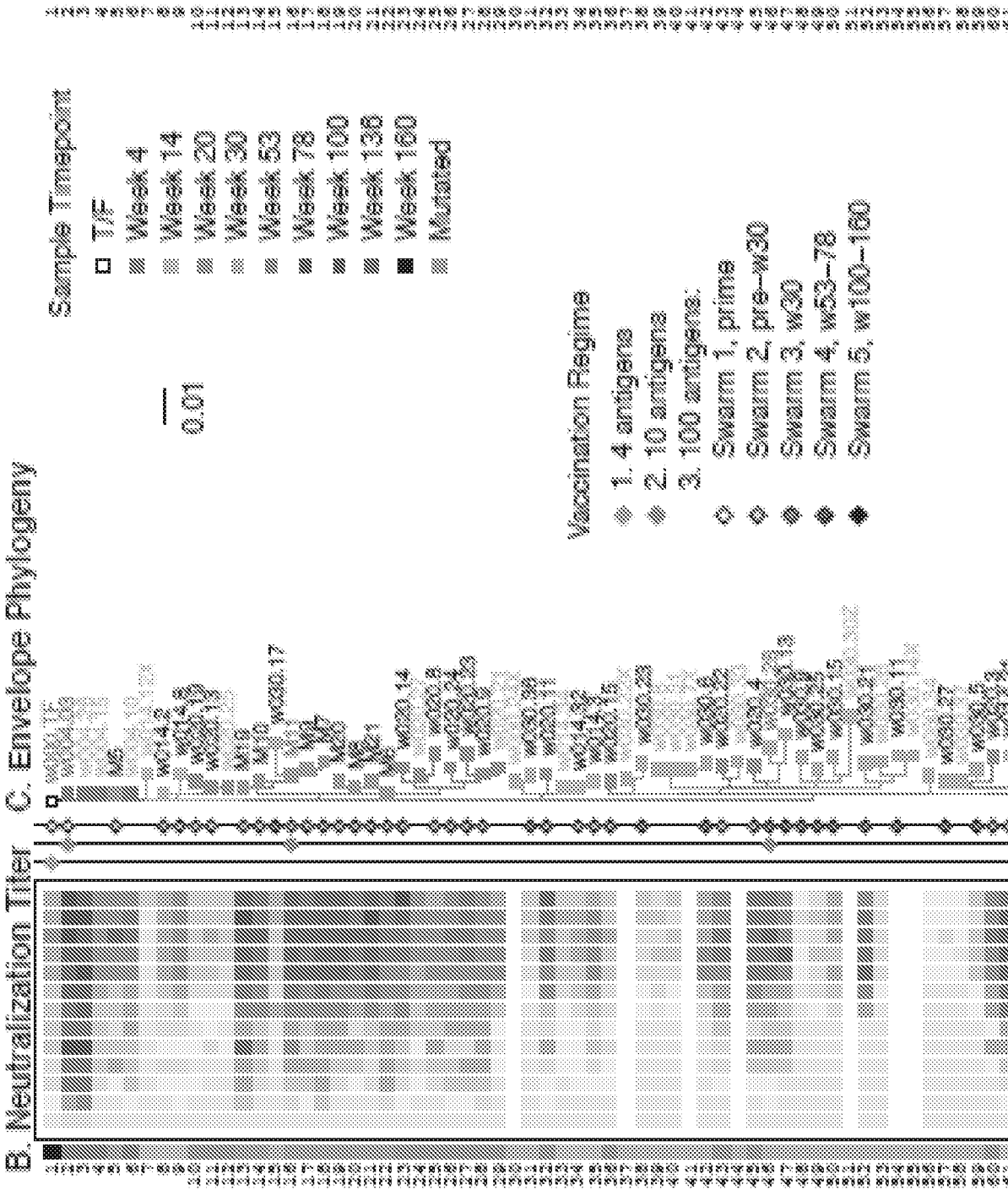
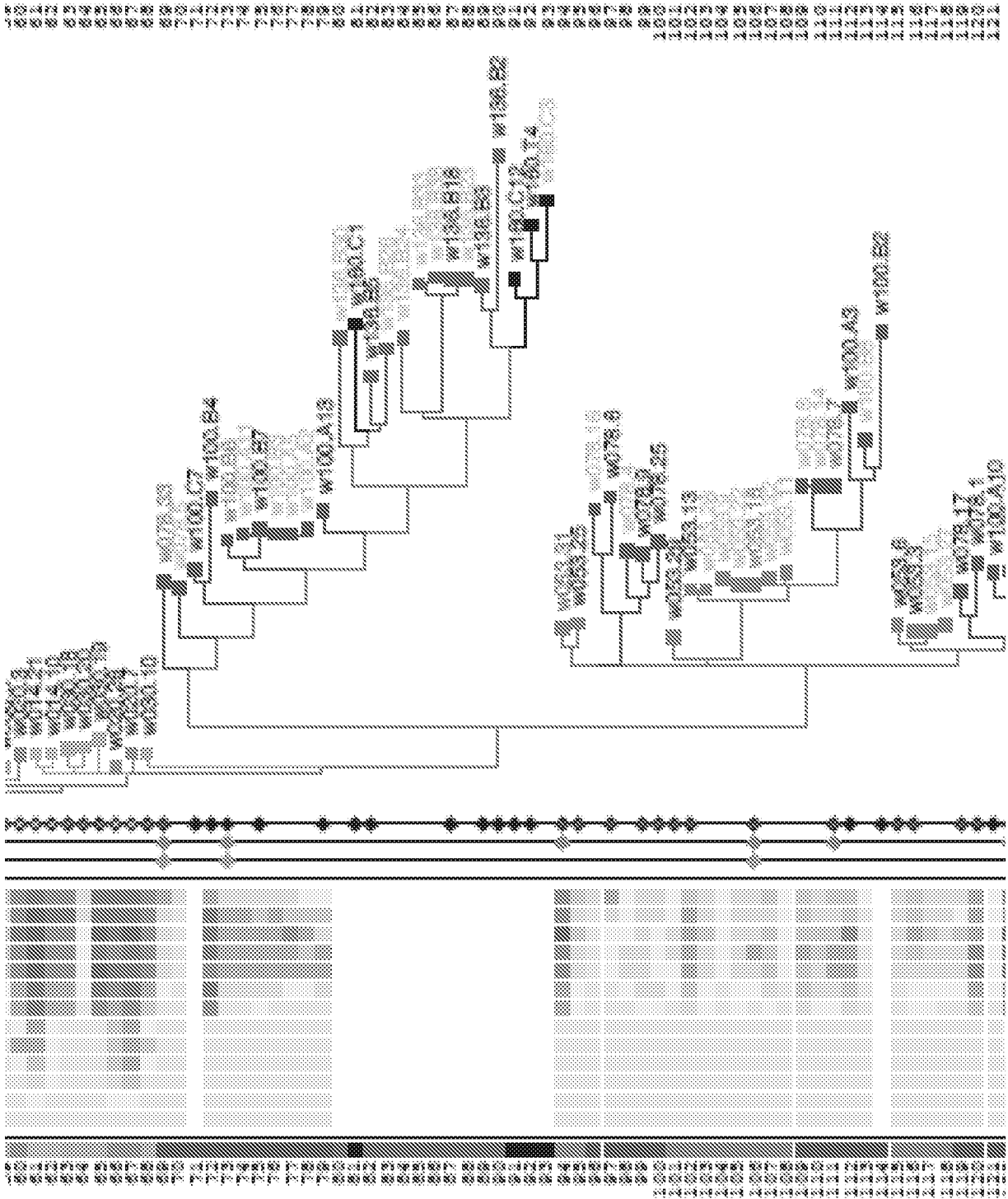


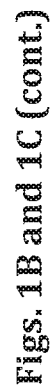
Fig. 1A (cont.)



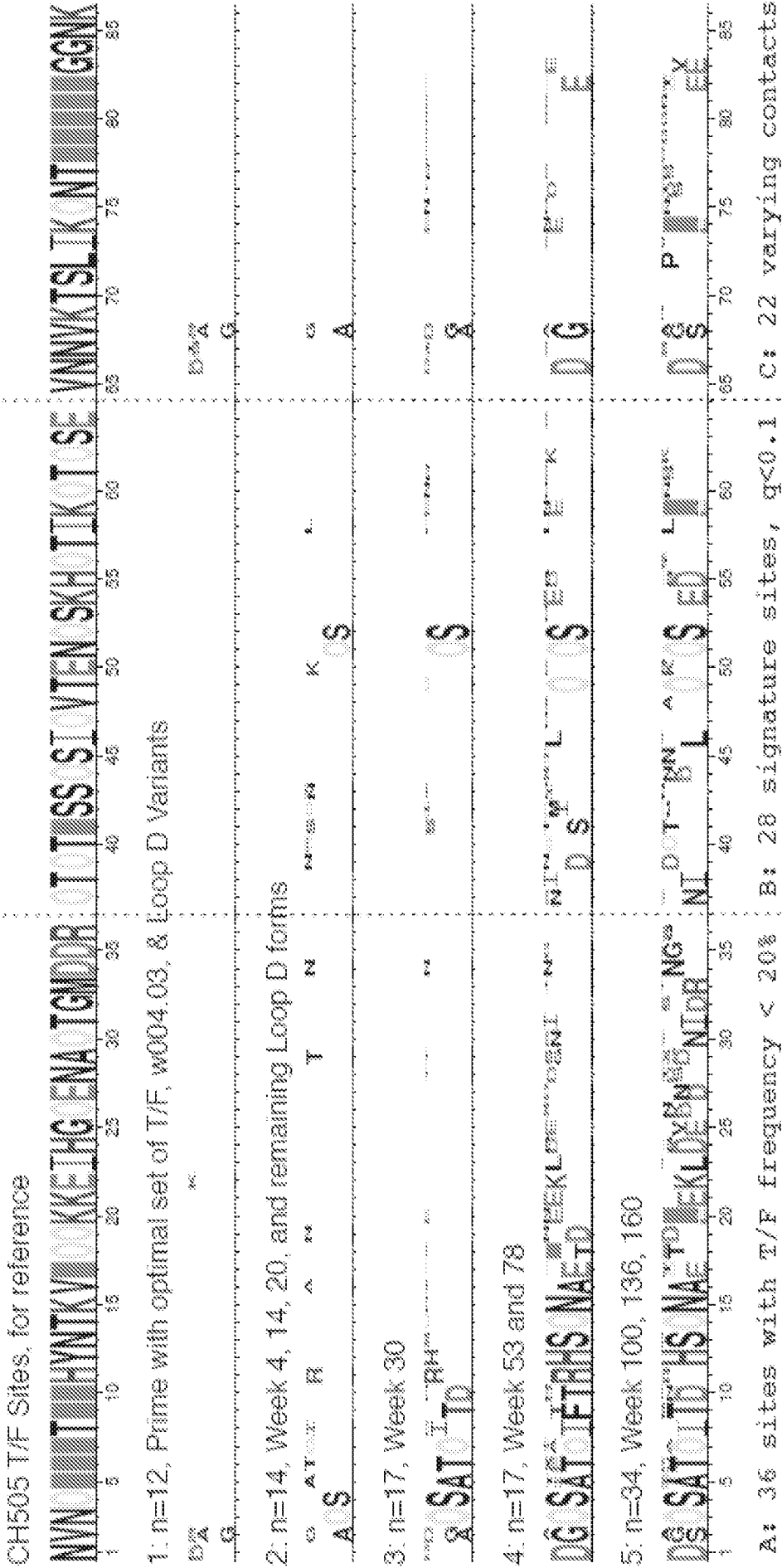
Figs. 1B and 1C



Figs. 1B and 1C (cont.)



Ex. B and C (cont.)



Figs. 2A-C

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| Col                                    | Aln | HXB2 | AA | CH505 | Feature   | Col                           | Aln | HXB2 | AA | CH505 | Feature |
|----------------------------------------|-----|------|----|-------|-----------|-------------------------------|-----|------|----|-------|---------|
| <b>A: 36 sites with T/F &lt; 20%</b>   |     |      |    |       |           | 43                            | 219 | 148  | I  | S     | V1      |
| 1                                      | 357 | 279  | D  | N     | Loop D    | 44                            | 218 | 147  | M  | O     | V1      |
| 2                                      | 359 | 281  | A  | V     | Loop D    | 45                            | 220 | 149  | M  | S     | V1      |
| 3                                      | 412 | 332  | O  | N     | PGT121    | 46                            | 222 | 151  | K  | I     | V1      |
| 4                                      | 414 | 334  | S  | O     | 2G12      | 47                            | 231 | 160  | O  | O     | PG9     |
| 5                                      | 214 | 144+ | —  | —     | V1        | 48                            | 278 | 200  | V  | V     |         |
| 6                                      | 215 | 144+ | —  | —     | V1        | 49                            | 312 | 234  | O  | T     | 8ANC195 |
| 7                                      | 213 | 144+ | —  | —     | V1        | 50                            | 408 | 328  | Q  | E     | V3      |
| 8                                      | 495 | 413  | T  | T     | V4        | 51                            | 412 | 332  | O  | N     | PGT121  |
| 9                                      | 552 | 465  | S  | —     | V5        | 52                            | 414 | 334  | S  | O     | 2G12    |
| 10                                     | 551 | 464  | E  | —     | V5        | 53                            | 416 | 336  | A  | S     |         |
| 11                                     | 499 | 417  | P  | H     | V4        | 54                            | 427 | 347  | S  | K     |         |
| 12                                     | 410 | 330  | H  | Y     | V3        | 55                            | 437 | 356  | O  | H     |         |
| 13                                     | 379 | 300  | N  | N     | V3        | 56                            | 439 | 358  | T  | O     |         |
| 14                                     | 312 | 234  | O  | T     | 8ANC195   | 57                            | 441 | 360  | I  | T     |         |
| 15                                     | 381 | 302  | N  | K     | V3        | 58                            | 498 | 416  | L  | I     | V4      |
| 16                                     | 844 | 756  | I  | V     |           | 59                            | 542 | 460  | N  | K     | V5      |
| 17                                     | 550 | 463+ | —  | —     | V5        | 60                            | 543 | 461  | S  | O     | V5      |
| 18                                     | 480 | 398  | S  | O     | V4        | 61                            | 545 | 463  | O  | T     | V5      |
| 19                                     | 196 | 133  | D  | O     | V1        | 62                            | 831 | 743  | D  | O     | Kennedy |
| 20                                     | 542 | 460  | N  | K     | V5        | 63                            | 833 | 745  | S  | S     | Epitope |
| 21                                     | 427 | 347  | S  | K     |           | 64                            | 926 | 831  | E  | E     | LLP-1   |
| 22                                     | 353 | 275  | V  | E     | Loop D    | <b>C: 22 varying contacts</b> |     |      |    |       |         |
| 23                                     | 222 | 151  | K  | I     | V1        | 65                            | 190 | 127  | V  | V     | CD4     |
| 24                                     | 437 | 356  | O  | H     |           | 66                            | 357 | 279  | D  | N     | Loop D  |
| 25                                     | 558 | 471  | G  | G     | beta24    | 67                            | 358 | 280  | N  | N     | Loop D  |
| 26                                     | 218 | 147  | M  | O     | V1        | 68                            | 359 | 281  | A  | V     | Loop D  |
| 27                                     | 727 | 640  | S  | E     | *         | 69                            | 360 | 282  | K  | K     | Loop D  |
| 28                                     | 544 | 462  | N  | N     | V5        | 70                            | 361 | 283  | T  | T     | Loop D  |
| 29                                     | 216 | 145  | G  | A     | V1        | 71                            | 446 | 365  | S  | S     | CD4     |
| 30                                     | 193 | 130  | K  | O     |           | 72                            | 450 | 369  | P  | L     | CD4     |
| 31                                     | 195 | 132  | T  | T     | V1        | 73                            | 452 | 371  | I  | I     | CD4     |
| 32                                     | 707 | 620  | E  | G     |           | 74                            | 542 | 460  | N  | K     | V5      |
| 33                                     | 4   | 4    | K  | M     | SignalPep | 75                            | 543 | 461  | S  | O     | V5      |
| 34                                     | 405 | 325  | N  | D     | V3        | 76                            | 544 | 462  | N  | N     | V5      |
| 35                                     | 259 | 185  | D  | D     | V2        | 77                            | 545 | 463  | O  | T     | V5      |
| 36                                     | 494 | 412  | D  | R     | V4        | 78                            | 546 | 463+ | —  | —     | V5      |
| <b>B: 28 signature sites, q&lt;0.1</b> |     |      |    |       |           | 79                            | 547 | 463+ | —  | —     | V5      |
| 37                                     | 193 | 130  | K  | O     |           | 80                            | 548 | 463+ | —  | —     | V5      |
| 38                                     | 195 | 132  | T  | T     | V1        | 81                            | 549 | 463+ | —  | —     | V5      |
| 39                                     | 196 | 133  | D  | O     | V1        | 82                            | 550 | 463+ | —  | —     | V5      |
| 40                                     | 198 | 135  | K  | T     | V1        | 83                            | 558 | 471  | G  | G     | beta24  |
| 41                                     | 200 | 137  | D  | —     | V1        | 84                            | 559 | 472  | G  | G     | CD4     |
| 42                                     | 217 | 146  | R  | S     | V1        | 85                            | 561 | 474  | D  | N     | CD4     |
|                                        |     |      |    |       |           | 86                            | 563 | 476  | R  | K     | CD4     |

Figs. 3A-C

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| Col                                    | Aln | HXB2 | AA | CH505 | Feature   | Col                           | Aln | HXB2 | AA | CH505 | Feature |
|----------------------------------------|-----|------|----|-------|-----------|-------------------------------|-----|------|----|-------|---------|
| <b>A: 36 sites with T/F &lt; 20%</b>   |     |      |    |       |           | 43                            | 219 | 148  | I  | S     | V1      |
| 1                                      | 357 | 279  | D  | N     | Loop D    | 44                            | 218 | 147  | M  | O     | V1      |
| 2                                      | 359 | 281  | A  | V     | Loop D    | 45                            | 220 | 149  | M  | S     | V1      |
| 3                                      | 412 | 332  | O  | N     | PGT121    | 46                            | 222 | 151  | K  | I     | V1      |
| 4                                      | 414 | 334  | S  | O     | 2G12      | 47                            | 231 | 160  | O  | O     | PG9     |
| 5                                      | 214 | 144+ | -  | -     | V1        | 48                            | 278 | 200  | V  | V     |         |
| 6                                      | 215 | 144+ | -  | -     | V1        | 49                            | 312 | 234  | O  | T     | 8ANC195 |
| 7                                      | 213 | 144+ | -  | -     | V1        | 50                            | 408 | 339  | Q  | E     | V3      |
| 8                                      | 495 | 413  | T  | T     | V4        | 51                            | 412 | 332  | O  | N     | PGT121  |
| 9                                      | 552 | 465  | S  | -     | V5        | 52                            | 414 | 324  | S  | O     | 2G12    |
| 10                                     | 551 | 464  | E  | -     | V5        | 53                            | 416 | 336  | A  | S     |         |
| 11                                     | 499 | 417  | P  | H     | V4        | 54                            | 427 | 347  | S  | K     |         |
| 12                                     | 410 | 330  | H  | Y     | V3        | 55                            | 437 | 356  | O  | H     |         |
| 13                                     | 379 | 300  | N  | N     | V3        | 56                            | 439 | 358  | T  | O     |         |
| 14                                     | 312 | 234  | O  | T     | 8ANC195   | 57                            | 441 | 360  | I  | T     |         |
| 15                                     | 381 | 302  | N  | K     | V3        | 58                            | 498 | 416  | L  | I     | V4      |
| 16                                     | 844 | 756  | I  | V     |           | 59                            | 542 | 460  | N  | K     | V5      |
| 17                                     | 550 | 463+ | -  | -     | V5        | 60                            | 543 | 461  | S  | O     | V5      |
| 18                                     | 480 | 398  | S  | O     | V4        | 61                            | 545 | 463  | O  | T     | V5      |
| 19                                     | 196 | 133  | D  | O     | V1        | 62                            | 831 | 743  | D  | O     | Kennedy |
| 20                                     | 542 | 460  | N  | K     | V5        | 63                            | 833 | 745  | S  | S     | Epitope |
| 21                                     | 427 | 347  | S  | K     |           | 64                            | 926 | 831  | E  | E     | LLP-1   |
| 22                                     | 353 | 275  | V  | E     | Loop D    | <b>C: 22 varying contacts</b> |     |      |    |       |         |
| 23                                     | 222 | 151  | K  | I     | V1        | 65                            | 190 | 127  | V  | V     | CD4     |
| 24                                     | 437 | 356  | O  | H     |           | 66                            | 357 | 279  | D  | N     | Loop D  |
| 25                                     | 558 | 471  | G  | G     | beta24    | 67                            | 358 | 280  | N  | N     | Loop D  |
| 26                                     | 218 | 147  | M  | O     | V1        | 68                            | 359 | 281  | A  | V     | Loop D  |
| 27                                     | 727 | 640  | S  | E     | *         | 69                            | 360 | 282  | K  | K     | Loop D  |
| 28                                     | 544 | 462  | N  | N     | V5        | 70                            | 361 | 283  | T  | T     | Loop D  |
| 29                                     | 216 | 145  | G  | A     | V1        | 71                            | 446 | 365  | S  | S     | CD4     |
| 30                                     | 193 | 130  | K  | O     |           | 72                            | 450 | 369  | P  | L     | CD4     |
| 31                                     | 195 | 132  | T  | T     | V1        | 73                            | 452 | 371  | I  | I     | CD4     |
| 32                                     | 707 | 620  | E  | G     |           | 74                            | 542 | 460  | N  | K     | V5      |
| 33                                     | 4   | 4    | K  | M     | SignalPep | 75                            | 543 | 461  | S  | O     | V5      |
| 34                                     | 405 | 325  | N  | D     | V3        | 76                            | 544 | 462  | N  | N     | V5      |
| 35                                     | 259 | 185  | D  | D     | V2        | 77                            | 545 | 463  | O  | T     | V5      |
| 36                                     | 494 | 412  | D  | R     | V4        | 78                            | 546 | 463+ | -  | -     | V5      |
| <b>E: 28 signature sites, q&lt;0.1</b> |     |      |    |       |           | 79                            | 547 | 463+ | -  | -     | V5      |
| 37                                     | 193 | 130  | K  | O     |           | 80                            | 548 | 463+ | -  | -     | V5      |
| 38                                     | 195 | 132  | T  | T     | V1        | 81                            | 549 | 463+ | -  | -     | V5      |
| 39                                     | 196 | 133  | D  | O     | V1        | 82                            | 550 | 463+ | -  | -     | V5      |
| 40                                     | 198 | 135  | K  | T     | V1        | 83                            | 558 | 471  | G  | G     | beta24  |
| 41                                     | 200 | 137  | D  | -     | V1        | 84                            | 559 | 472  | G  | G     | CD4     |
| 42                                     | 217 | 146  | R  | S     | V1        | 85                            | 561 | 474  | D  | N     | CD4     |
|                                        |     |      |    |       |           | 86                            | 563 | 476  | R  | K     | CD4     |

Figs. 4A-C

| column                                                                                    | 1 | 1 | 2 | 2 | 3 | 3 | 4 | 4 | 5 | 5 | 6 | 6 | 7 | 7 | 8 | 8 |
|-------------------------------------------------------------------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1                                                                                         | 5 | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 5 | 0 | 5 | 0 |
| A: 36 sites with T/F frequency < 20% B: 28 signature sites, q<0.1 C: 22 varying contacts  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| w000.TF                                                                                   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| # NVNC---T--HYNTKV-OCKKEIHGOENAOIGMDDR OTOT-SSOSIOVTENOSKHOTIKOTOSE VNNVTSLIKONT-----GGNK |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| M10                                                                                       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| # .G.....G.....                                                                           |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| M11                                                                                       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| DG.....DG.....                                                                            |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| M6                                                                                        |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| # .A.....A.....                                                                           |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| w004.03                                                                                   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| # .....A.....                                                                             |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| w004.26                                                                                   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| # .....A.....                                                                             |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| w020.14                                                                                   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| # .AOS.....W.....KOS.....L.....A.....                                                     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| w030.28                                                                                   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| # .DOS.....RH.....I.....OS.....D.....                                                     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| w053.16                                                                                   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| # DGOSIEIIFTEHSONAETN.E.....G.....NO.M.....O.OS.E.....D.G.....E.....                      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

Fig. 5

|         |                                       |                              |                         |
|---------|---------------------------------------|------------------------------|-------------------------|
| w053.31 | WGOSAT.I..RHSON..-DN.....             | ..D.S.....O.OS.....N.K..     | ..D.G.....N.....        |
| w078.7  | WGOSIE.IFTRHSONAE.NEEKLD...G....G.    | ..NOTM...L..O.OS.ED...E..... | ..D.G.....E.....E....   |
| w078.15 | WGOSATOI..RHSONA.T..EKL.E..D.S.....   | S.....L..O.OS.E.....         | ..D.G.....D.....E....   |
| w078.25 | #DGOSTAAIFTRHSONAE.D.EK.N.S..S.....   | ..D.SO.SI...O.OS.EN.....K..  | ..D.G.....E....         |
| w078.33 | *.AGOSATO.T..HSONA..-DN.....O.NI.T... | ..WIDOT.....OKOS.....LN..... | ..SA.....N.O.....       |
| w100.A4 | WGOSATO.TDHSNA.....EKLDE.DD.NI.....   | ..NI.....L..O.OS.ED.....     | ..D.G.....D.....E....   |
| w100.B4 | #WGOSATOI..RHSON...DO.K.DEK.S...D.... | ..DOT.NK...O.OS..D...OS.K..  | ..D.G...P.OSS.....E.... |
| w100.B6 | *DSOSATO.TM.HSONAO-DE...DEKDT...R...  | ..DOT..K...O.OS..D..LENR...  | ..D.S...P.ENTRDGGMOE... |
| w100.C7 | #WGOSATOITM.HSONAO.DE.K.D.KD.....     | ..DOT..K...O.OS..D...EM...   | ..D.G...P.EN.-.GGKO.... |

(c) (5) DPP, (b) (5) ACP



|                |                                       |                                |                            |
|----------------|---------------------------------------|--------------------------------|----------------------------|
| <u>w030.28</u> | .DOS.....RH.....                      | .....I.....OS.....             | .....D.....                |
| <u>w053.16</u> | *DGOSIEIIFTRHSONAETN.E.....G.....     | .....NO.W.....O.OS.E.....      | .....D.G.....E.....        |
| <u>w053.31</u> | DGOSAT.I..RHSOM..-DN.....             | .....D.S.....O.OS.....N..K..   | .....D.G.....N.....        |
| <u>w078.7</u>  | DGOSIE.IFTRHSONAE.NEEKLD...G...G..    | .....NOTM...L..O.OS.ED..E..... | .....D.G.....E.....E.....  |
| <u>w078.15</u> | DGOSATOI..RHSONA.T..EKL.E.D.S.....    | S.....L..O.OS.E.....           | .....D.G.....D.....E.....  |
| <u>w078.25</u> | #DGOSTRAIFTRHSONAE.D.EK.N.S..S.....   | .....D.SO.SI...O.OS.EN.....K.. | .....D.G.....E.....        |
| <u>w078.33</u> | *.ACOSATO.T..HSONA.-DN.....O.NI.T...  | NIDOT.....OKOS.....LN.....     | .....SA.....N.O.....       |
| <u>w100.A4</u> | DGOSATO.TDSSHSONA...EKLDE.DD.NI.....  | NI.....L..O.OS.ED.....         | .....D.G.....D.....E.....  |
| <u>w100.B4</u> | #DGOSATOI..RHSOM...DO.K.DEK.S...D.... | .....DOT.NK...O.OS..D...OS.K.. | .....D.G...P.OSS.....E...  |
| <u>w100.B6</u> | *DGOSATO.TN.HSONAO-DE...DEKDT...R...  | .....DOT..K...O.OS..D..LENR... | .....D.S...P.ENTRDGENCE... |
| <u>w100.C7</u> | #DGOSATOIIN.HSONAO.DE.K.D.KD.....     | .....DOT..K...O.OS..D..EN...   | .....D.G...P.EN...GGKO.... |

Fig. 6 (cont.)

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1: Prime with T/F and optimal set of Loop D Variants

w000.TF, w004.03,

M10, M11, M19, M20, M21, M5, M6, M7, M8, M9

2: w014.10, w014.2, w014.21, w014.3, w014.32, w014.8

w020.3, w020.4, w020.7, w020.8, w020.9, w020.11, w020.13,

w020.14, w020.15, w020.19, w020.22, w020.23, w020.24, w020.26

3: w030.5, w030.6, w030.9, w030.10, w030.11, w030.13, w030.15,

w030.17, w030.18, w030.19, w030.20, w030.21, w030.23, w030.25,

w030.27, w030.28, w030.36

4: w053.3, w053.6, w053.13, w053.16, w053.25, w053.29, w053.31,

w078.1, w078.6, w078.7, w078.9, w078.10, w078.15, w078.17,

w078.25, w078.33, w078.38

5: w100.A3, w100.A4, w100.A6, w100.A10, w100.A12, w100.A13,

w100.B2, w100.B4, w100.B6, w100.B7, w100.C7,

w136.B2, w136.B3, w136.B4, w136.B5, w136.B8, w136.B10,

w136.B12, w136.B18, w136.B20, w136.B27, w136.B29, w136.B36,

w160.A1, w160.C1, w160.C2, w160.C4, w160.C11, w160.C12,

w160.C14, w160.D1, w160.D5, w160.T2, w160.T4

**Fig. 7**

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1: Prime with T/F and optimal set of Loop D Variants  
w000.TF, w004.03, w004.26,  
M10, M11, M19, M20, M21, M5, M6, M7, M8, M9  
2: w014.10, w014.2, w014.21, w014.3, w014.32, w014.8  
w020.3, w020.4, w020.7, w020.8, w020.9, w020.11, w020.13,  
w020.14, w020.15, w020.19, w020.22, w020.23, w020.24, w020.26  
3: w030.5, w030.6, w030.9, w030.10, w030.11, w030.13, w030.15,  
w030.17, w030.18, w030.19, w030.20, w030.21, w030.23, w030.25,  
w030.27, w030.28, w030.36  
4: w053.3, w053.6, w053.13, w053.16, w053.25, w053.29, w053.31,  
w078.1, w078.6, w078.7, w078.9, w078.10, w078.15, w078.17,  
w078.25, w078.33, w078.38  
5: w100.A3, w100.A4, w100.A6, w100.A10, w100.A12, w100.A13,  
w100.B2, w100.B4, w100.B6, w100.B7, w100.C7,  
w136.B2, w136.B3, w136.B4, w136.B5, w136.B8, w136.B10,  
w136.B12, w136.B18, w136.B20, w136.B27, w136.B29, w136.B36,  
w160.A1, w160.C1, w160.C2, w160.C4, w160.C11, w160.C12,  
w160.C14, w160.D1, w160.D5, w160.T2, w160.T4

**Fig. 8**

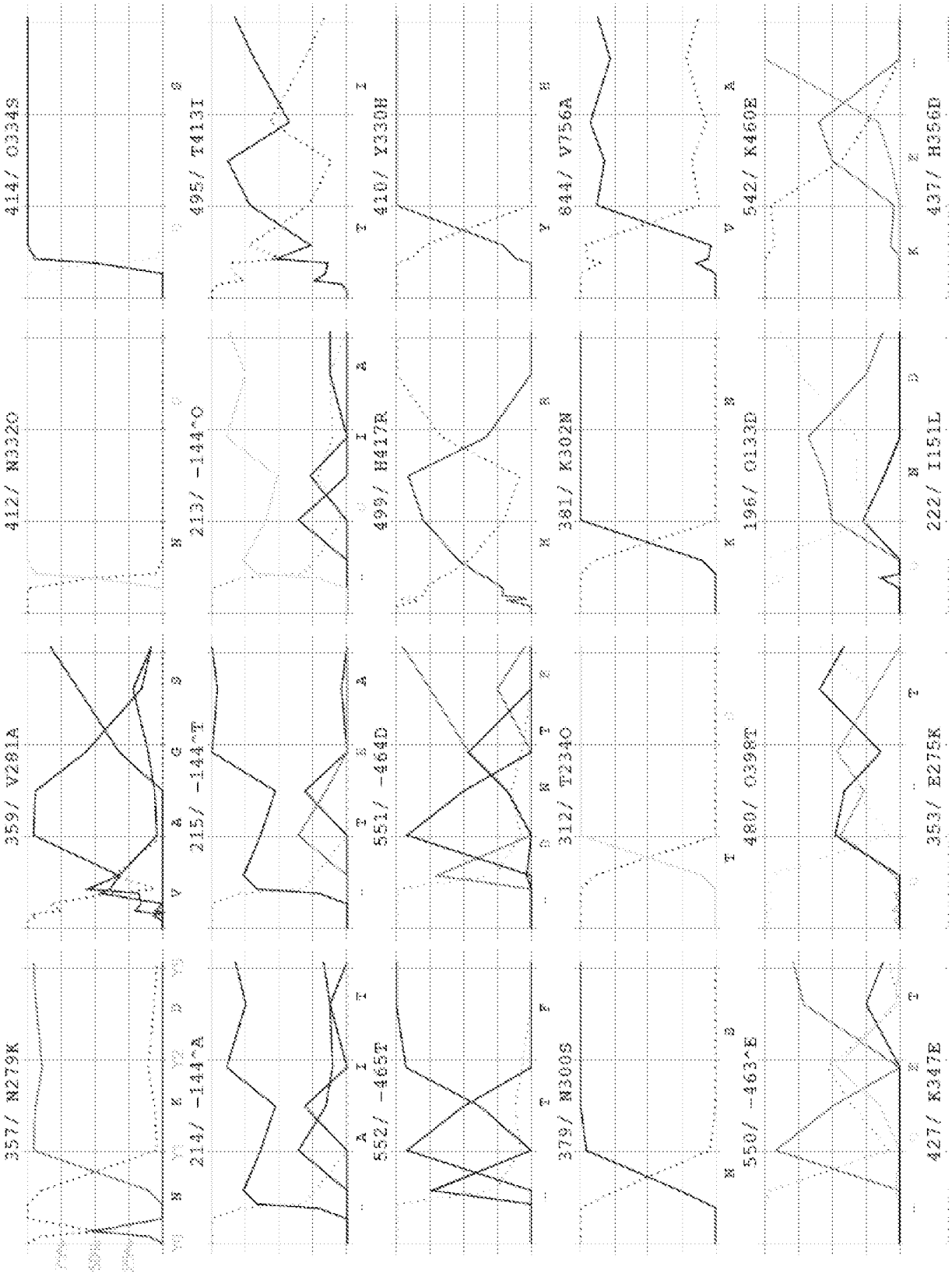


Fig. 9

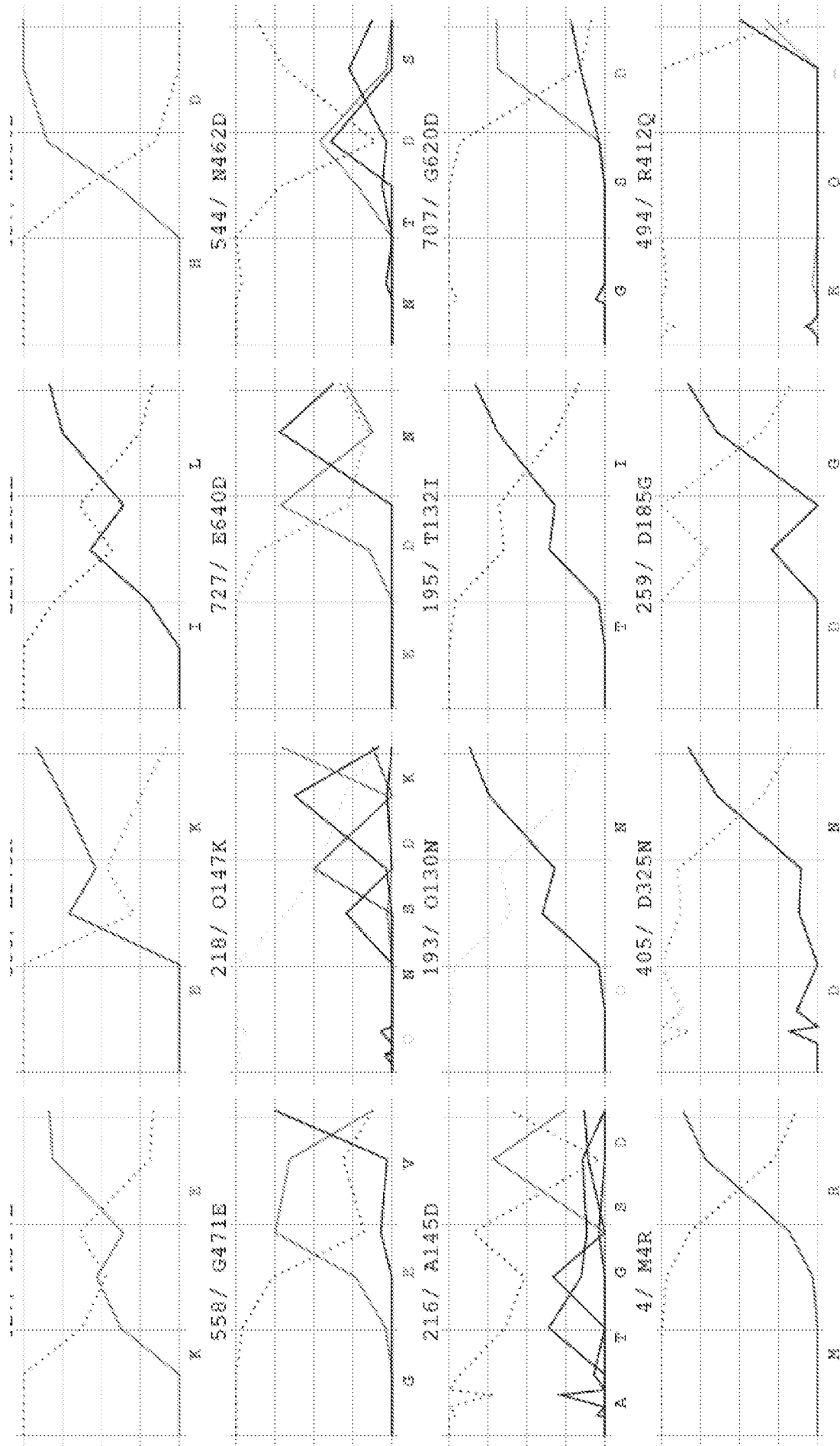


Fig. 9 (cont.)

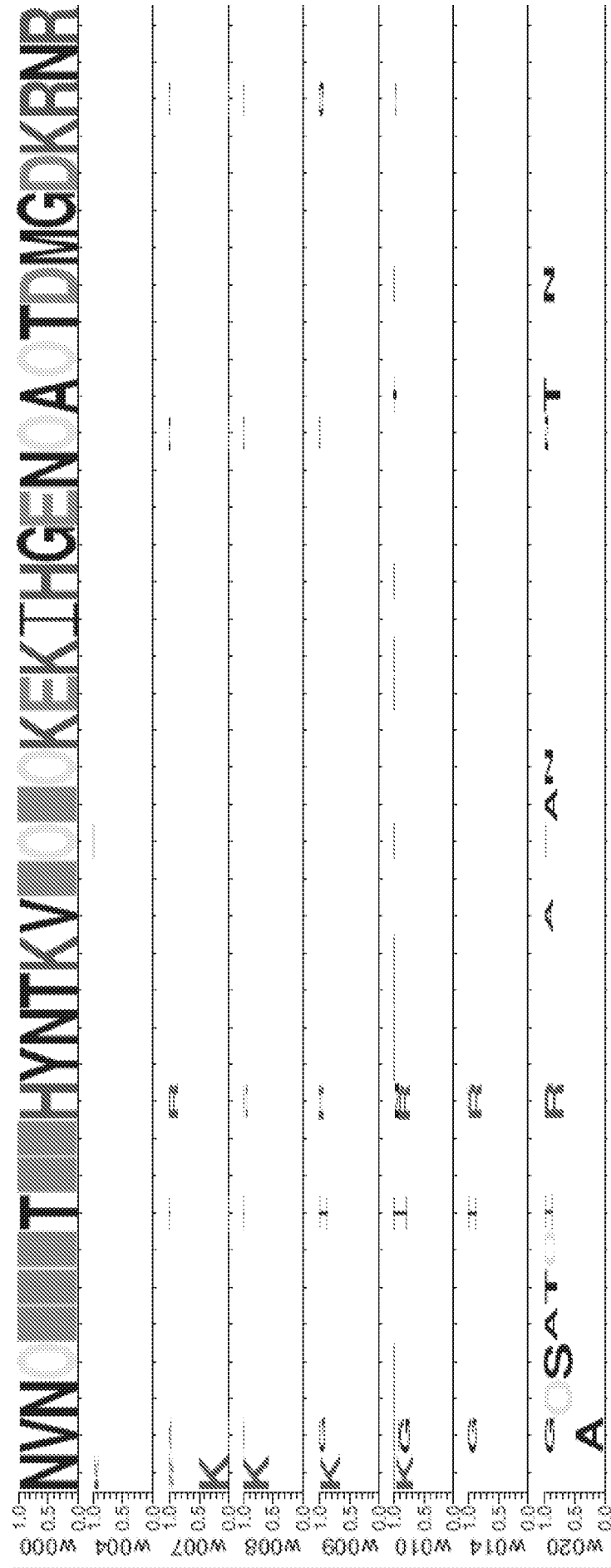


Fig. 10

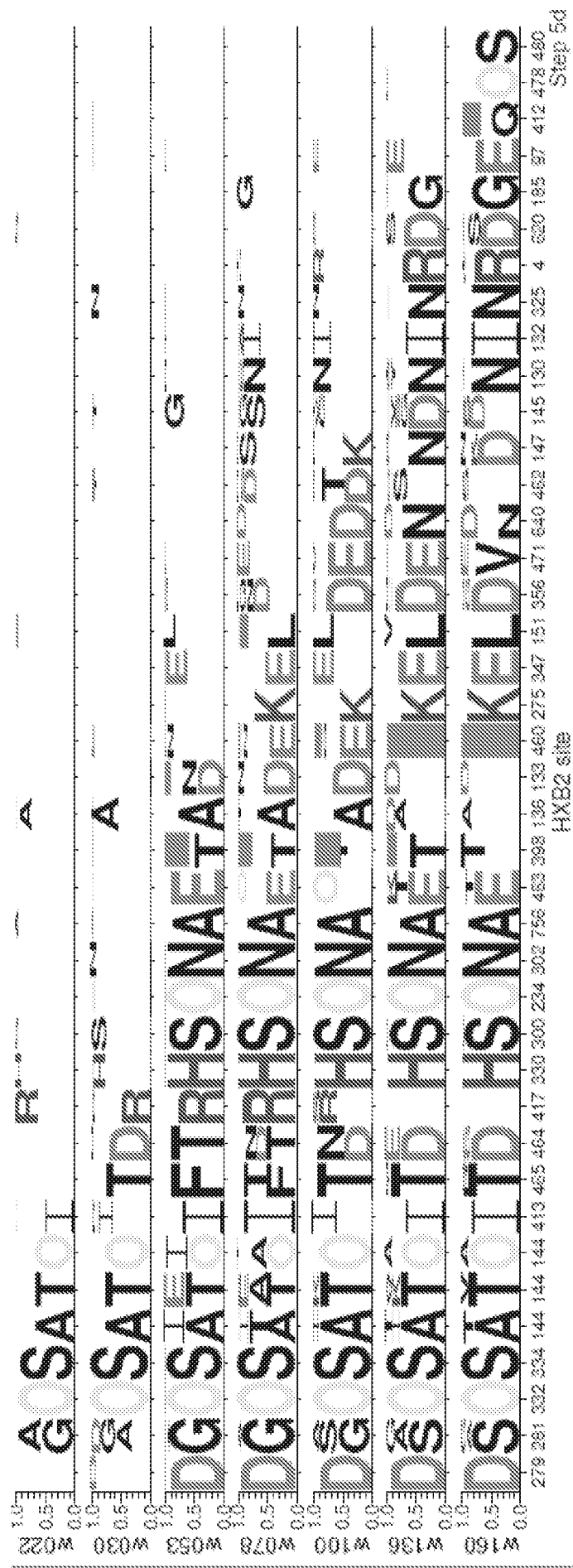


Fig. 10 (cont.)

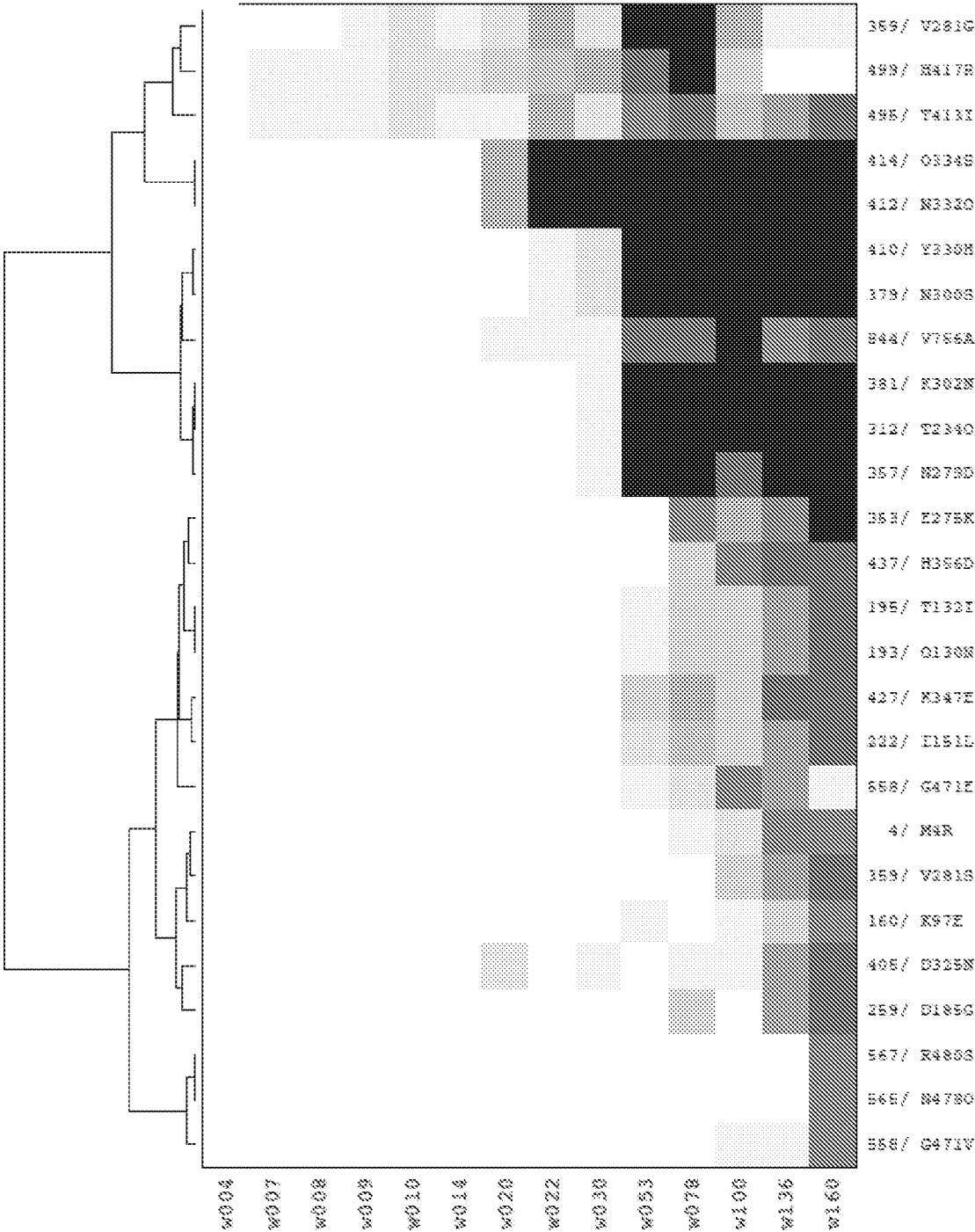


Fig. 11

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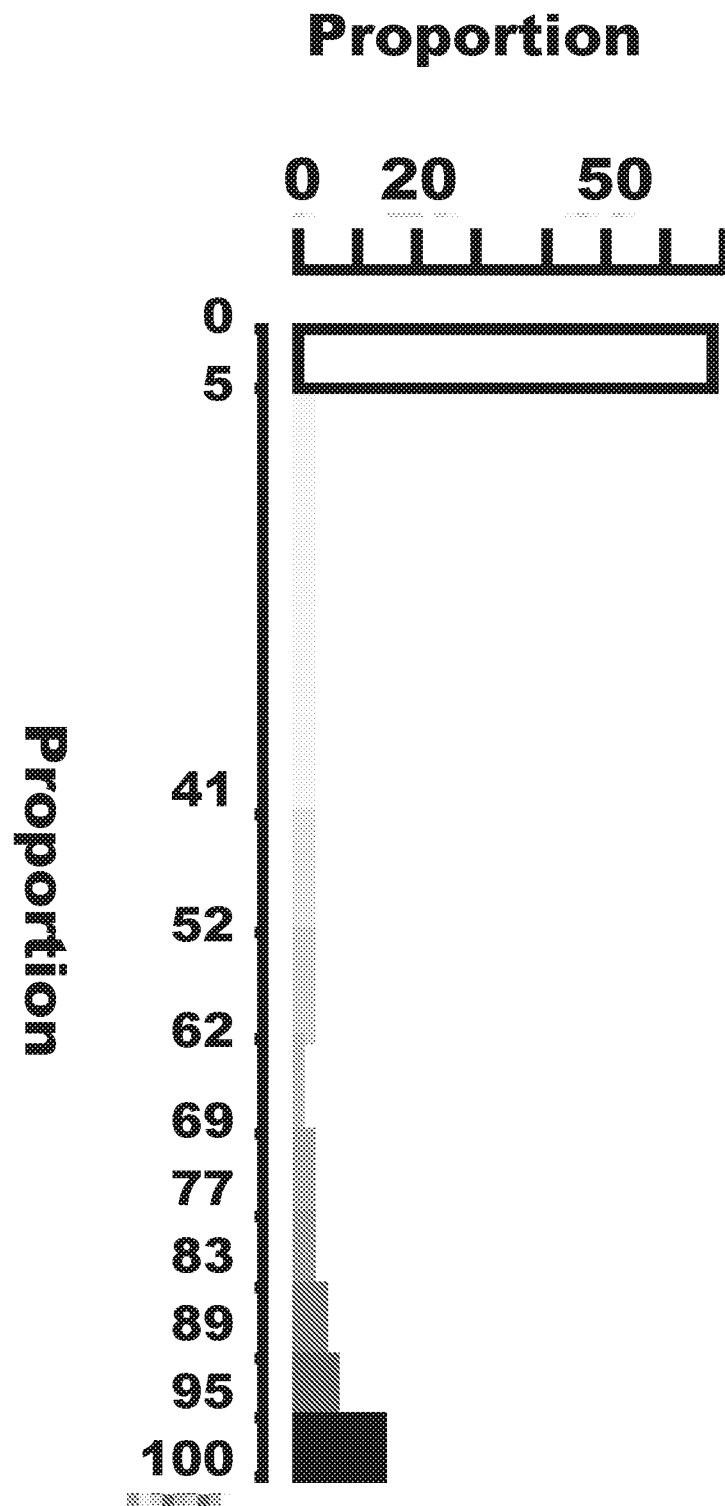


Fig. 11 (cont.)

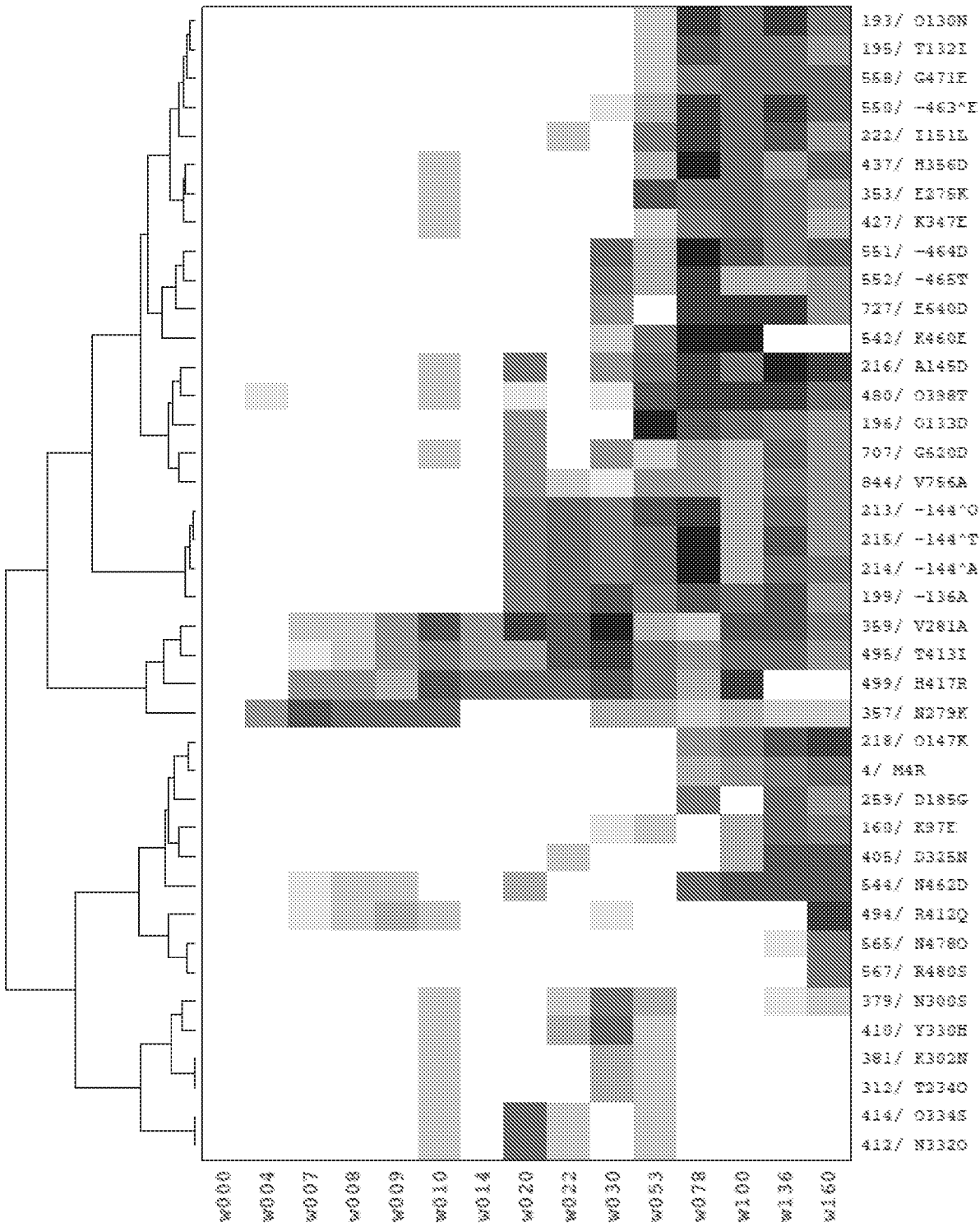


Fig. 12

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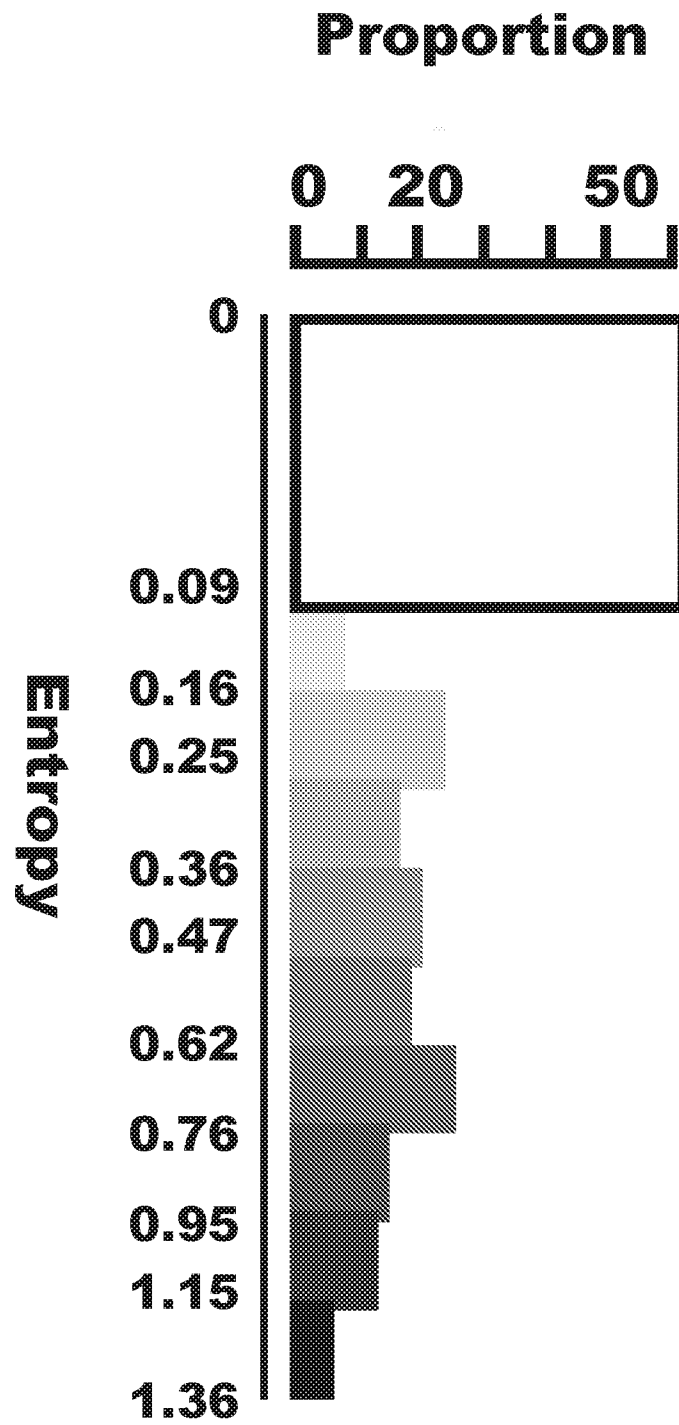
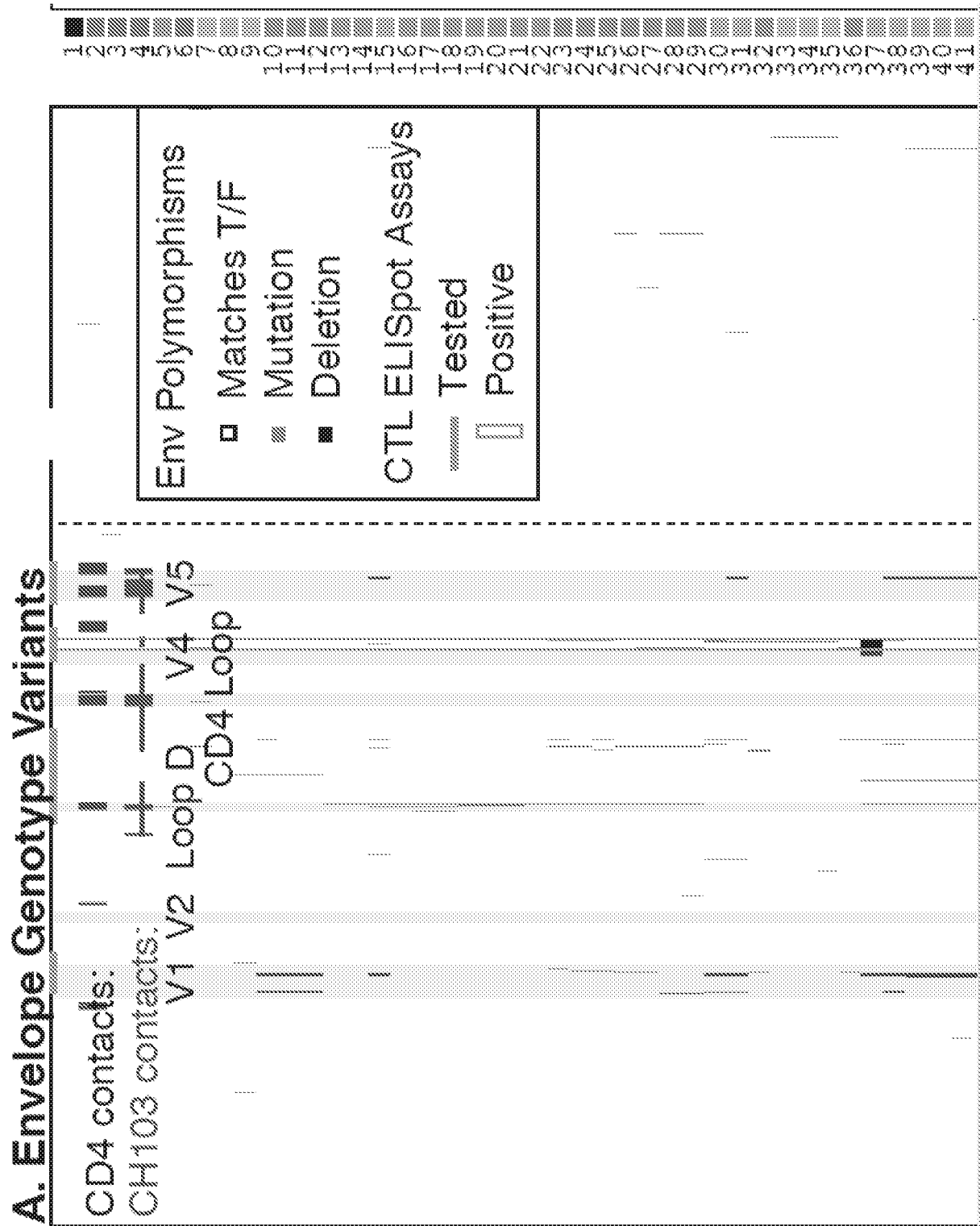


Fig. 12 (cont.)





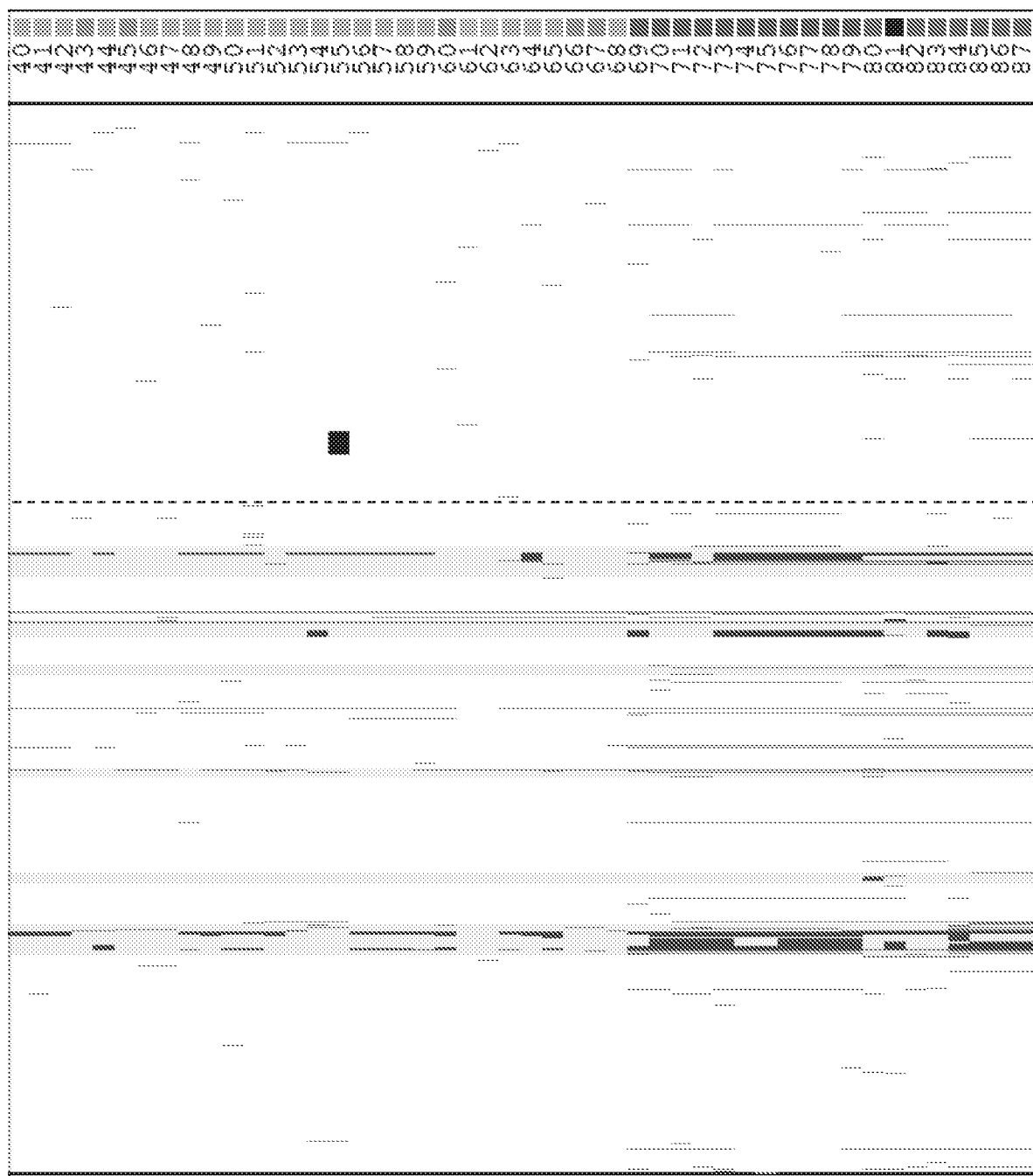


Fig. 13A (cont.)

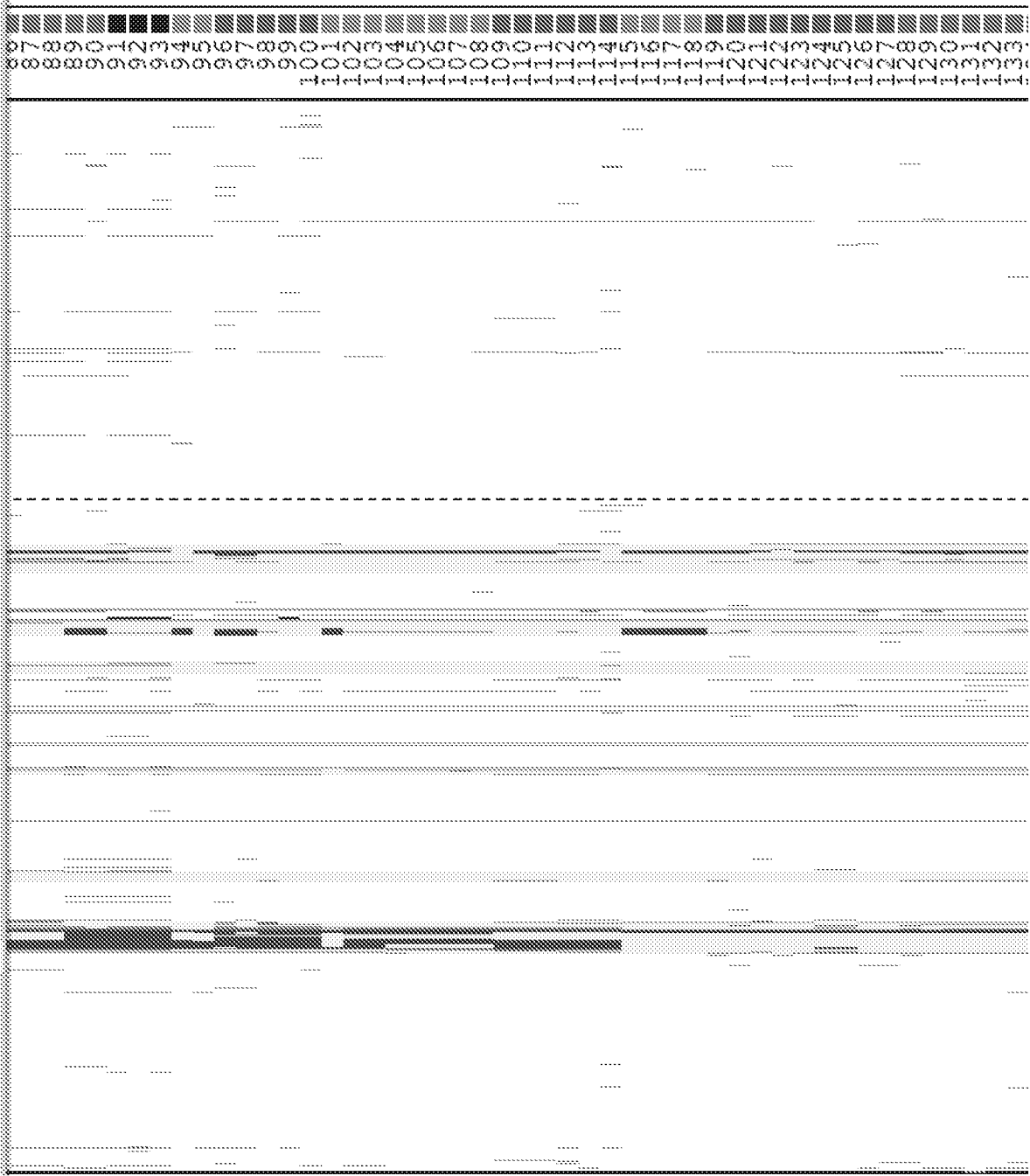


Fig. 13A (cont.)

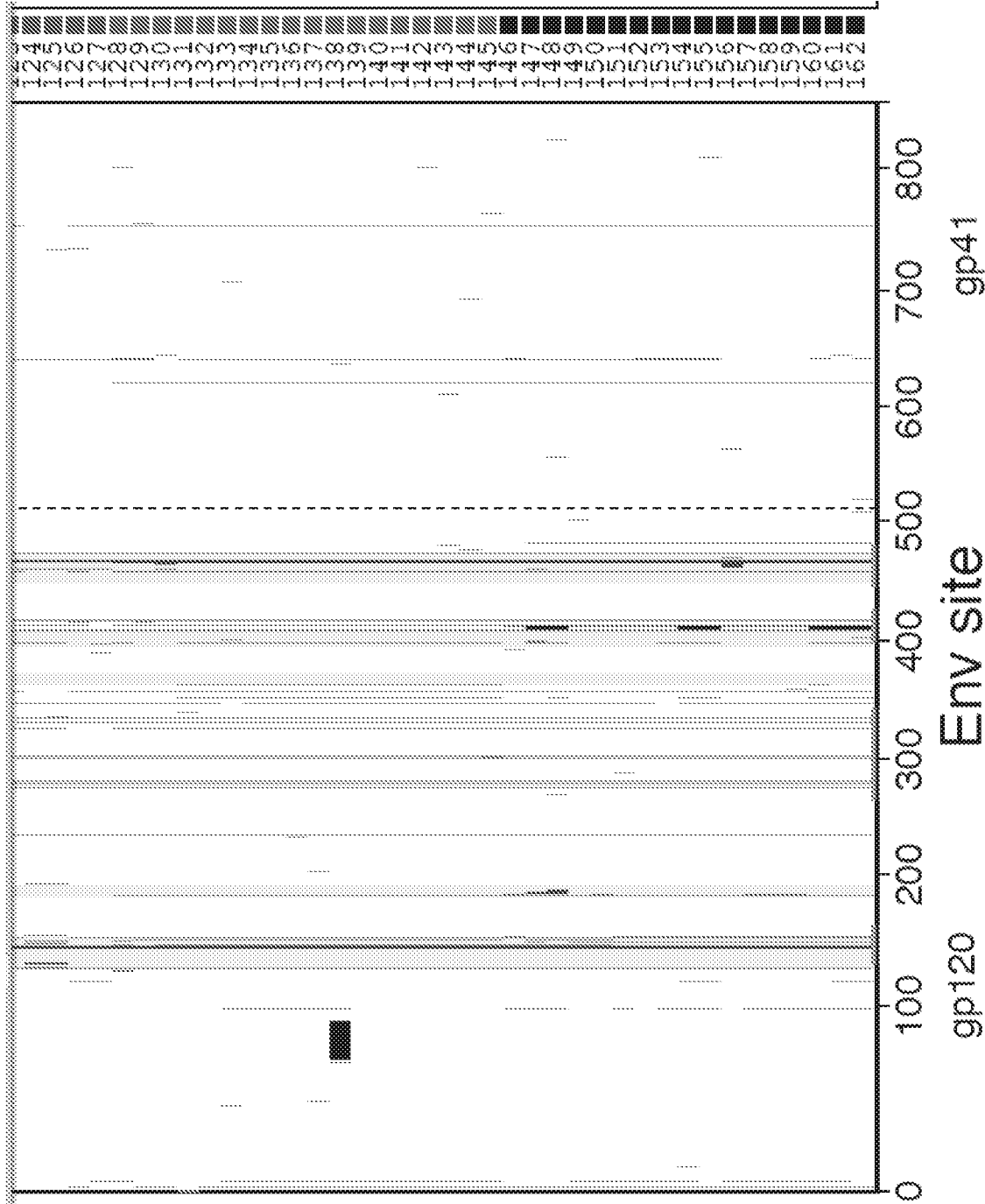
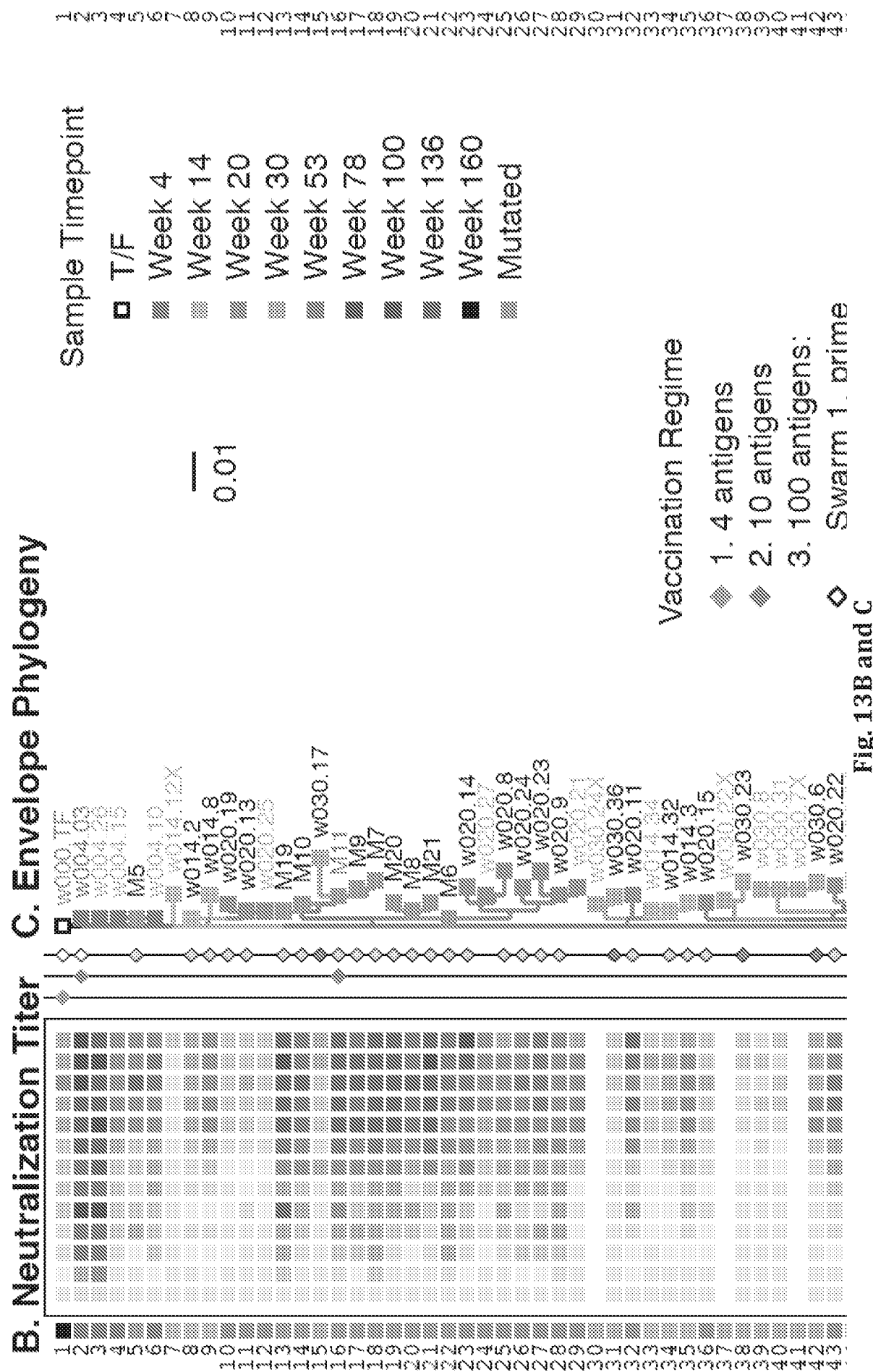
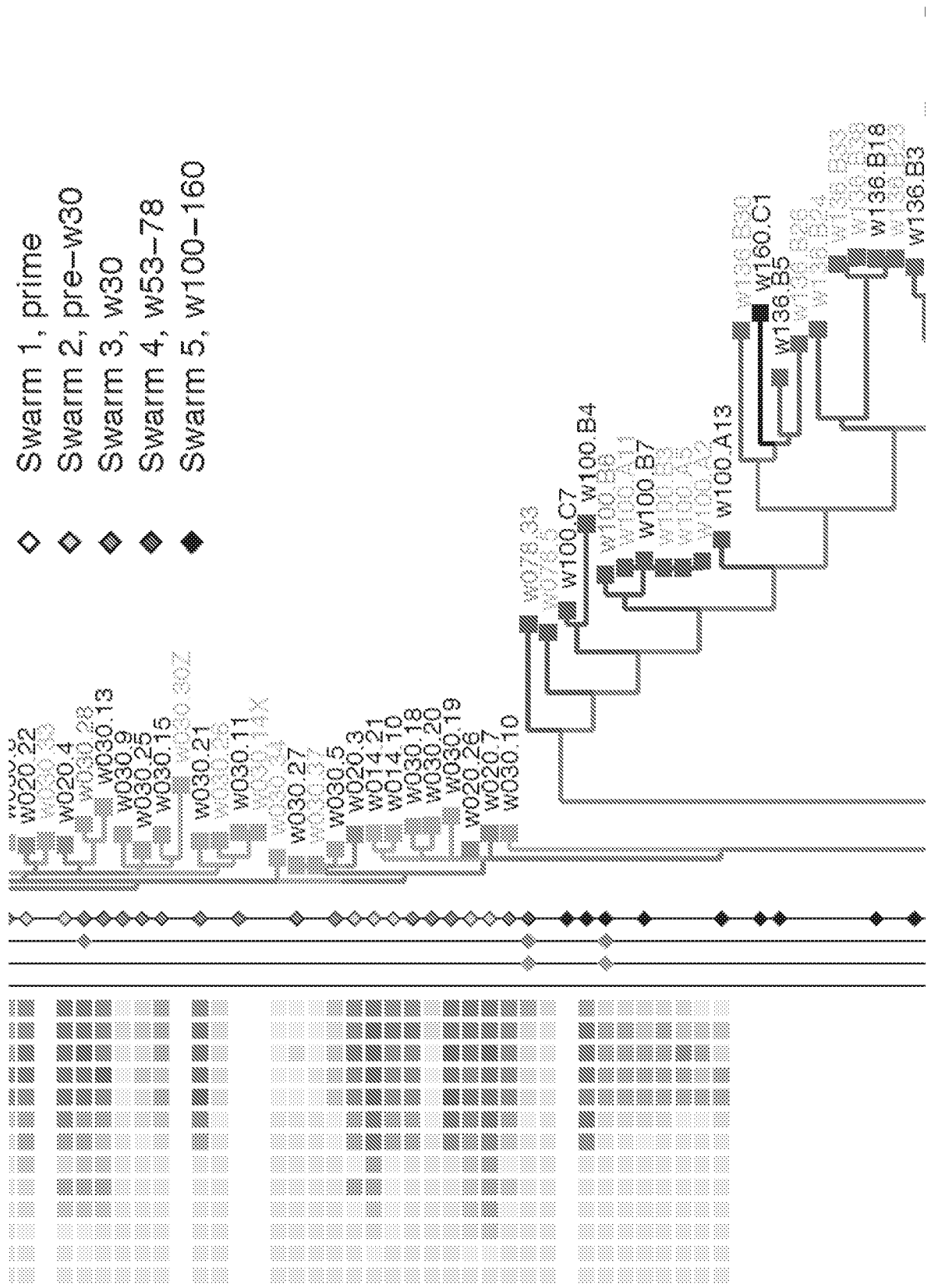
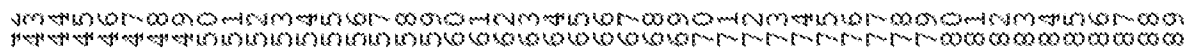
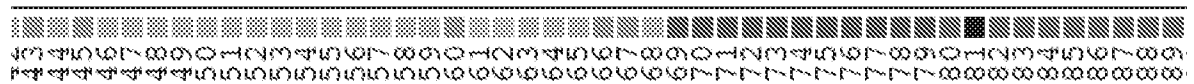


Fig. 13A (cont.)





Ex. 50-13 and C (cont.)



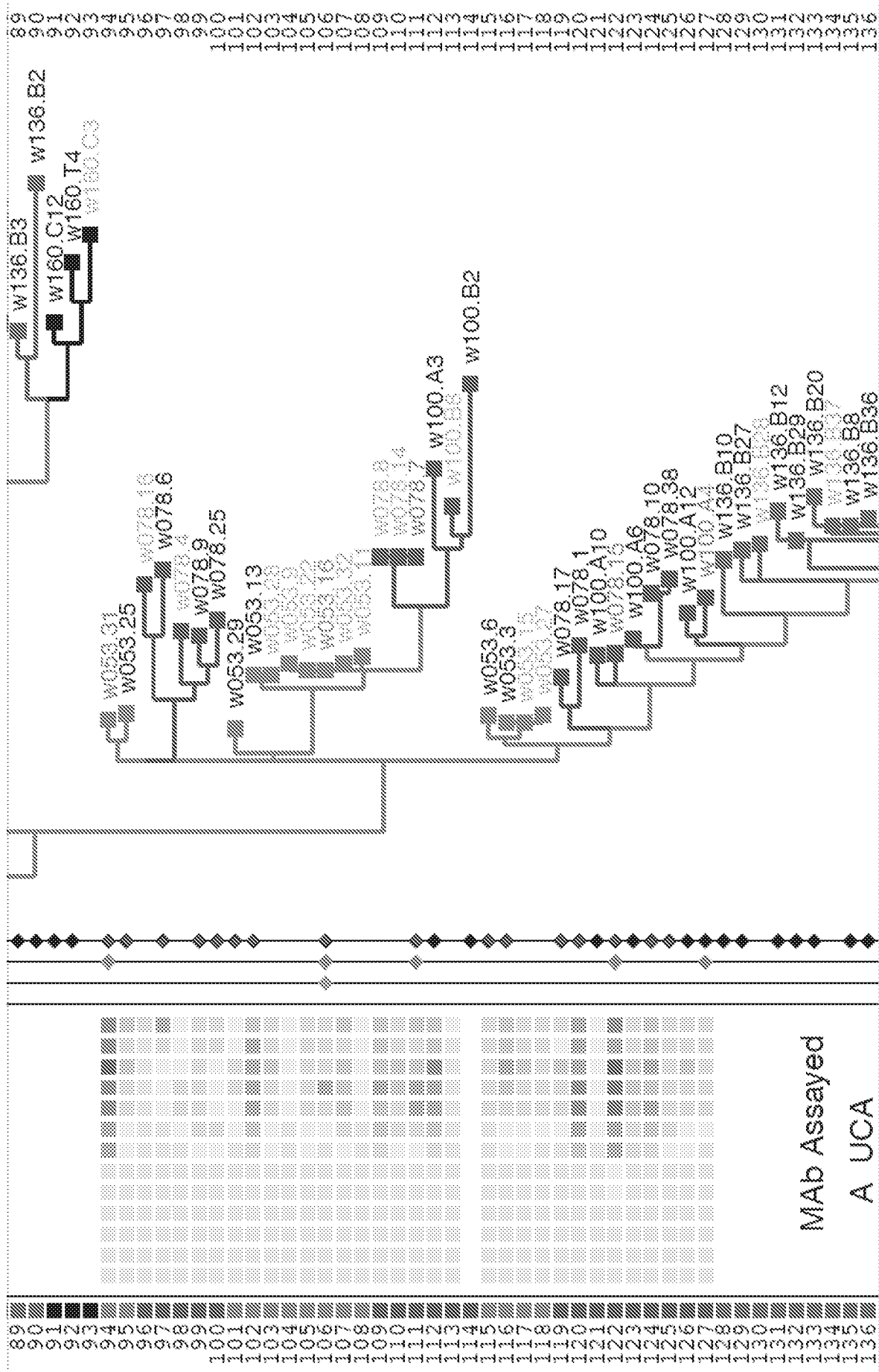


Fig. 13B and C (cont.)

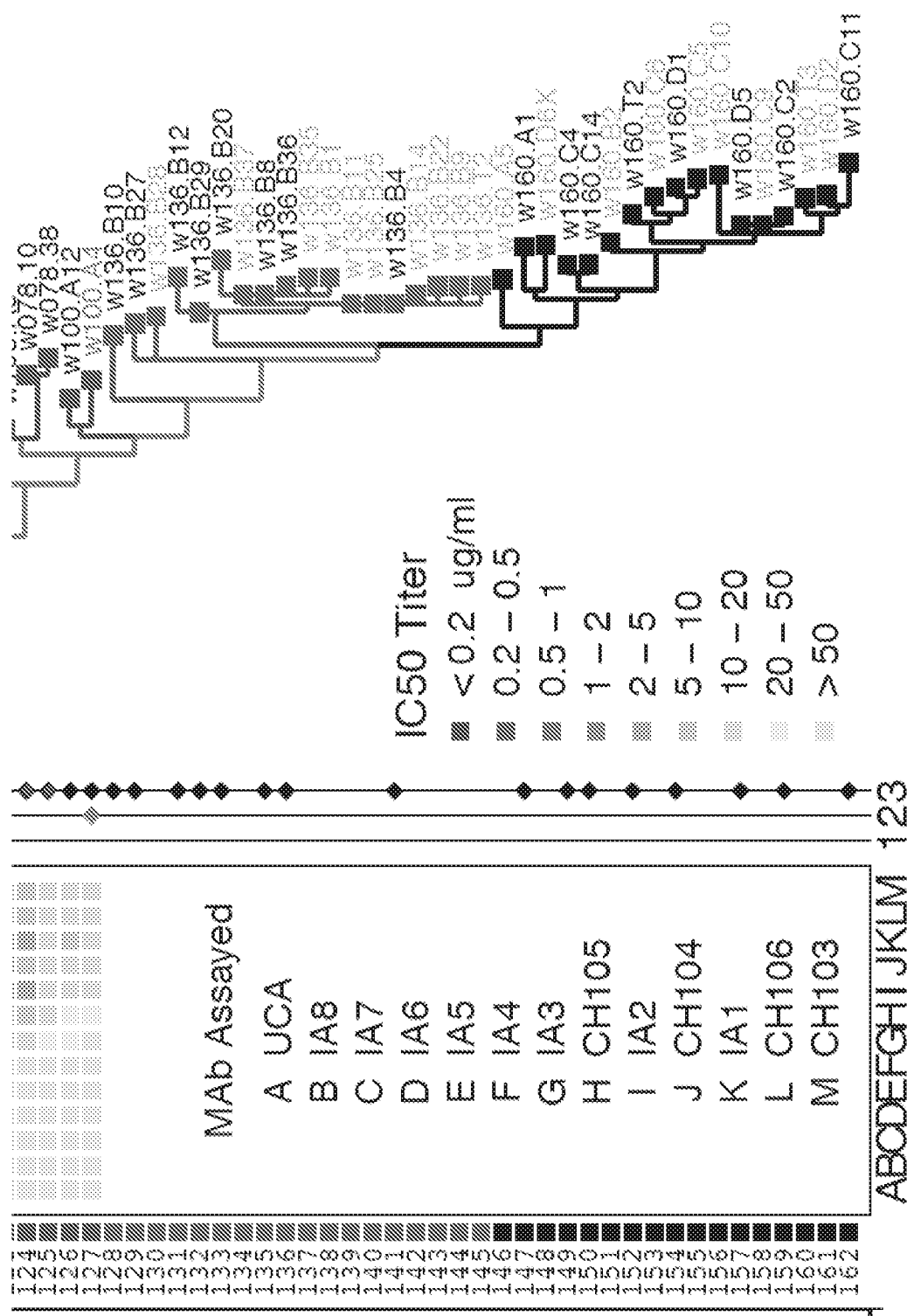
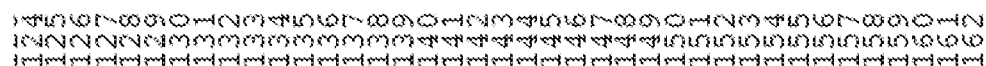


Fig. 13B and C (cont.)

# OSU Title







```

TF      GENCGEFFYCNTSSLFNRTYMANSTDMANSTETNSTRITITHCRIKQIINMWQEVGGRAMYAPP
M11
w4.03
w30.28
w53.16
w53.31
w78.33
w78.15
w100.B6
703010505.TF_M24
703010505.TF_M14
w100.A4-optional
w78.7-optional

```

```

IAGNITCISNITGLLTRDGGKNT...ETFRPGCGNMKDNWRSELYKKVVEVKPL
...
...
...ETF...
...N...
...N...T...I...
...D...D...E...
...E-TRDGGNNNT...E...
...E...
...D...DT...E...
...E...ETF...

```

500

Fig. 14A (cont.)

TF  
M11  
w4.03  
w30.28  
w53.16  
w53.31  
w78.33  
w78.15  
w100.B6  
703010505.TF\_M24  
703010505.TF\_M14  
w100.A4-optional  
w78.7-optional

GVAFTHARRRVVEREKRAVCGNCAVFLGFLGAAGSTMCAASITLTVOAROLLSCIVQQQSNLLKA

IEAQQHMLKLTVWGKQLQARVLALERYLKDQQLGNWGCGLICTTNWVWSSWSNKTY

S

G

TF  
M11  
w4.03  
w30.28  
w53.16  
w53.31  
w78.33  
w78.15  
w100.B6  
703010505.TF\_M24  
703010505.TF\_M14  
w100.A4-optional  
w78.7-optional

LWYKIFINIVGGLIGLRIIFAVLSLVNRVROGYSPLSLQTLIPSRGPDPRPGGIEEGGEQDRNR

--G--

--K--

T--

750

Fig. 14A (cont.)



w4.03 MRVNGIQENYPQWNIWSMLGFWMLMICNGMWVTVYGVVPWKEAKTILFCASDAKAYEKEVHNV  
CH0505.TF.M11  
w14.32  
w14.12  
w30.28  
w53.31  
w53.16  
w78.33  
w78.15  
w78.7  
w100.A4  
w100.B6

WATHACVPTDPNPQEMVLKNVTENFNWKNDMVDQMHEDVISLWDQSLKPCVKLTPLCVTL

-----M-----  
-----|-----  
-----A-----  
-----R-----  
-----E-----A-----

```
w4.03
CH0505.TF.M11
w14.32
w14.12
w30.28
w53.31
w53.16
w78.33
w78.15
w78.7
w100.A4
w100.B6
```

CNTSVITQACPKVSEDPFIHYCAPAGYALKCNKFTTGTGPCNNVSTVQC

|   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |     |
|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|
| 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 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 |
| 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 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 |
| 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 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 |
| 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 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 |
| 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 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 |
| 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 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 8  |    |    |    |    |    |    |    |    |    |    |    |     |

Fig. 14B (cont.)

w4.03 THGIKPVVSTQLLNGSLAEGEIIIRSENITNNVKTIIVHLNESVKIECTRPNNKTRTSIRIGPGQ.  
 CH0505.TF.M11 -----D-G-----  
 w14.32 -----  
 w14.12 -----  
 w30.28 -----D-----  
 w53.31 -----D-G-----S-N-----  
 w53.16 -----D-G-----S-N-----  
 w78.33 -----SA-----S-N-----  
 w78.15 -----K--D-G-----S-N-----  
 w78.7 -----K--D-G-----S-N-----  
 w100.A4 -----K--D-G-----S-N-----  
 w100.B6 -----D-S-----S-N-----

AFYATQQVIGDIREAYCNINESKWNETLQRVSKKLKEYFPFKNITFQPSSGGDLEITH  
 -----  
 -----  
 -----A-----P-----  
 -----H--S-----  
 -----H--S-----  
 -----H--S-----E-----  
 -----K-H--S-----  
 -----H--S-----E-----  
 -----H--S-----E-----D-----  
 -----H--S-----E-----D-----  
 -----H--S-----D-----P-----



w4.03                   GVAPTNARRRVVEREKRAVGMGAVFLGFLGAAGSTMGAASITLTVQARQLLSGIVQQQSNLLKAIEA  
CH0505.TF.M11                   -----  
w14.32                   -----  
w14.12                   -----  
w30.28                   -----  
w53.31                   -----G-  
w53.16                   -----  
w78.33                   -----  
w78.15                   -----  
w78.7                    -----  
w100.A4                  -----  
w100.B6                  -----K-----

QQHMLKLTWGIKQLQARVLALERYLKDQQLGMWGCCKLICITINVYWNSSWSNKTY  
-----  
-----  
-----  
-----S-----  
-----  
-----  
-----+-----  
-----  
-----  
-----  
-----  
-----  
-----  
-----  
-----  
-----



w4.03 STRLVSGELALVWDDLRLSLCLFIYHRLRDFILIAARAGELLGRSSSLKGLERGWAEALKYLGSLVQY  
CH0505.TF.M11  
w14.32  
w14.12  
w30.28  
w53.31  
w53.16  
w78.33  
w78.15  
w78.7  
w100.A4  
w100.B6

WGLELKRSALSLLDTLAIAVGEGTDRILEFVLGICRAIRNIPTRIRQGFETALL\*  
K  
A

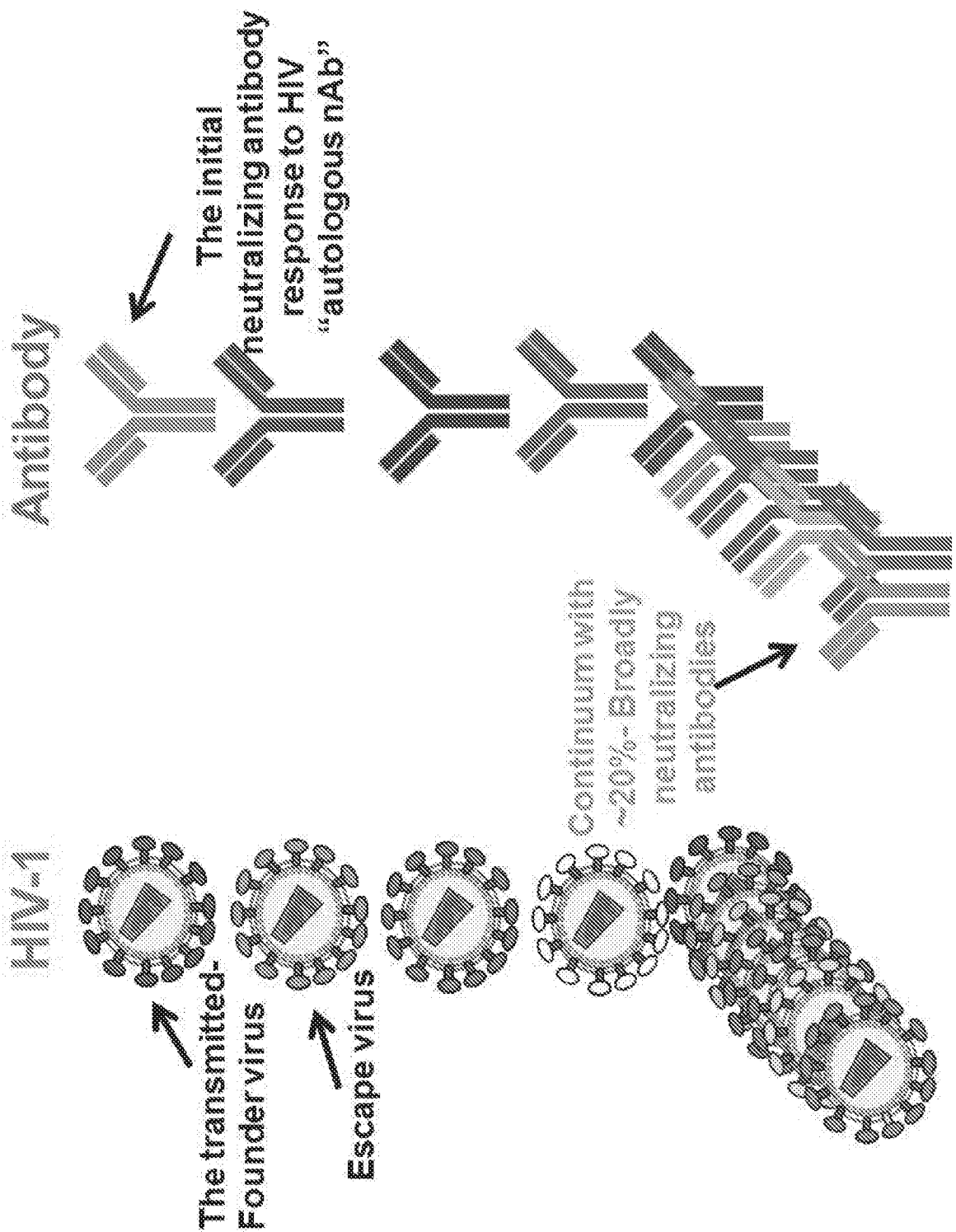


Fig. 15

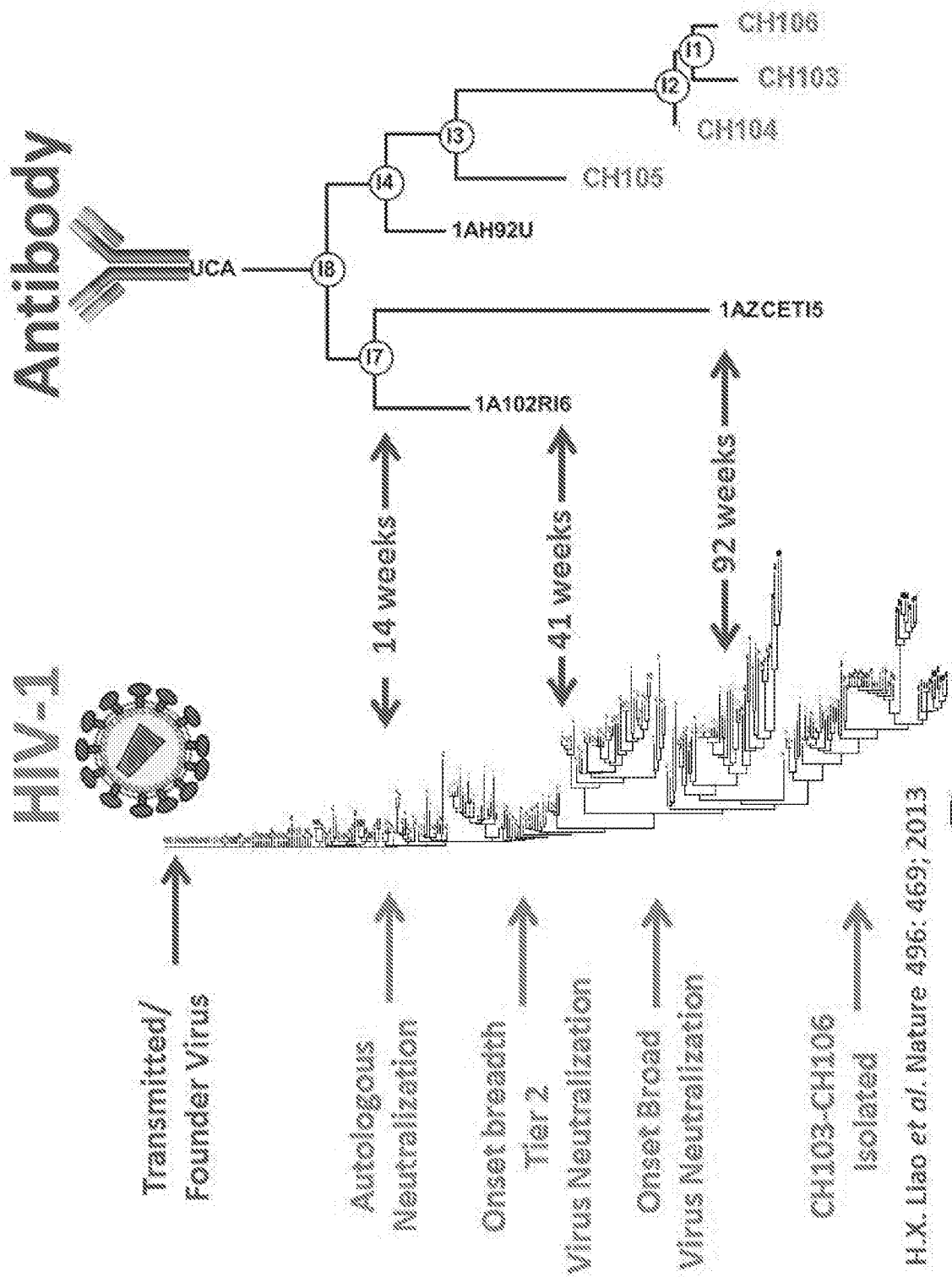


Fig. 16

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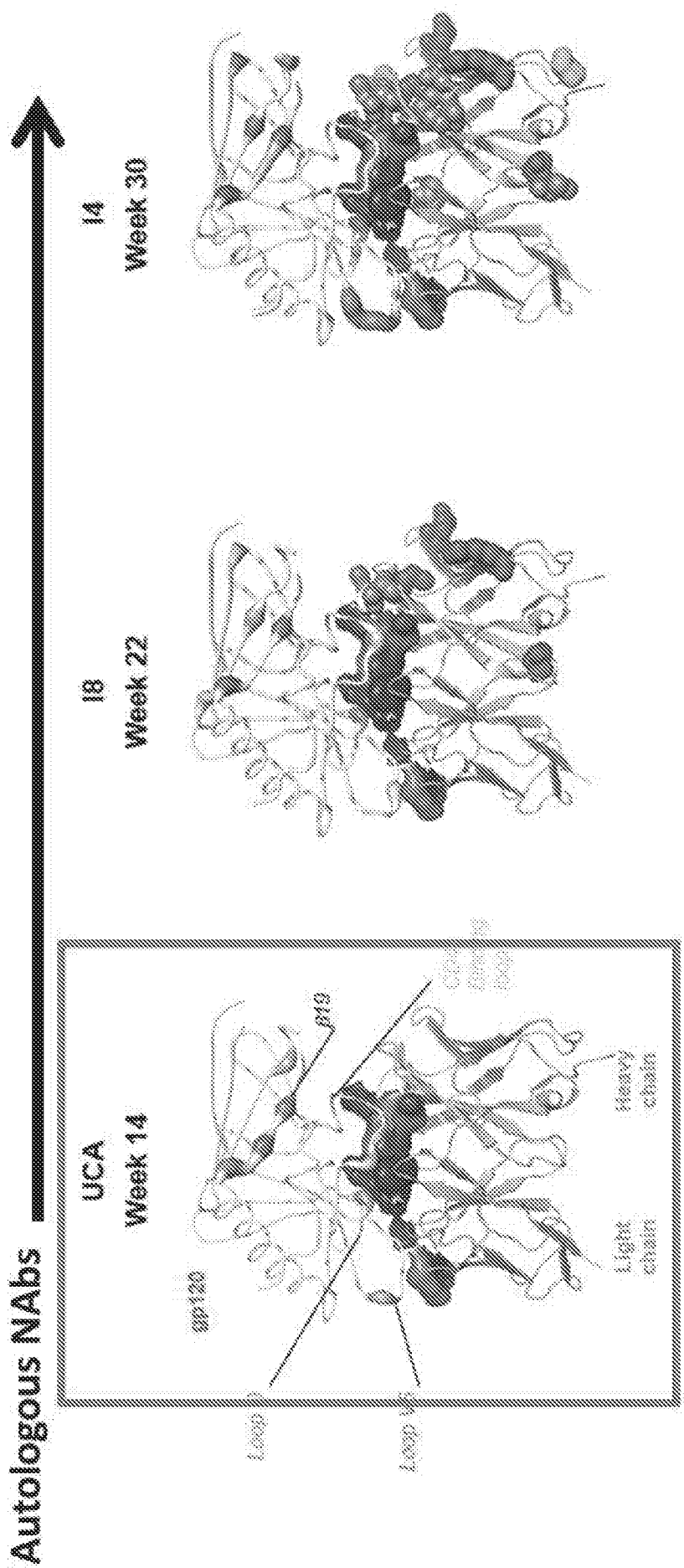


Fig. 17

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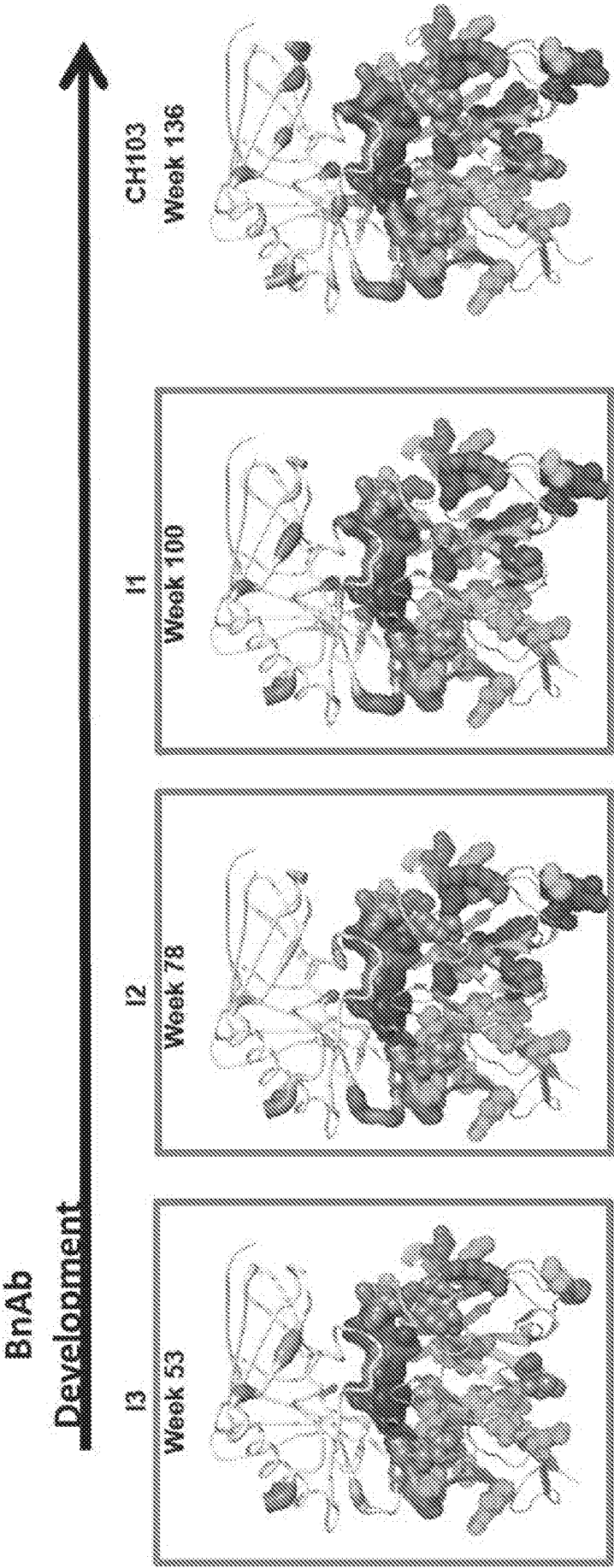


Fig. 17 (cont.)

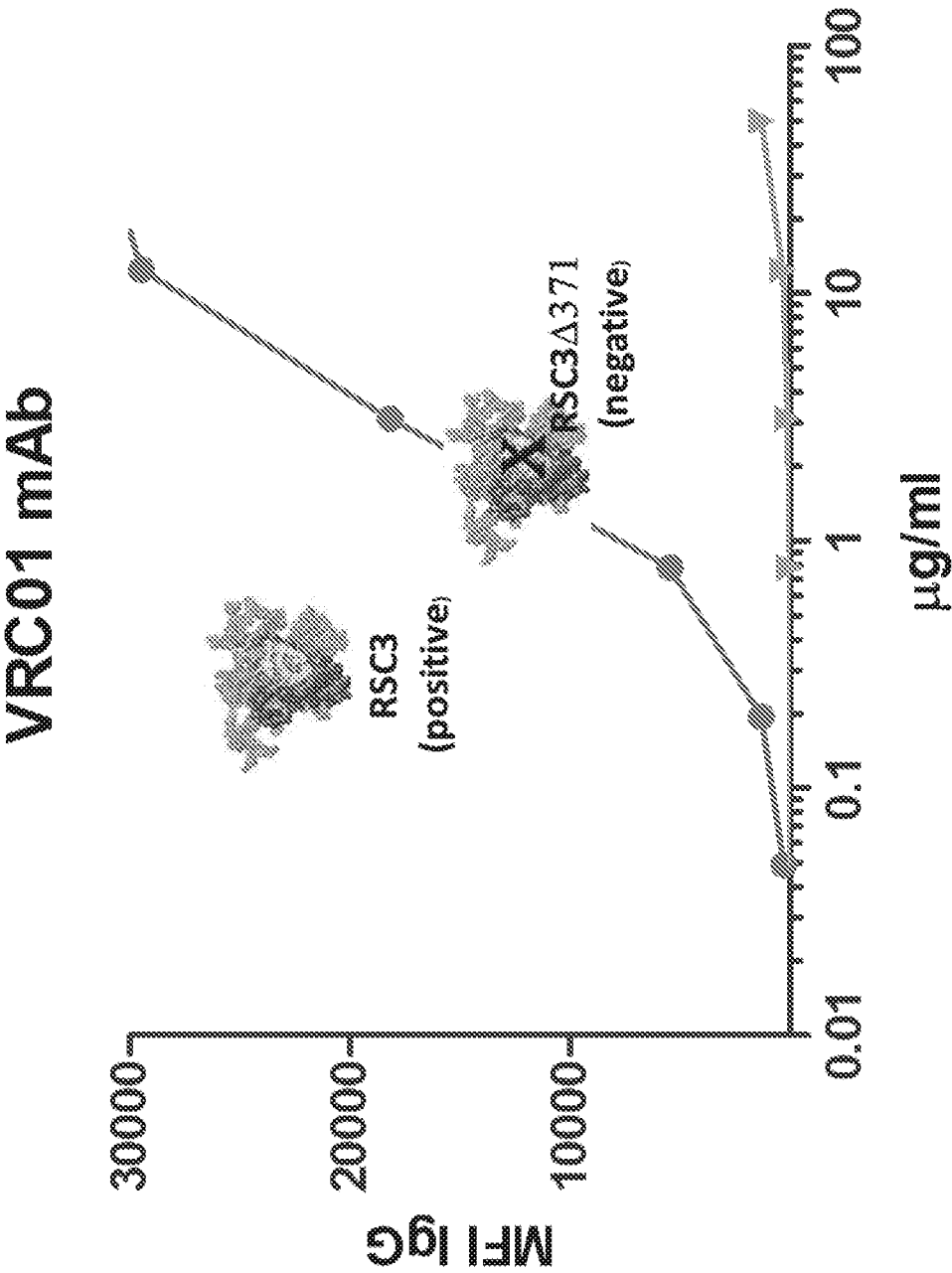


Fig. 18

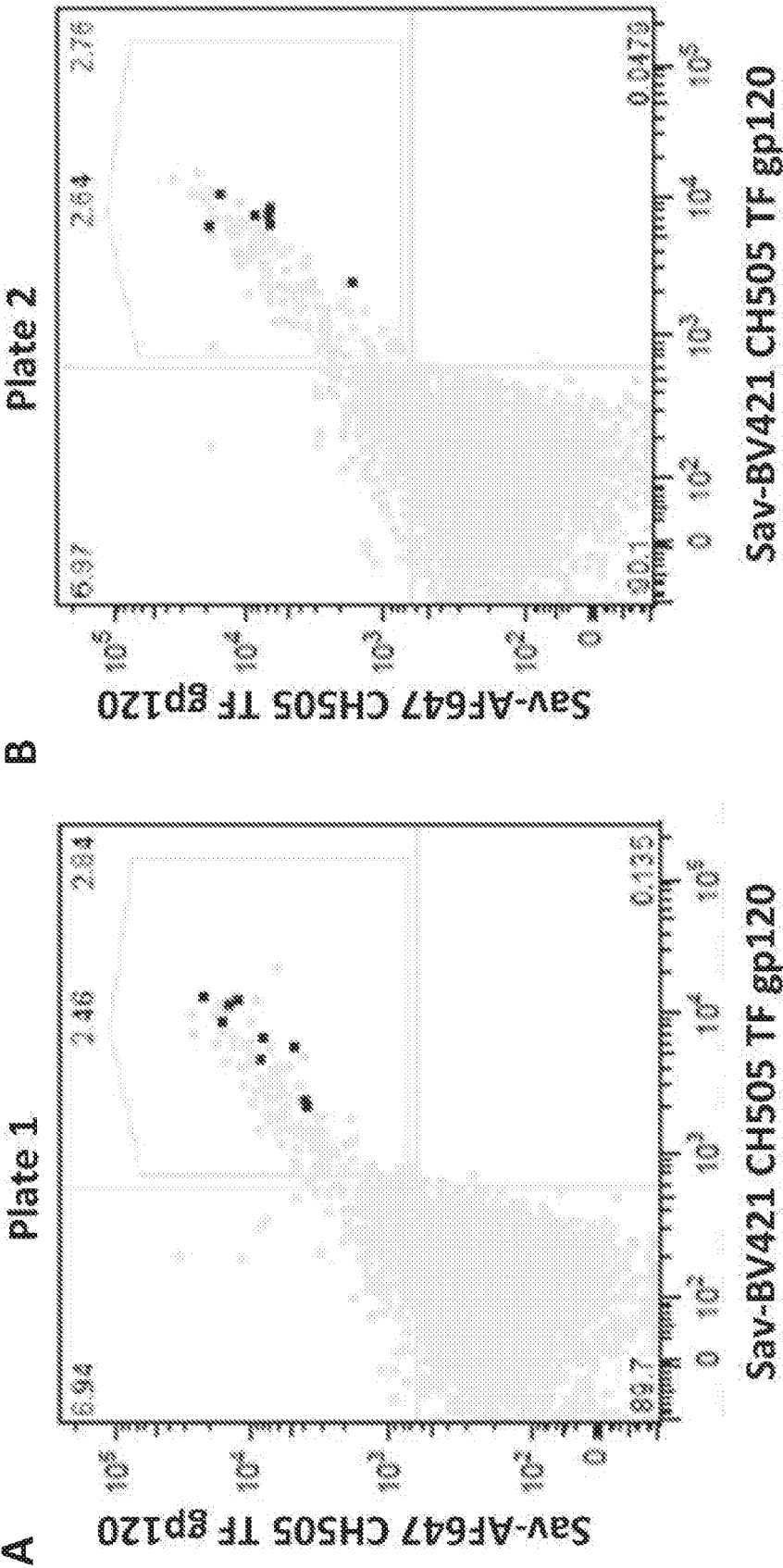


Fig. 19-A-B

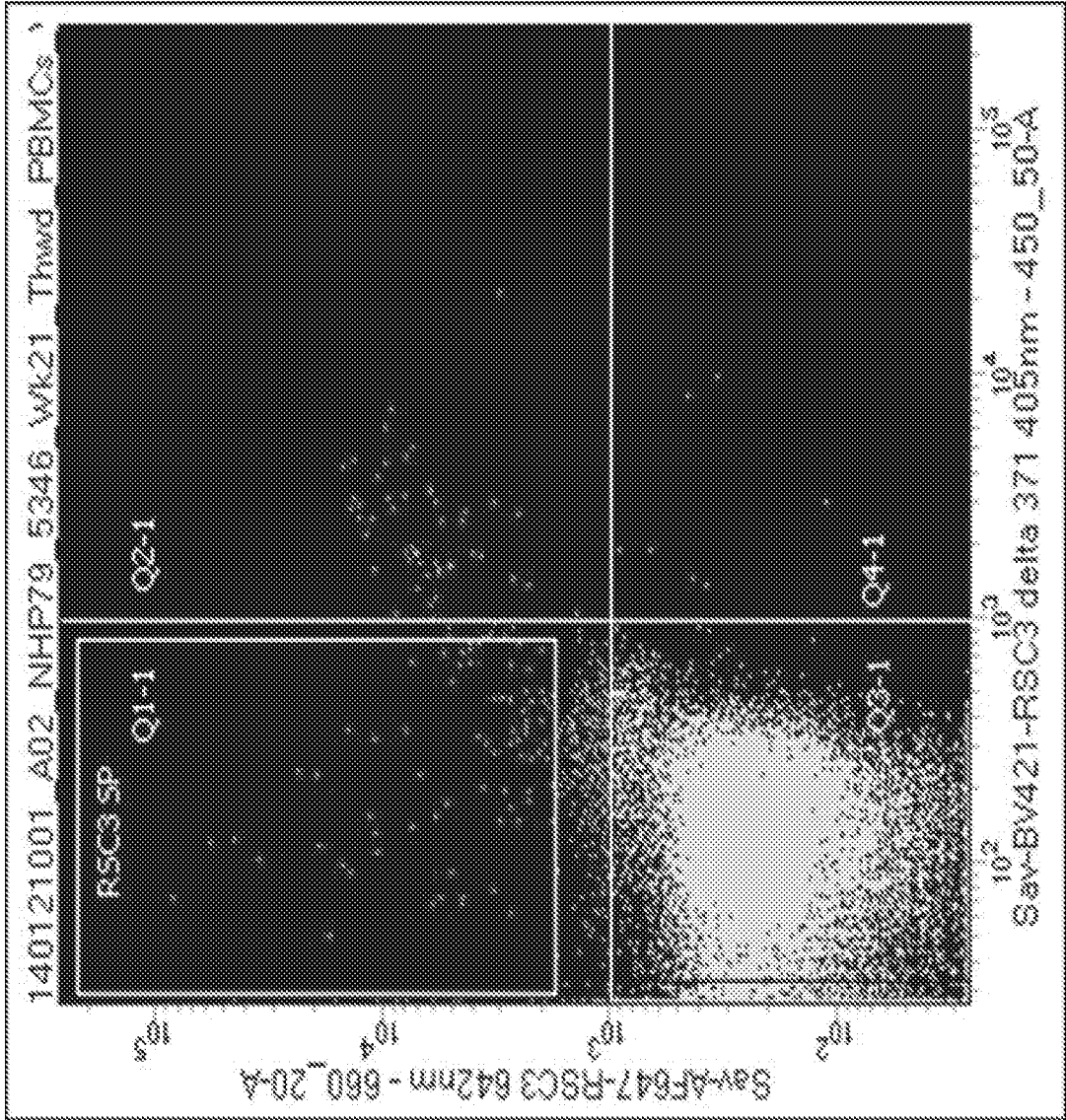


Fig. 20

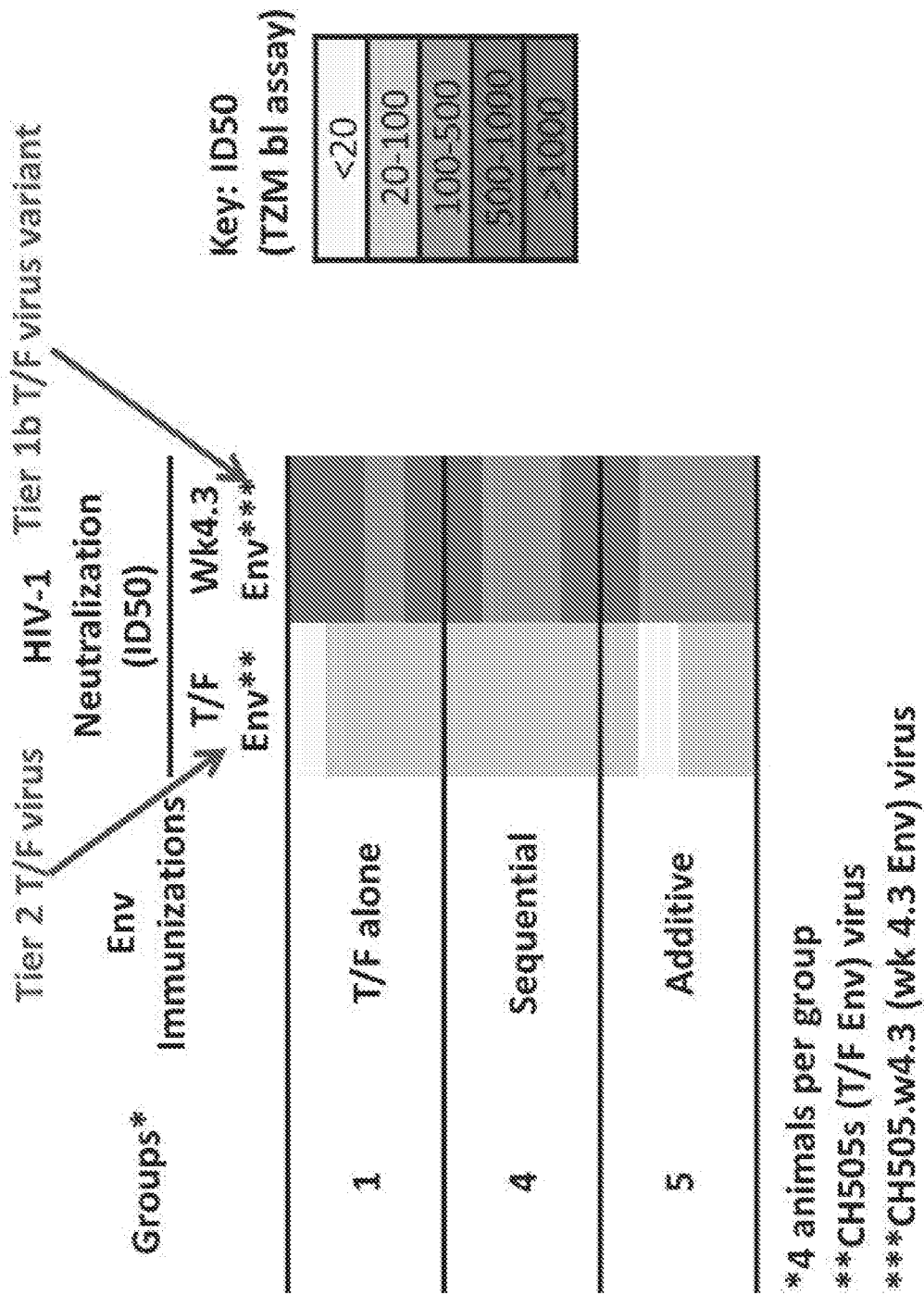


Fig. 21

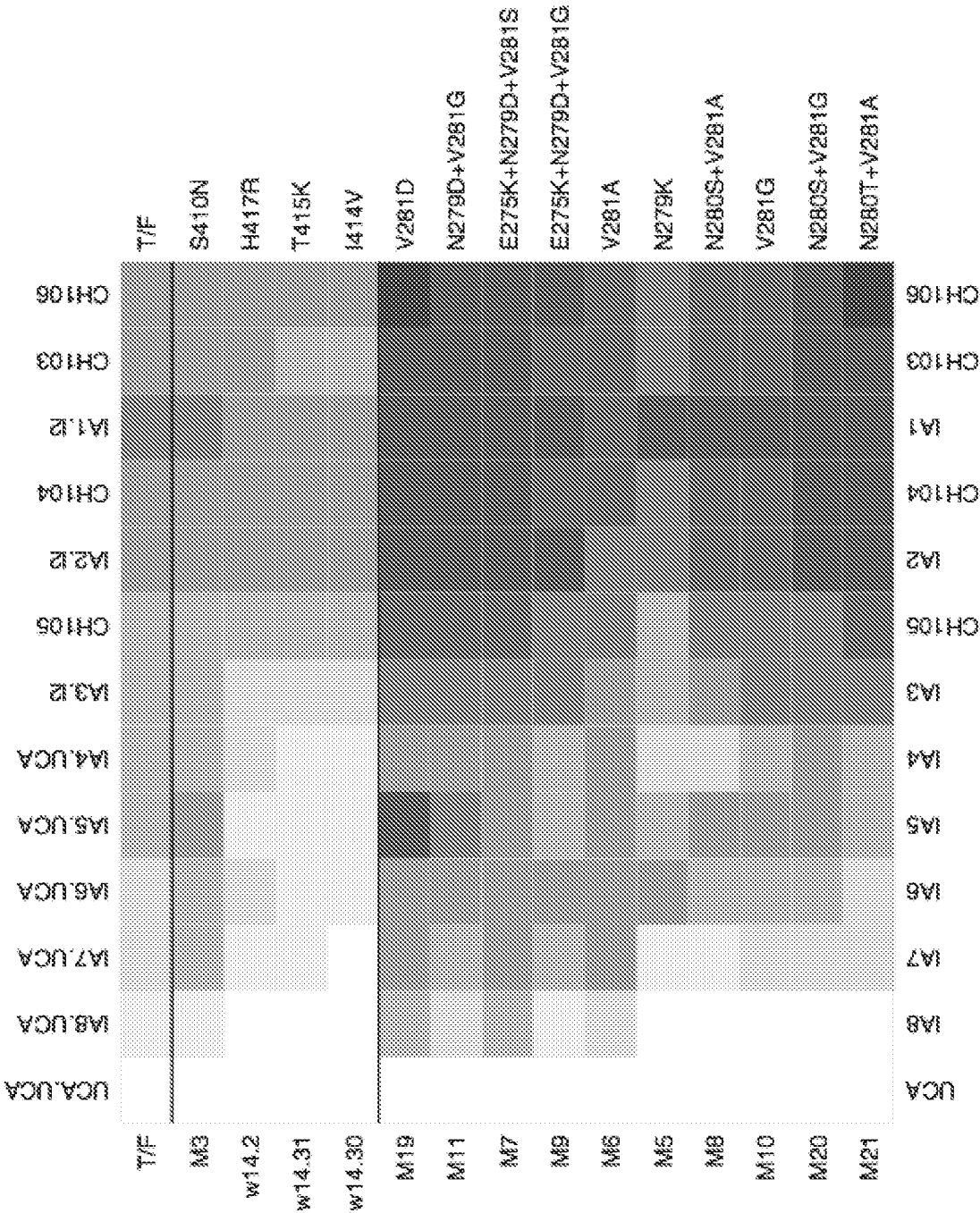


Fig. 22

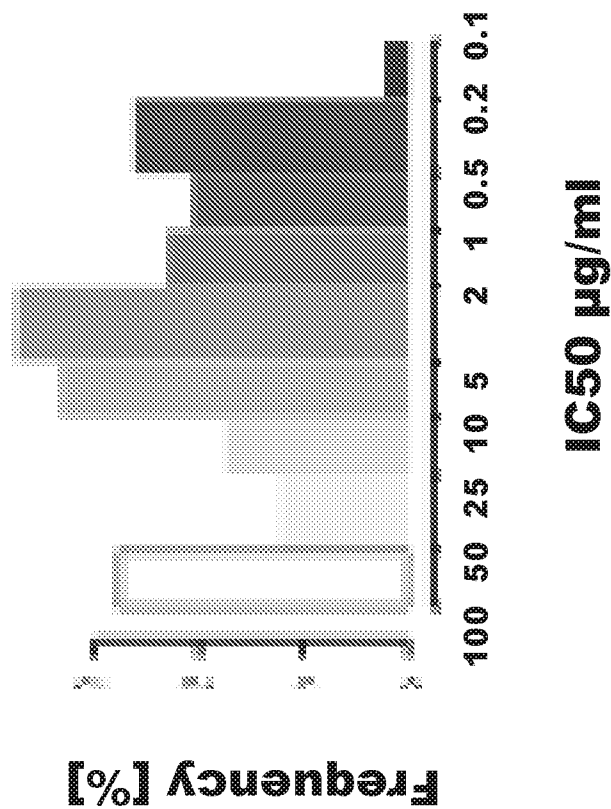


Fig. 22 (cont.)

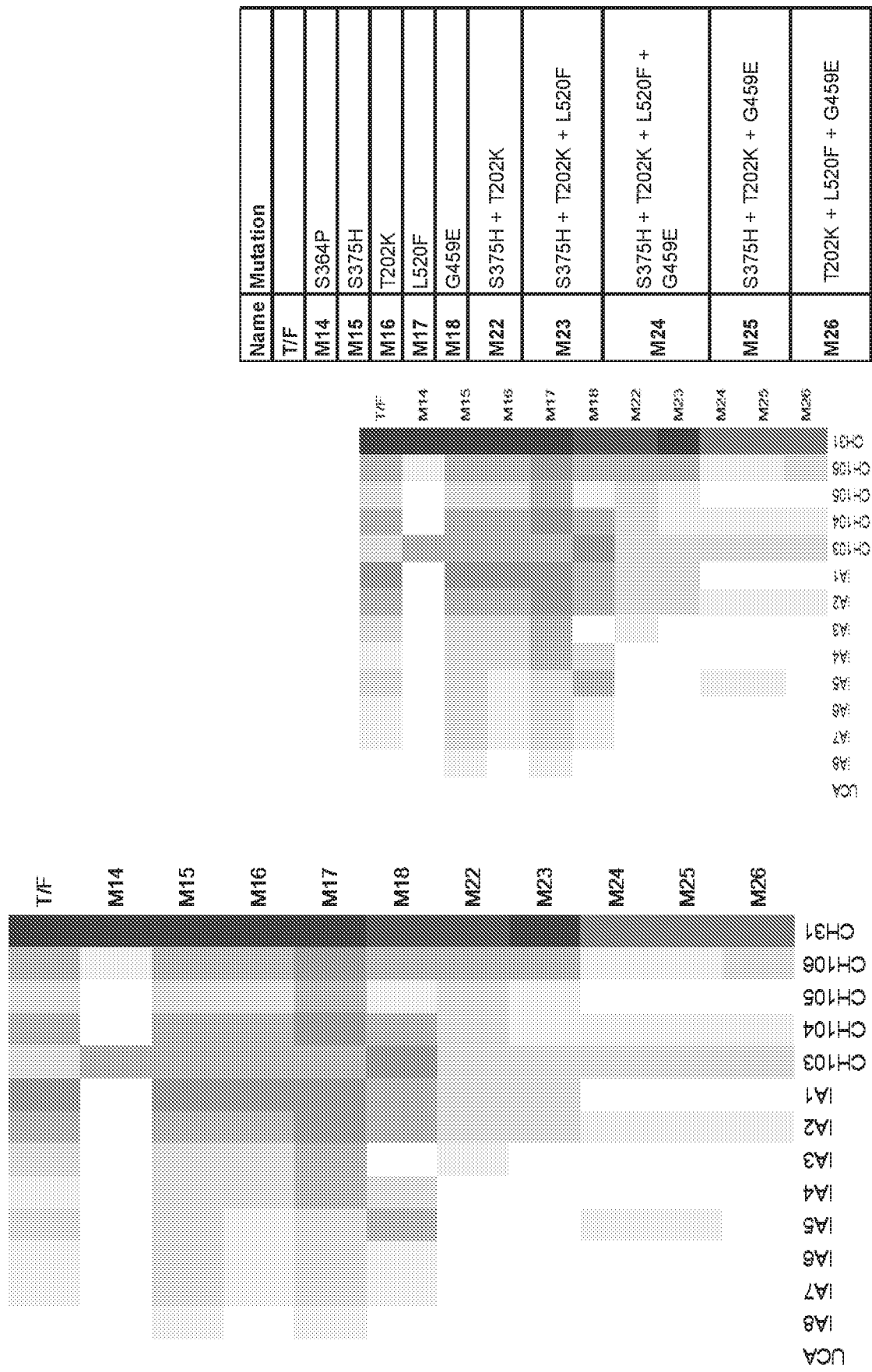


Fig. 23

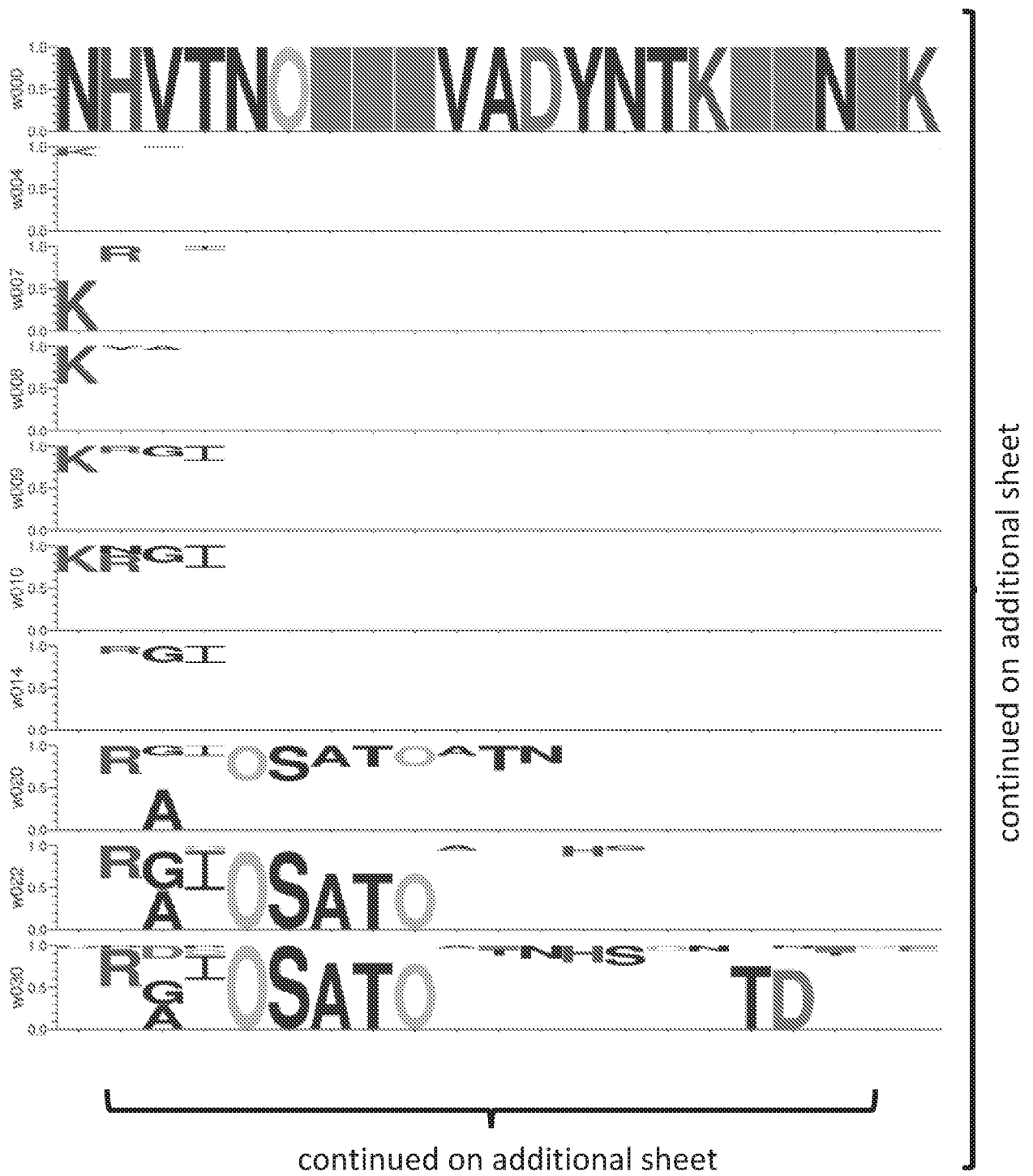
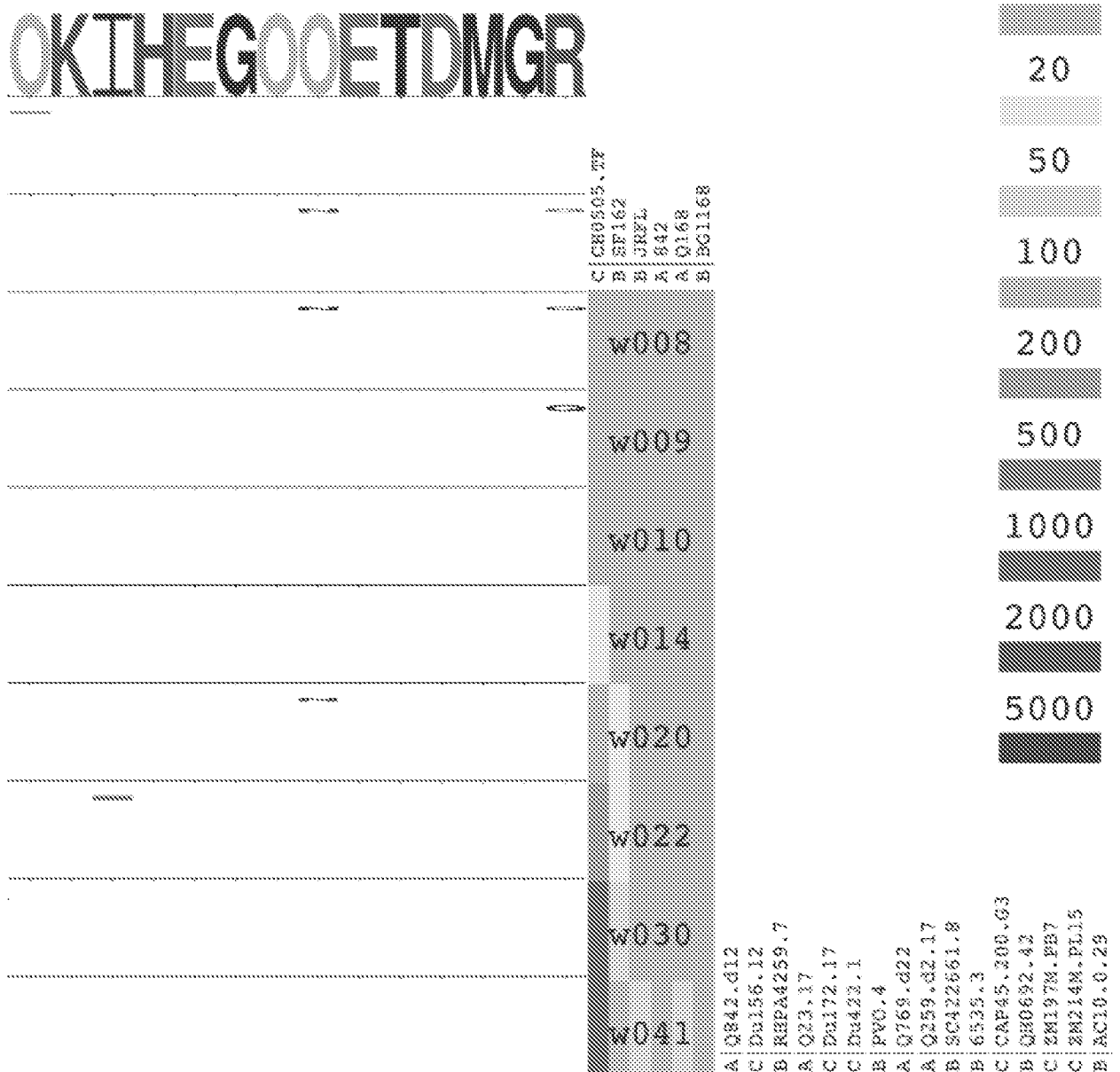


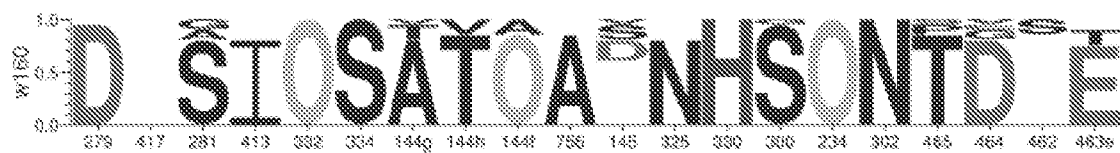
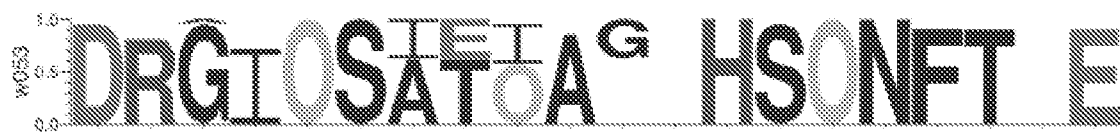
Fig. 24



continued on additional sheet

Fig. 24 (cont.)

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continued on additional sheet

Fig. 24 (cont.)

60/83

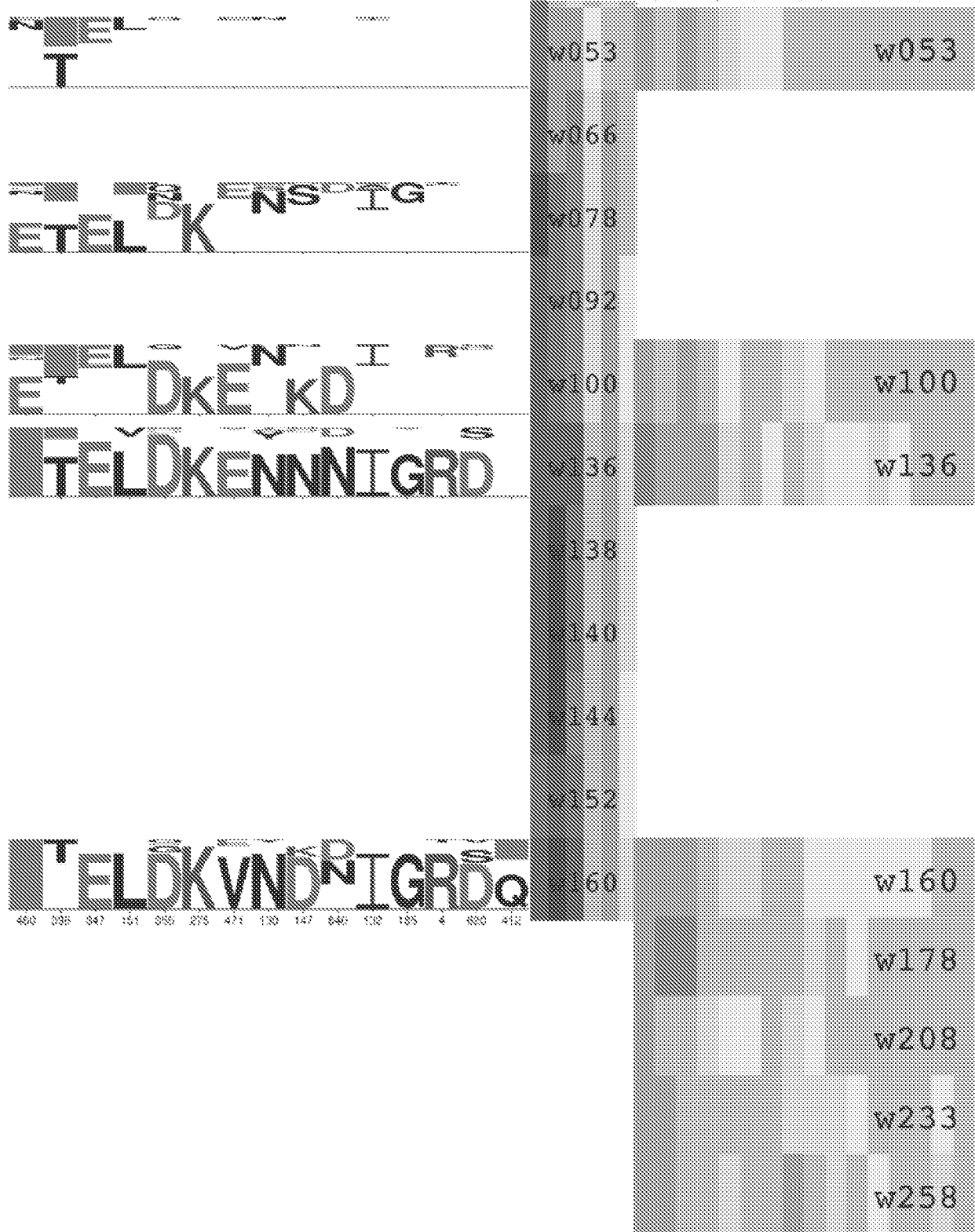
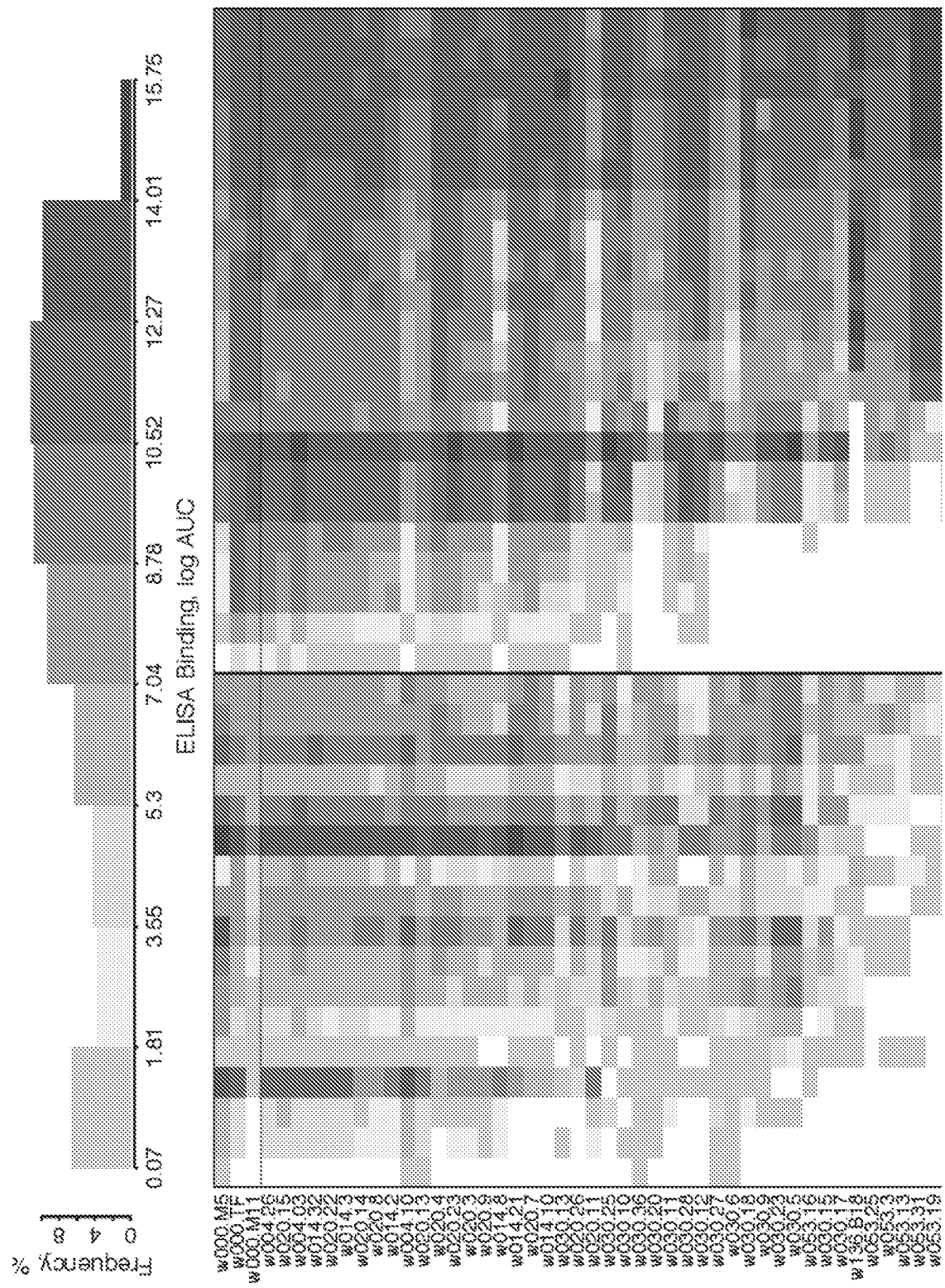


Fig. 24 (cont.)



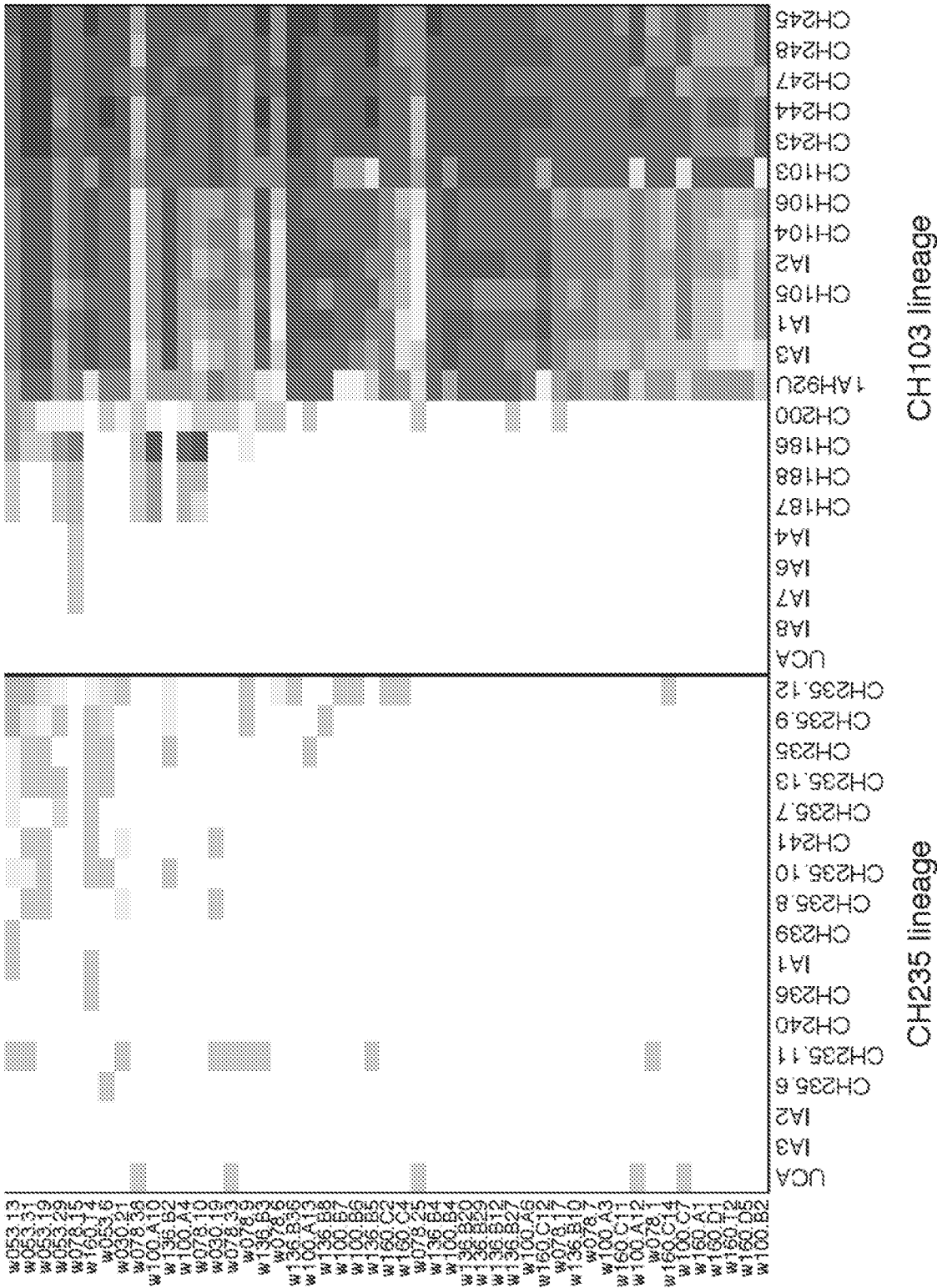
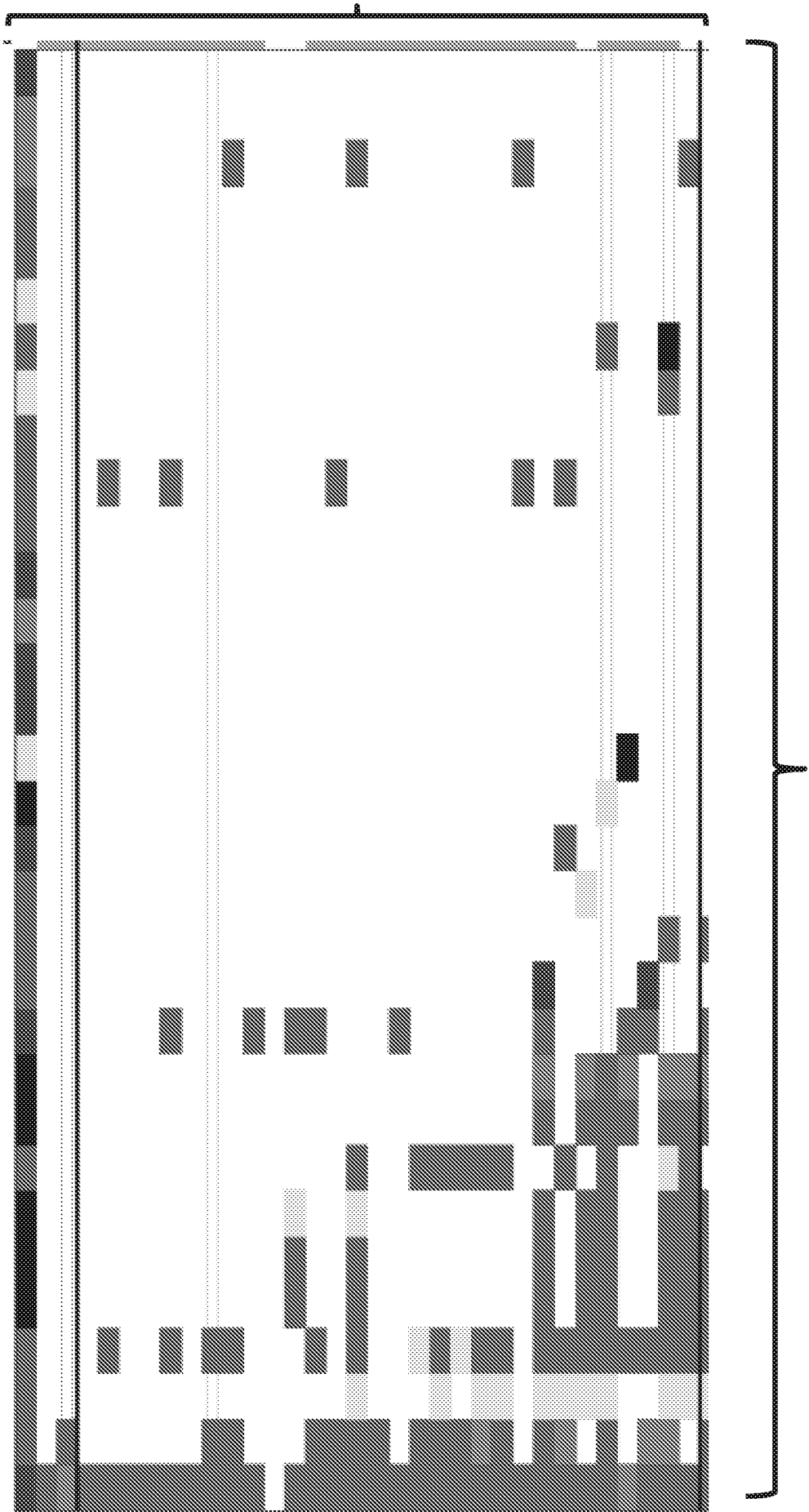


Fig. 25 (cont.)

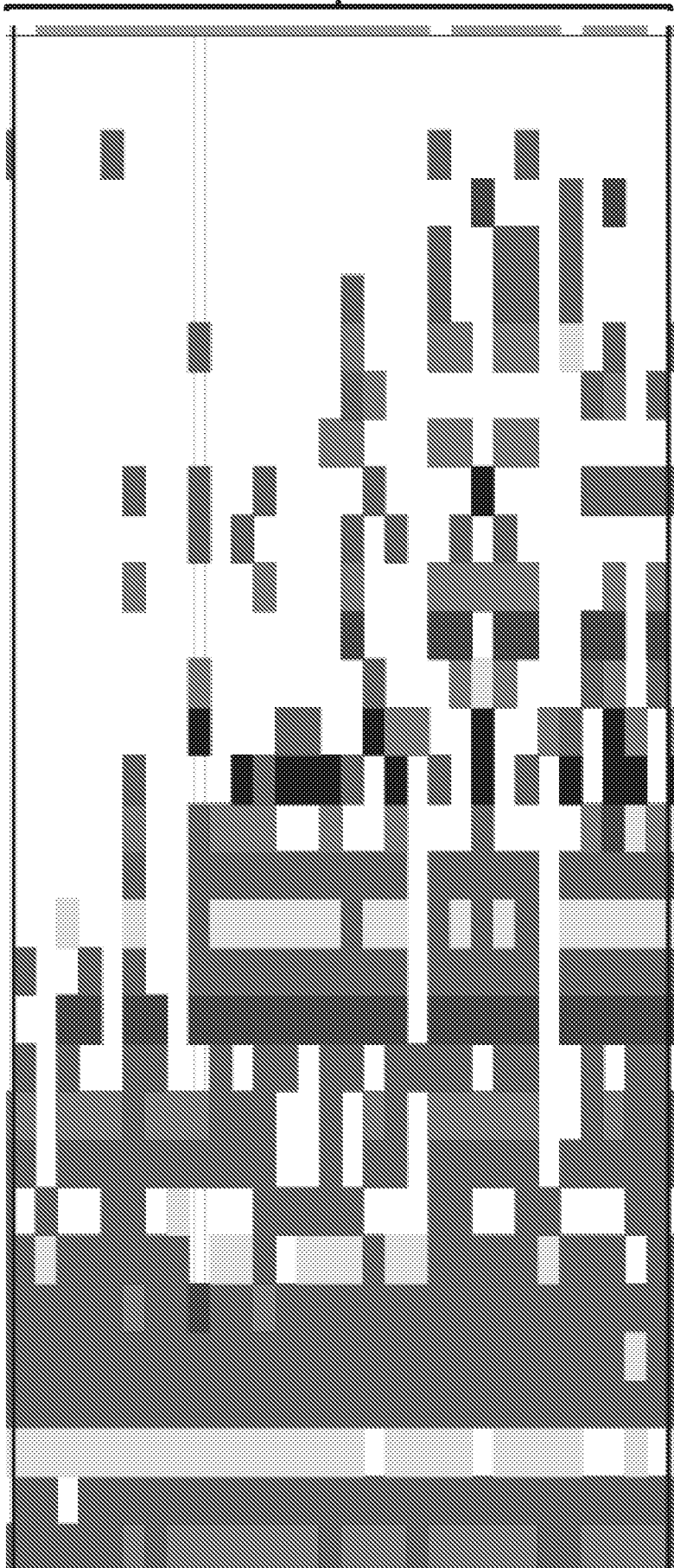
continued on Figure 26B



continued on additional sheet

Fig. 26A

continued on Figure 26B



continued on additional sheet

Fig. 26A (cont.)

continued on Figure 26B

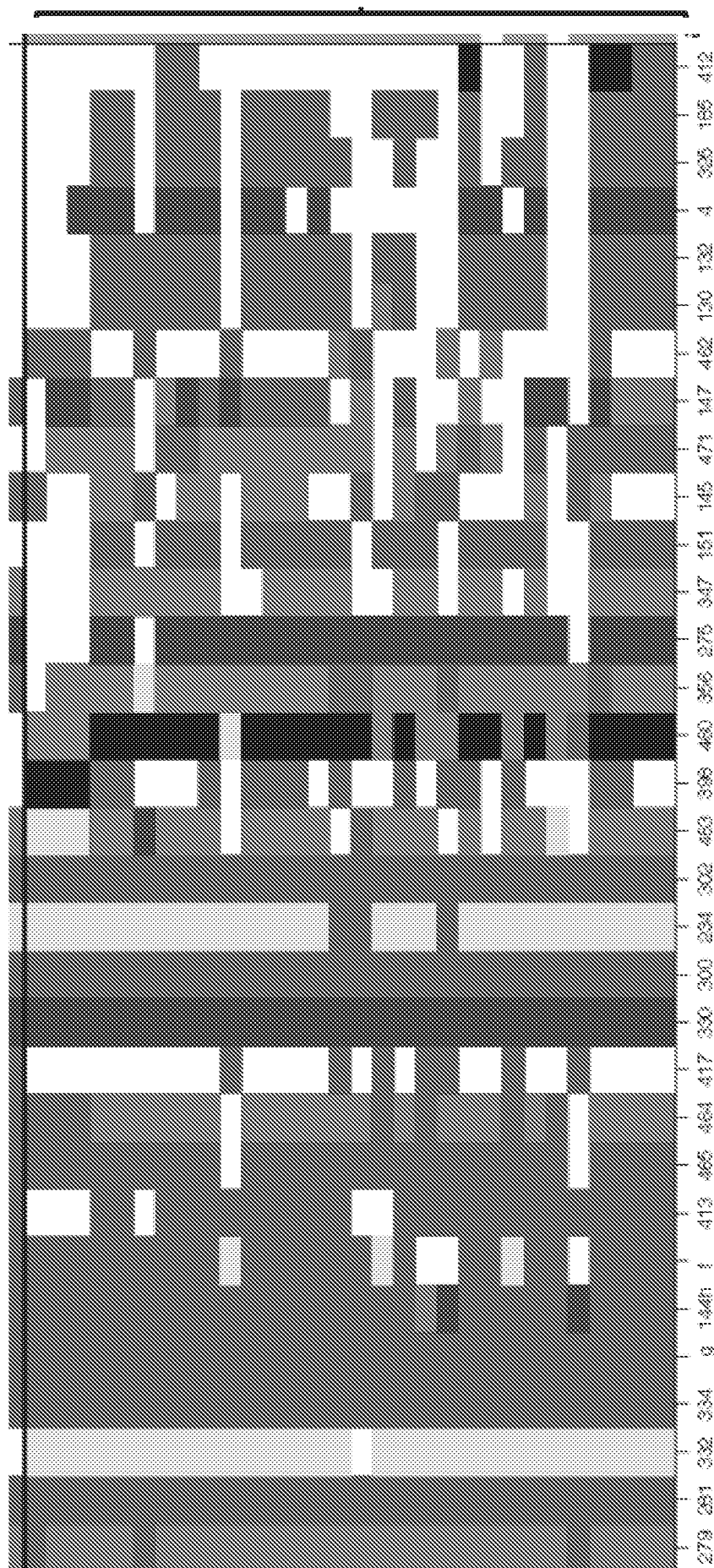
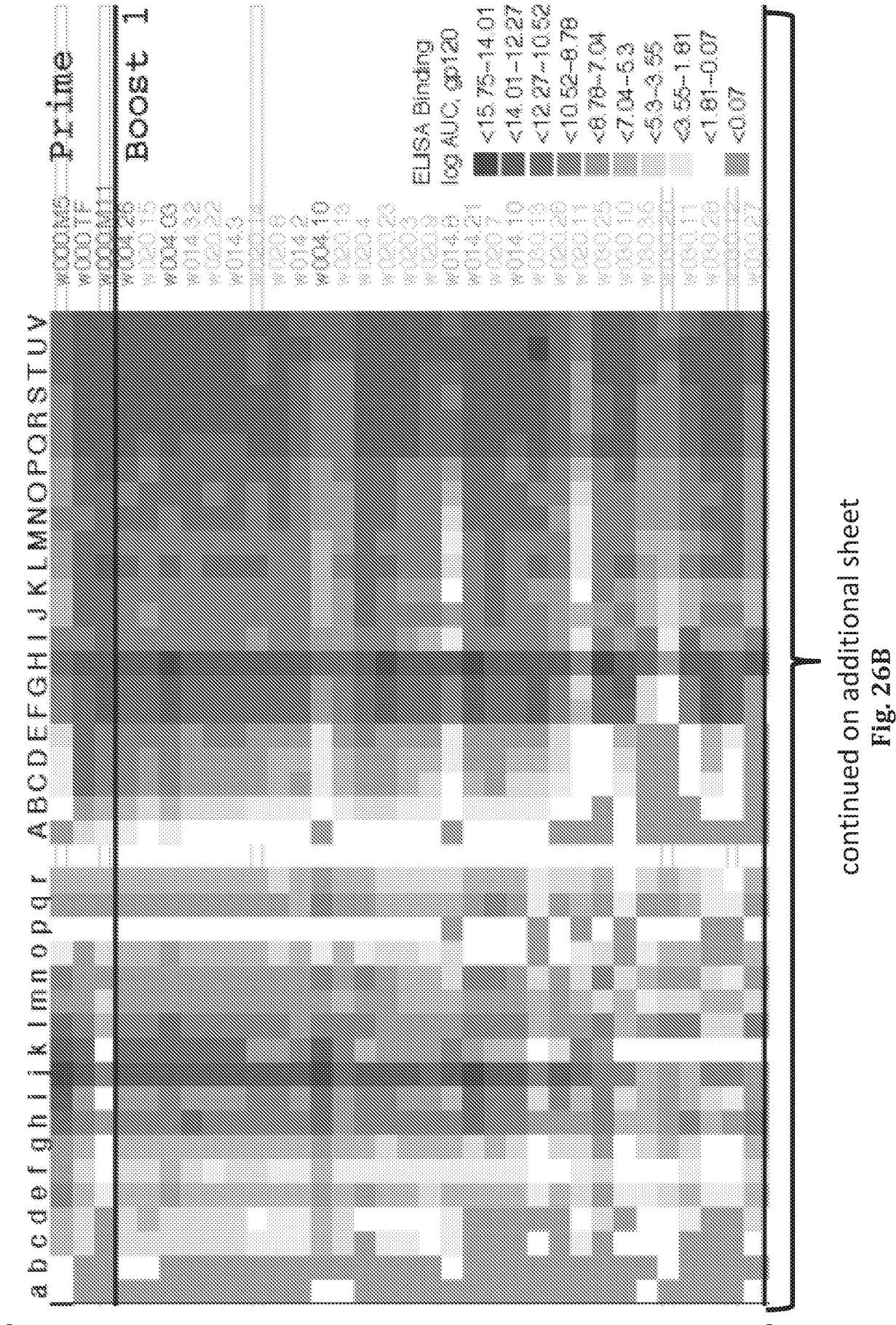
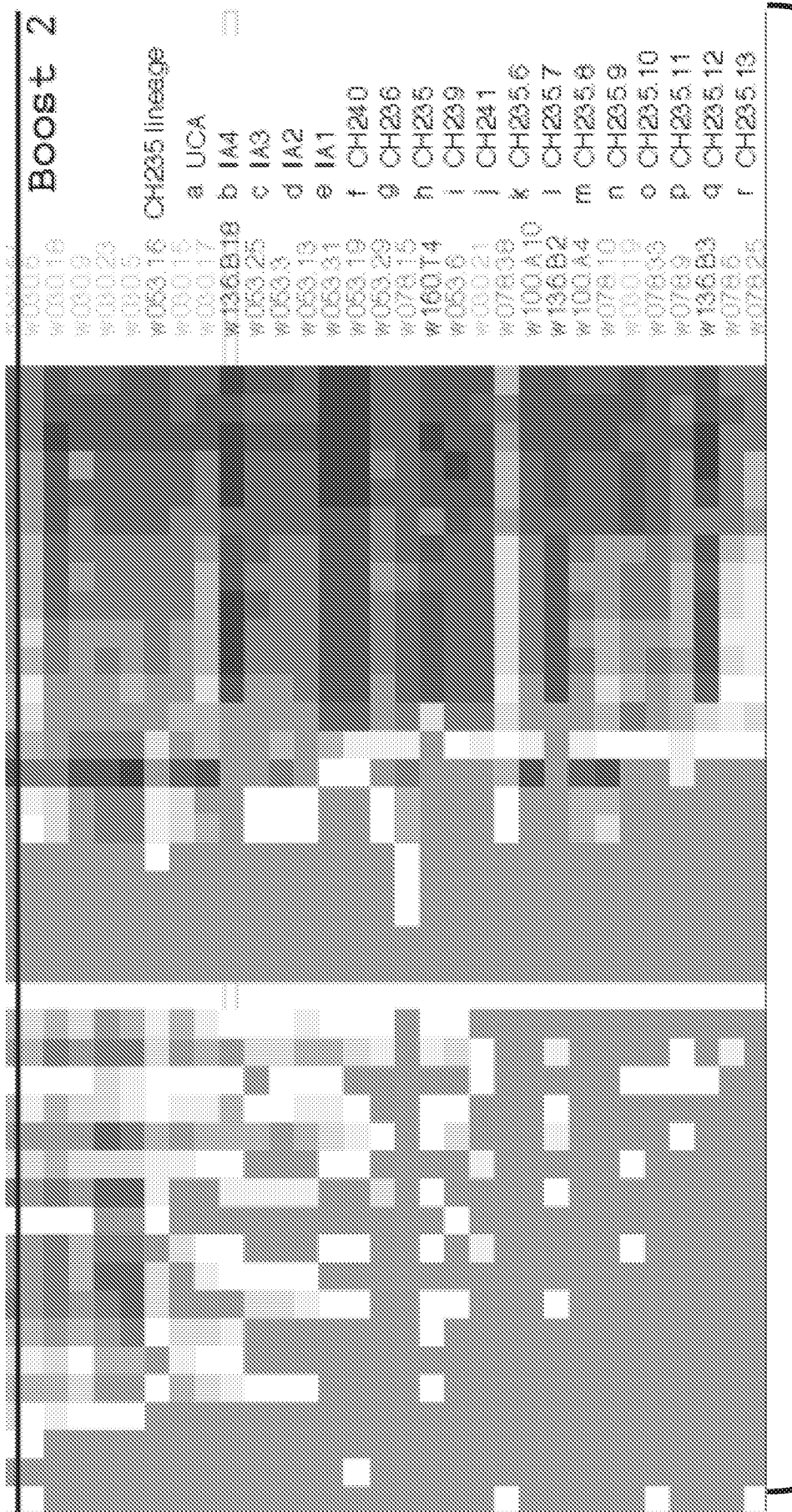


Fig. 26A (cont.)



continued on Figure 26A



continued on additional sheet

Fig. 26B (cont.)

continued on Figure 26A

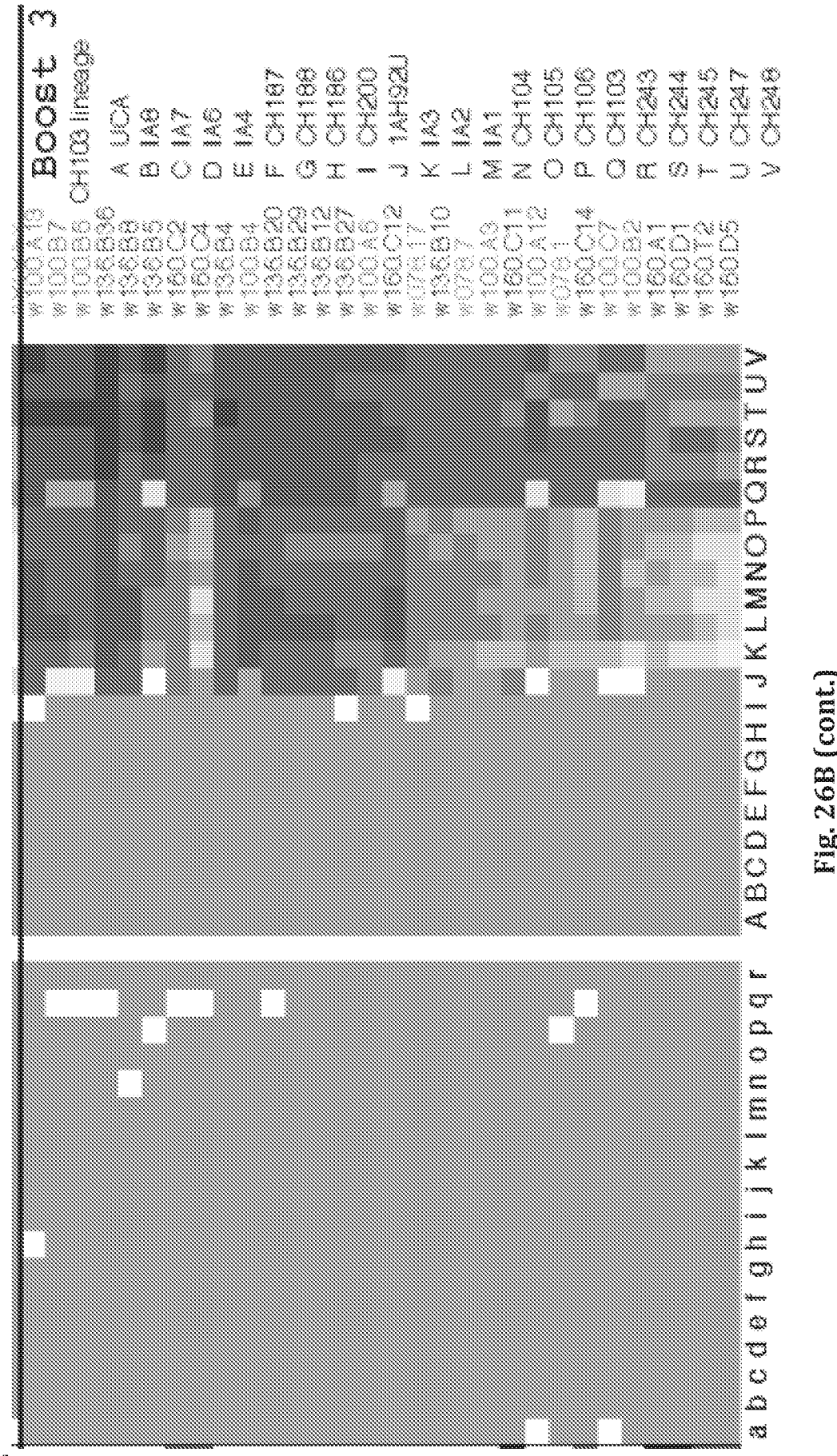
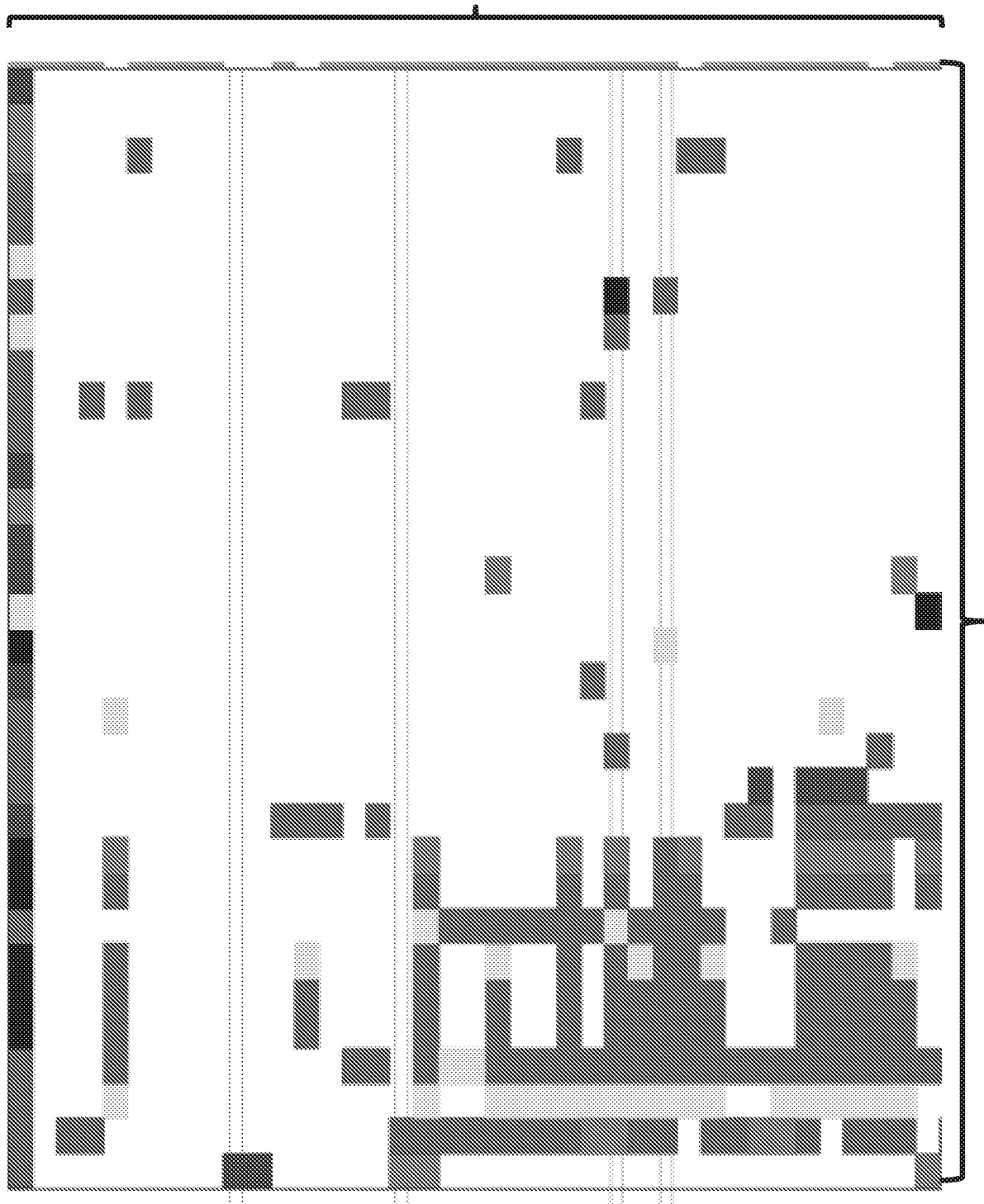


Fig. 26B (cont.)

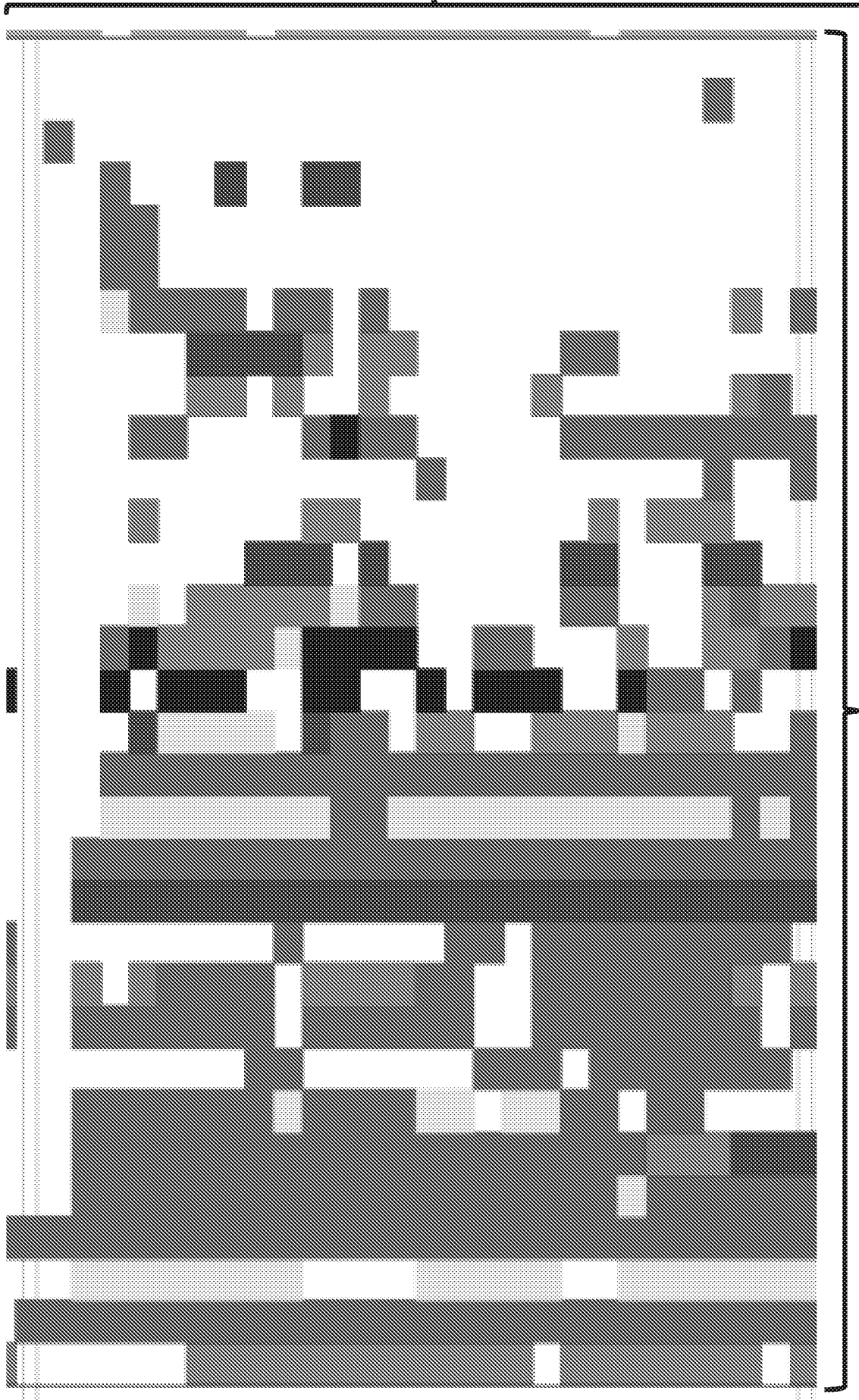
continued on additional sheet



continued on additional sheet

Fig. 27A

continued on additional sheet



continued on additional sheet

Fig. 27A (cont.)

continued on additional sheet

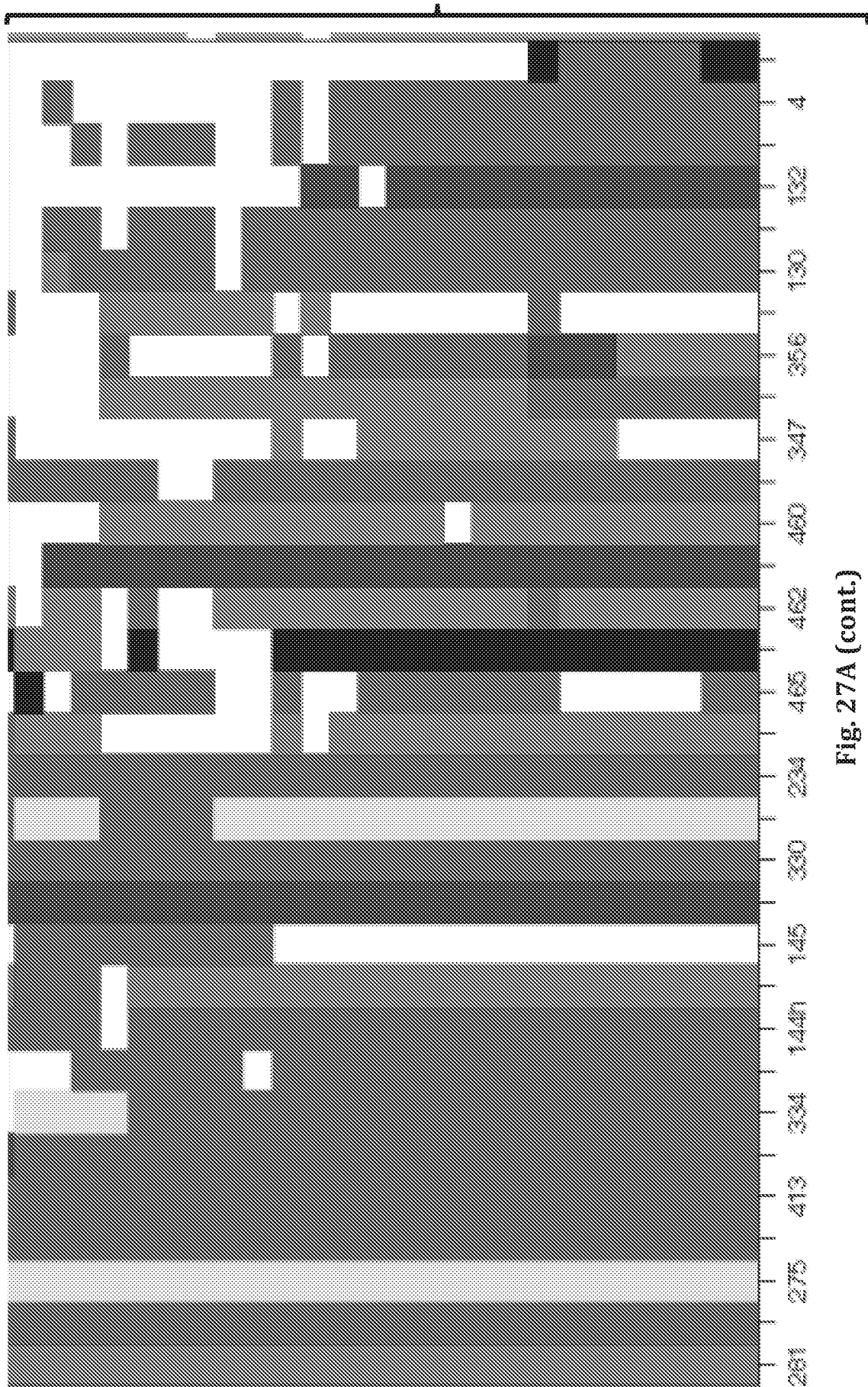


Fig. 27A (cont.)

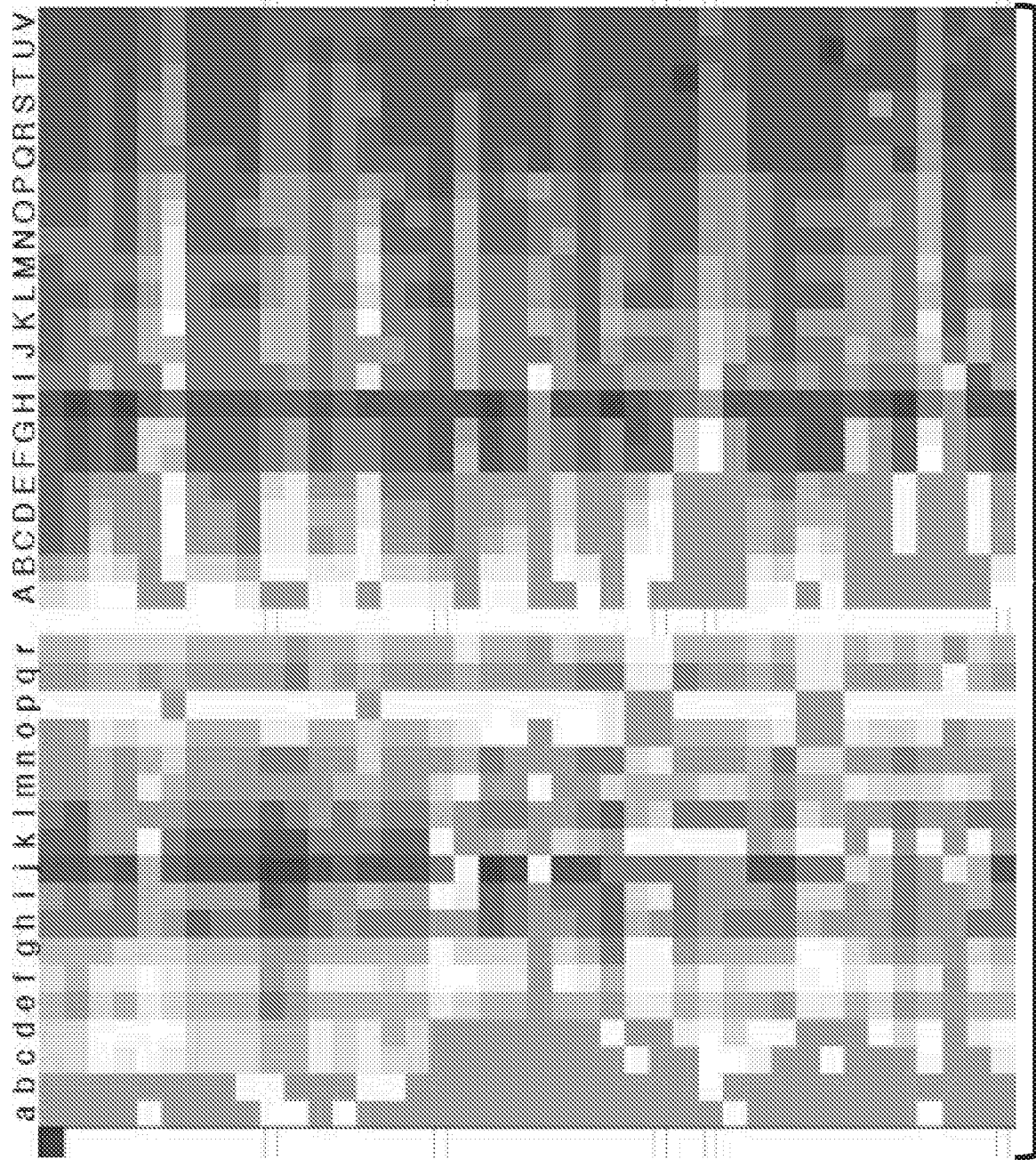


Fig. 27A (cont.)

continued on additional sheet

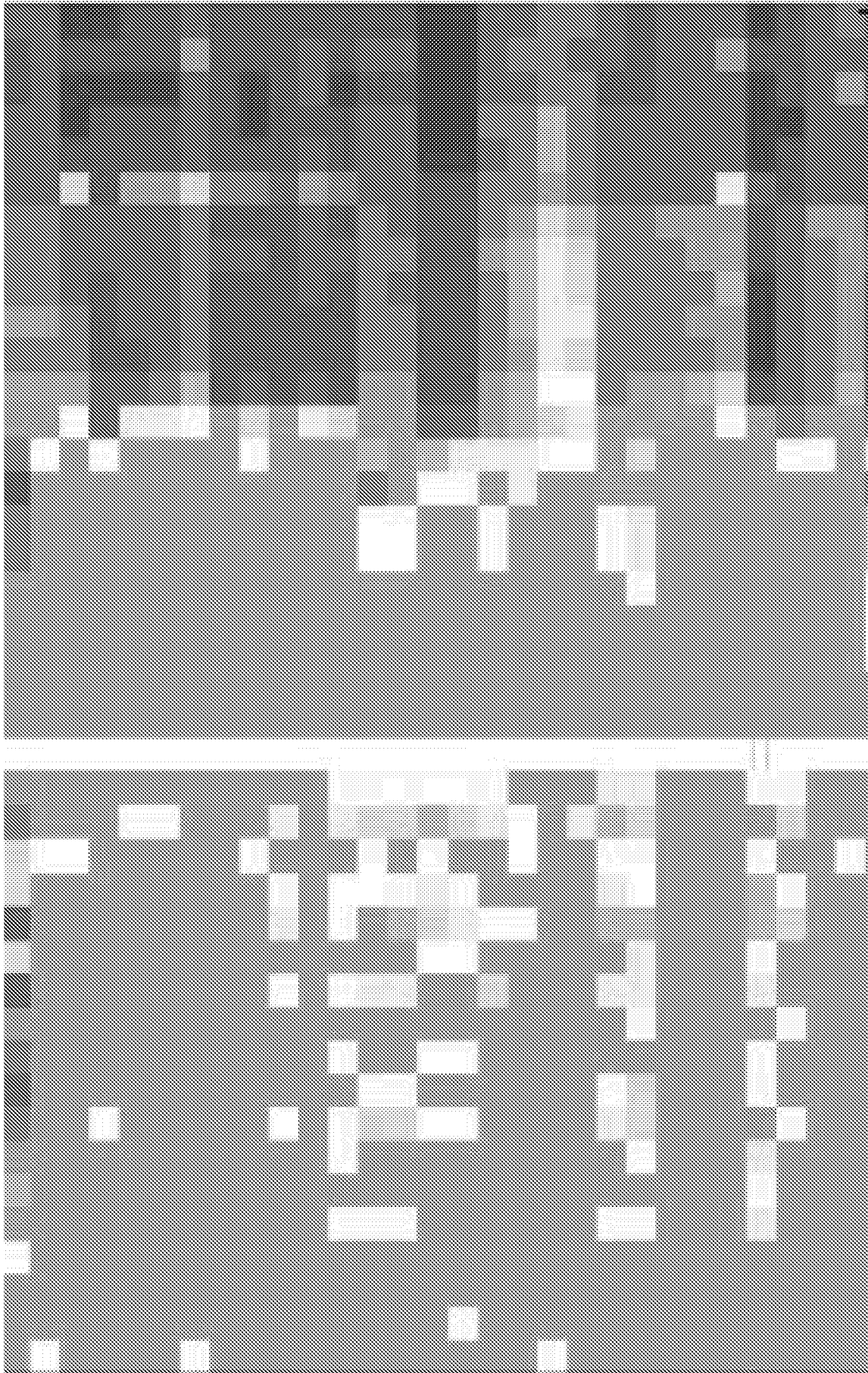


Fig. 27A (cont.)

continued on additional sheet

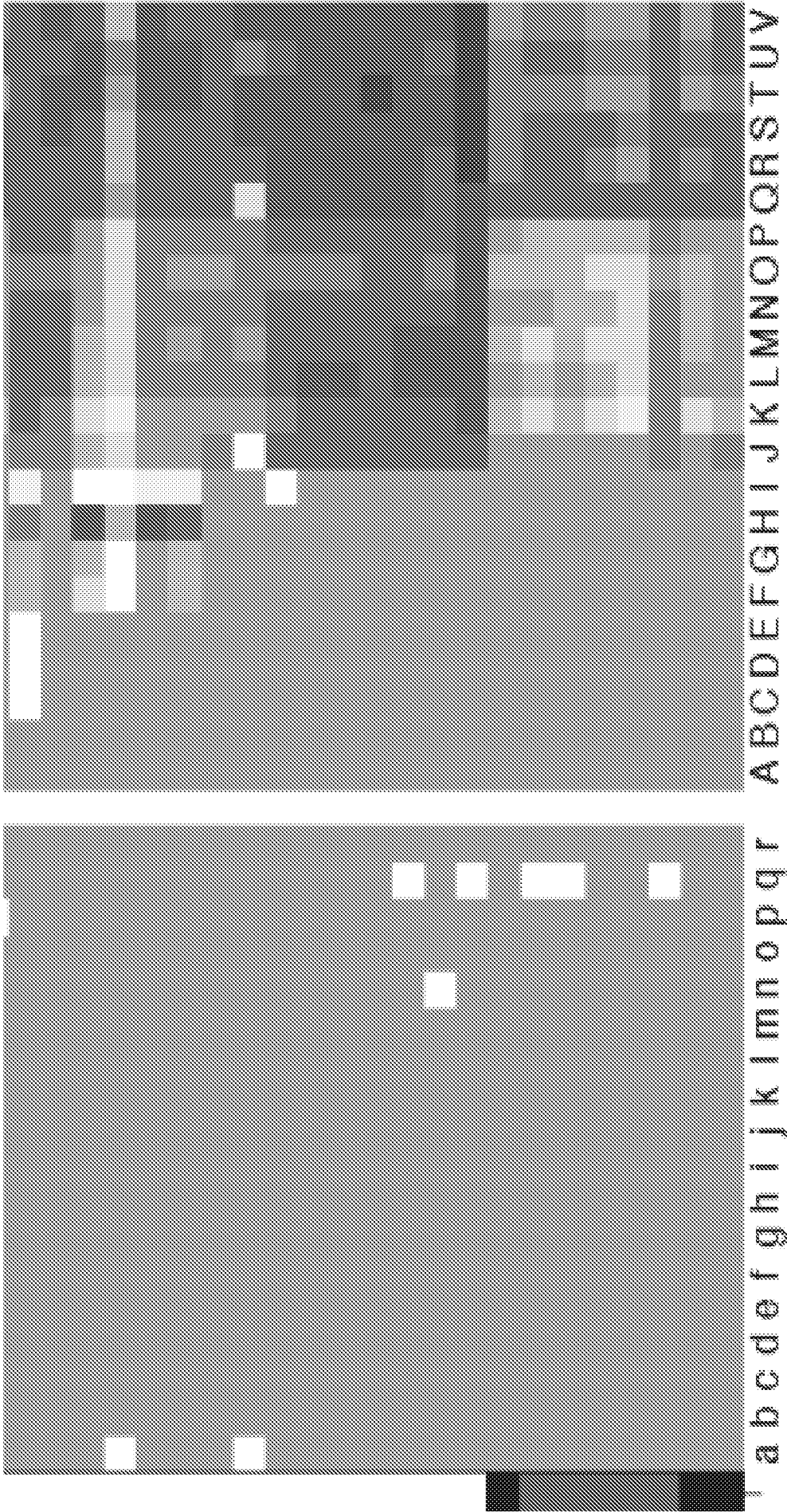
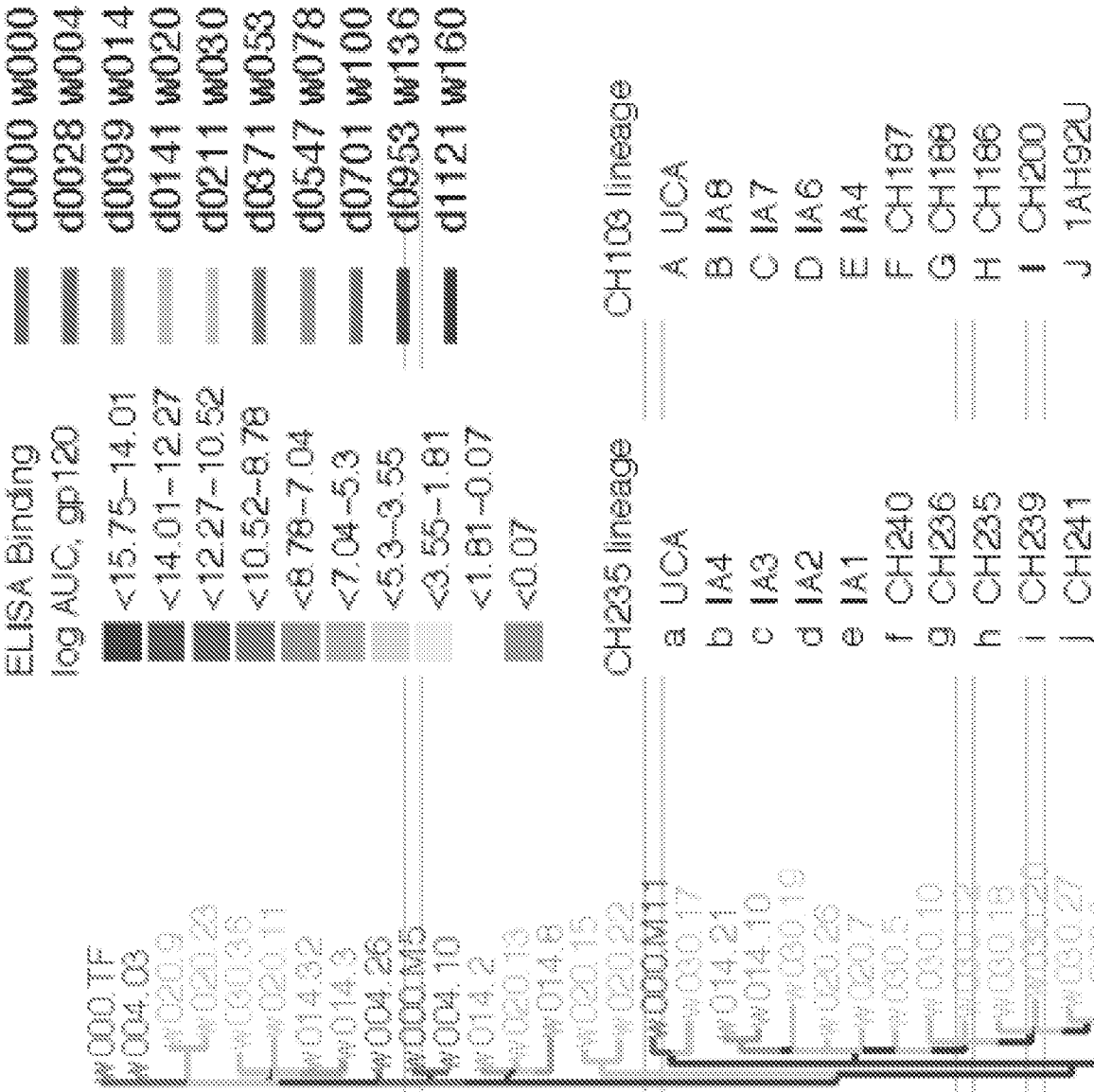


Fig. 27A (cont.)



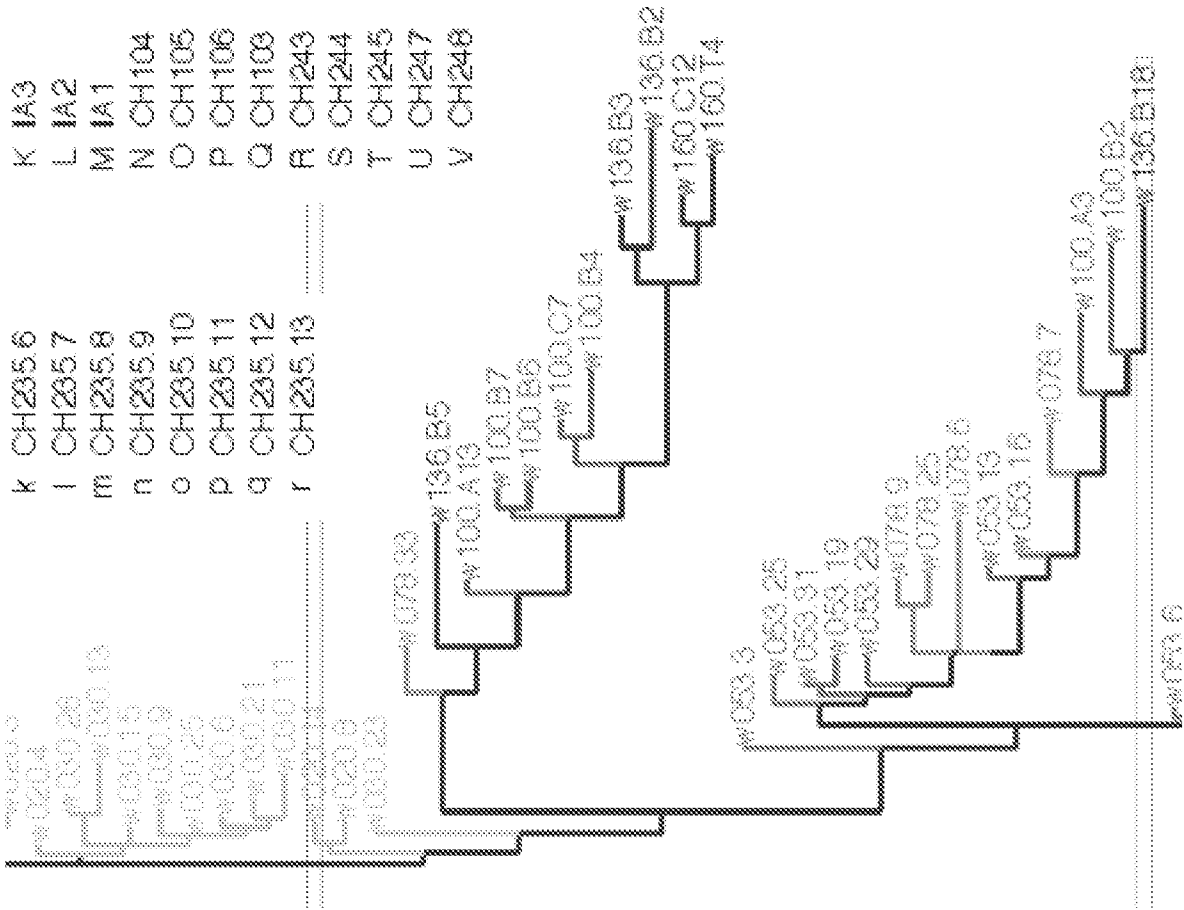


Fig. 27B (cont.)

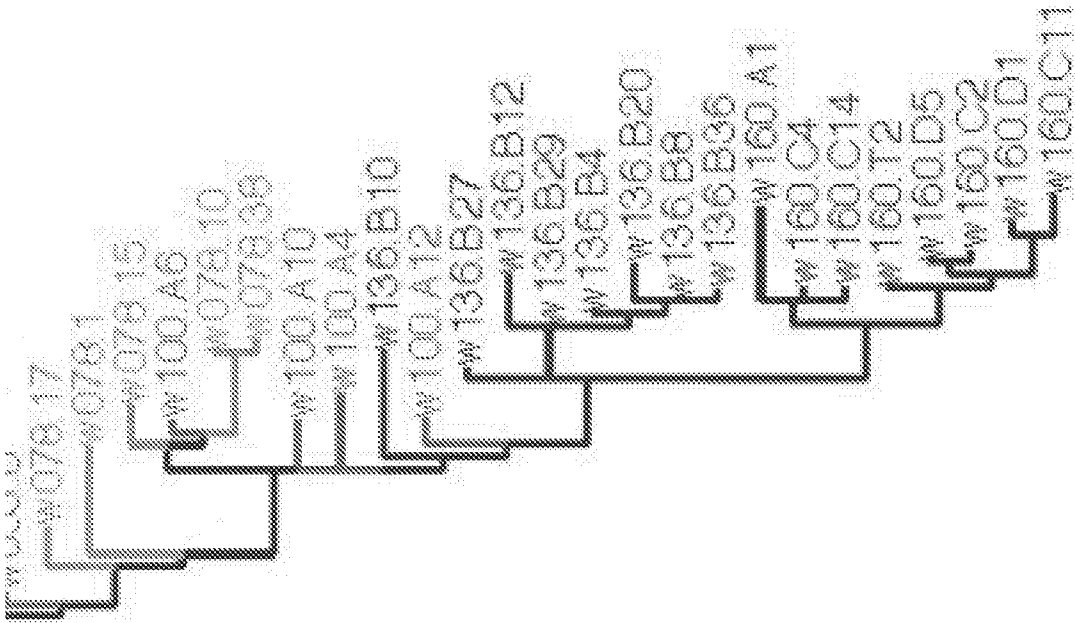
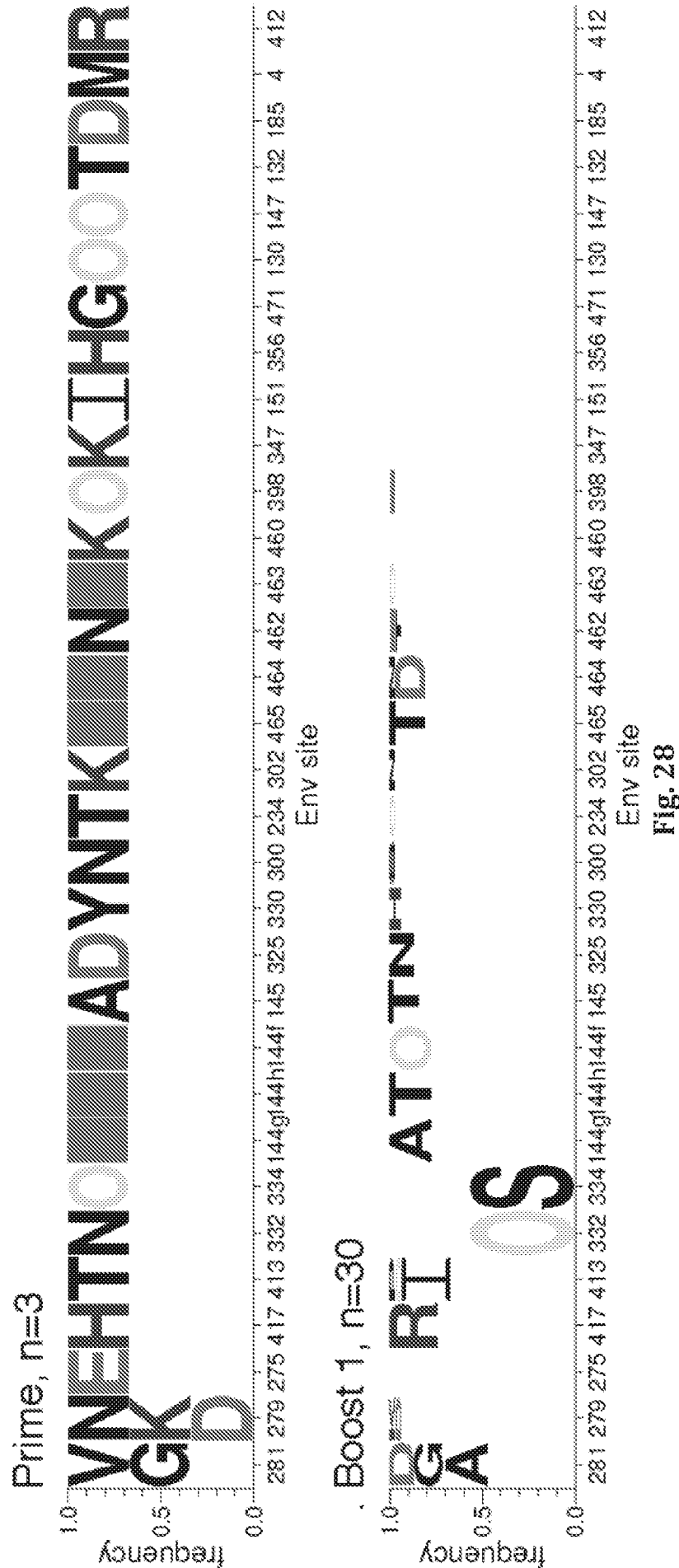


Fig. 27B (cont.)



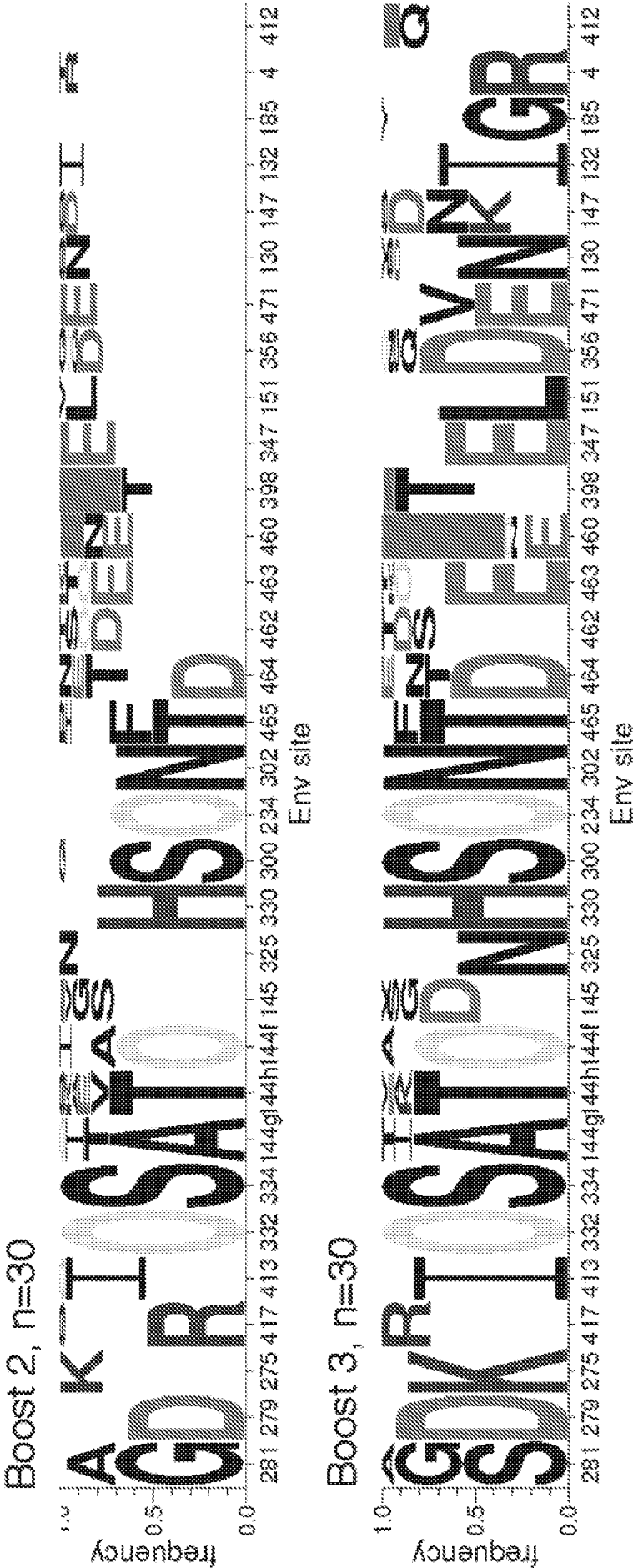
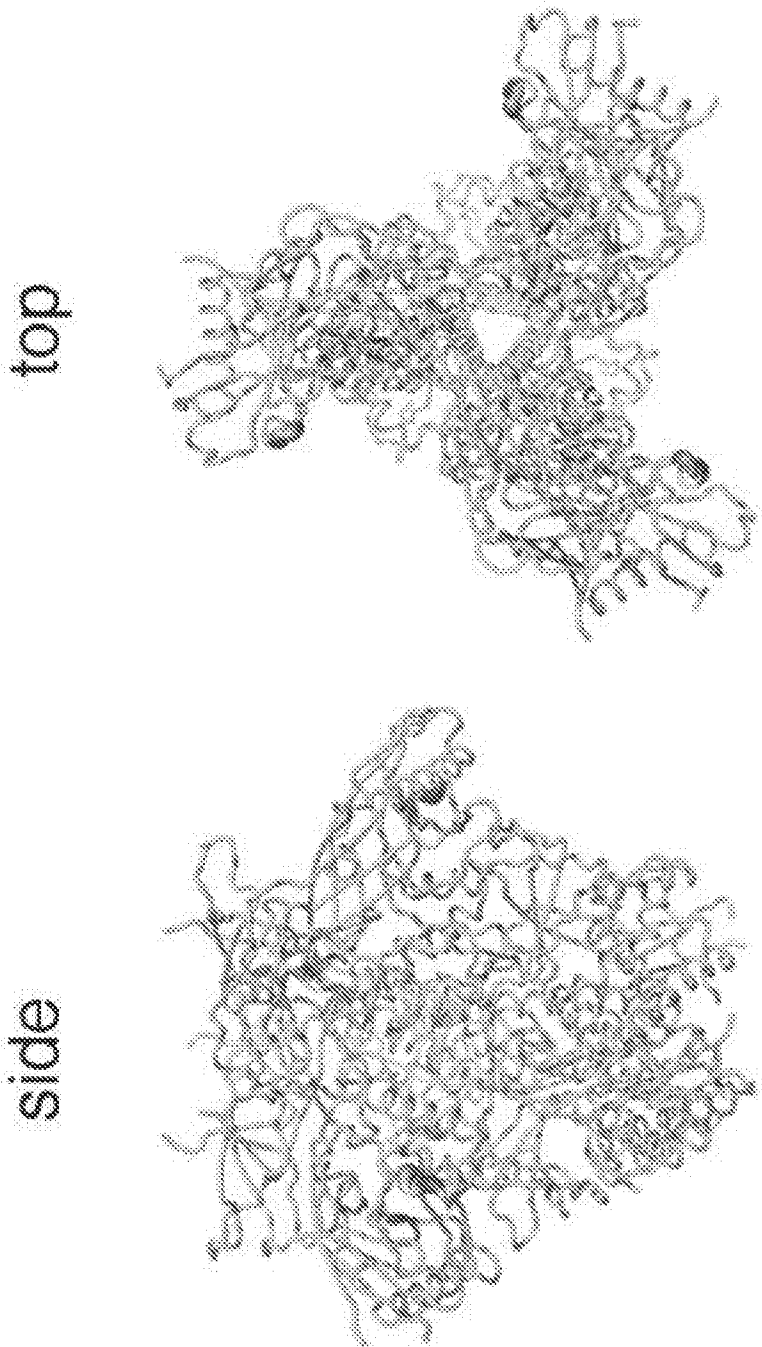


Fig. 28 (cont.)



Prime,  $n=3$

Fig. 29A

Boost 1, n=30



Fig. 29B

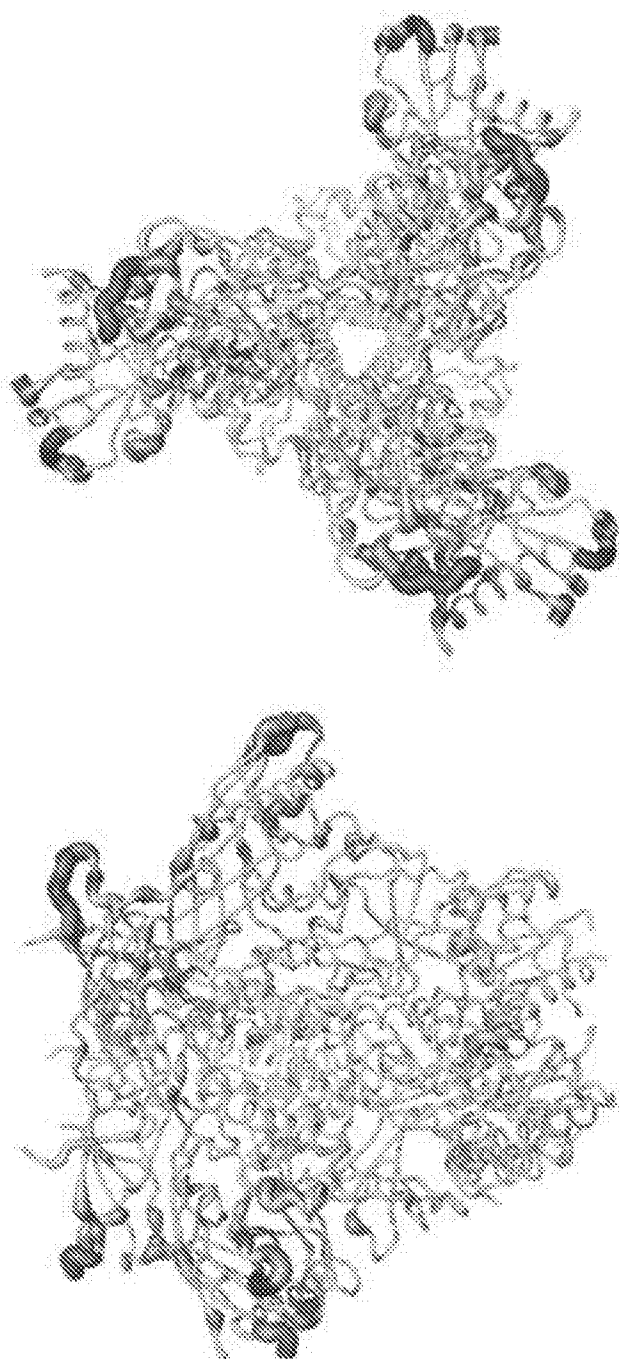


Fig. 29C

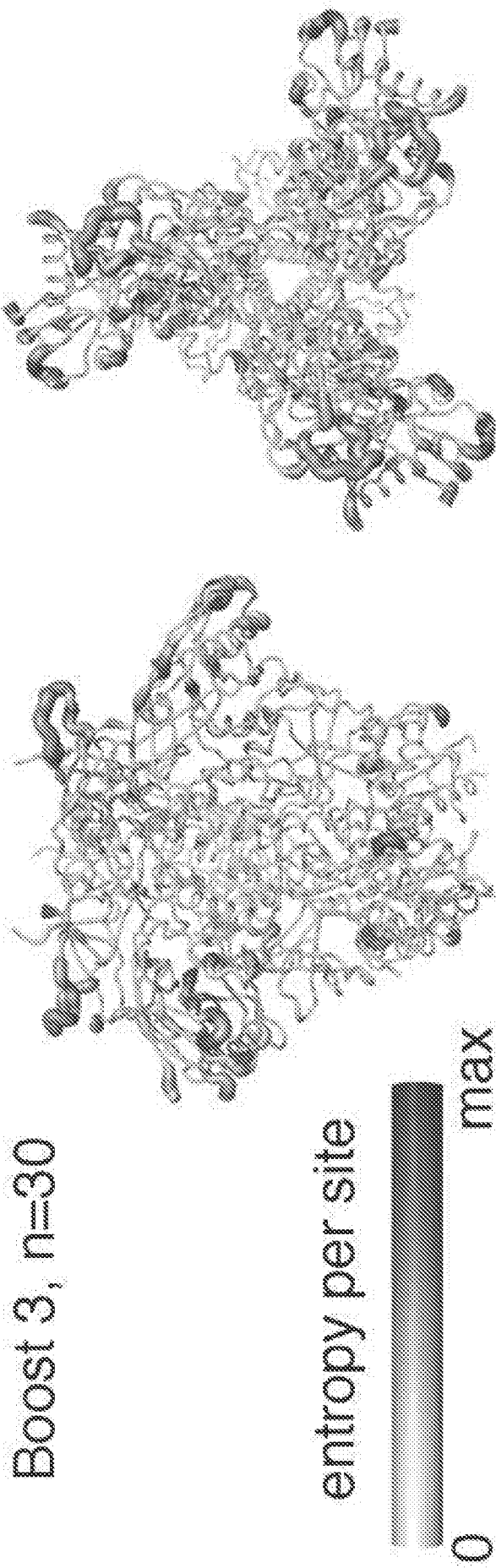


Fig. 29D

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/20821

## A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 39/12; C07K 16/10; C12Q1/68 (2017.01)

CPC - A61K 39/12; C07K 16/10, 16/1045, 16/1063; C12Q 1/703

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)

See Search History document

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

See Search History document

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

See Search History document

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                 | Relevant to claim No.                |
|-----------|------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|
| X         | WO 2016/014721 A2 (DUKE UNIVERSITY) January 28, 2016; paragraphs [0005], [0006], [0008], [0009], [0062], [0070], [0130]; figure 18 | 1-2, 3/1-2, 6/1-2, 7/1, 8, 12, 16-20 |
| A         | US 2006/0246088 A1 (THIBODEAU, L et al.) November 2, 2006; entire document                                                         | 1-2, 3/1-2, 6/1-2, 7/1, 8, 12, 16-20 |
| A         | US 2002/0081566 A1 (BERETTA, A) June 27, 2002; entire document                                                                     | 1-2, 3/1-2, 6/1-2, 7/1, 8, 12, 16-20 |

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

\* 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

"&amp;" document member of the same patent family

Date of the actual completion of the international search

15 June 2017 (15.06.2017)

Date of mailing of the international search report

01 AUG 2017

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300  
PCT OSP: 571-272-7774

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/20821

## 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:

- a. ☒ forming part of the international application as filed:
  - ☒ in the form of an Annex C/ST.25 text file.
  - ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13*ter*. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
  - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*. 1(a)).
  - ☐ on paper or in the form of an image file (Rule 13*ter*. 1(b) and Administrative Instructions, Section 713).

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 forming part of 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/US17/20821

## 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.:  
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:

---Please See Supplemental Page---

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.:  
1-2, 3/1-2, 6/1-2, 7/1, 8, 12, 16-20; SEQ ID NOS.: 102, 307

### 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**  
Information on patent family members

International application No.  
PCT/US17/20821

-\*\*\*-Continued from Box No. III Observations where unity of invention is lacking: -\*\*\*-

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 1-20 and SEQ ID NOs: 102 and 307 are directed toward a composition comprising a nucleic acid encoding an HIV-1 envelope; or an HIV-1 envelope polypeptide; and a method of inducing an immune response therewith.

The composition and method will be searched to the extent they encompass a Gp160 nucleic acid sequence encompassing SEQ ID NO: 102 (first exemplary envelope-encoding nucleic acid), encoding an amino acid sequence encompassing SEQ ID NO: 307 (first exemplary envelope polypeptide). Applicant is invited to elect additional envelope polypeptide(s) with corresponding encoding nucleic acid(s), with specified SEQ ID NO: for each polypeptide and specified SEQ ID NO: for each nucleic acid, to be searched. Additional envelope polypeptide sequence(s) and encoding nucleic acid sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 3 (in-part), 6 (in-part), 7 (in-part), 8 (in-part), 12 (in-part), 16 (in-part), 17 (in-part), 18 (in-part), 19 (in-part) and 20 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 102 envelope-encoding nucleic acid), and SEQ ID NO: 307 (envelope polypeptide). Applicants must specify the claims that encompass any additionally elected envelope polypeptide sequence(s) and/or envelope-encoding nucleic acid sequence(s). Applicants must further indicate, if applicable, the claims which encompass 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 an envelope polypeptide encompassing SEQ ID NO: 447 (first exemplary elected envelope polypeptide), encoded by a nucleic acid encompassing SEQ ID NO: 551 (first exemplary elected envelope-encoding nucleic acid).

No technical features are shared between the envelope sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a composition comprising: (a) a nucleic acid encoding a HIV-1 envelope; and/or (b) an HIV-1 envelope polypeptide; and a method of inducing an immune response in a subject comprising administering a composition comprising a nucleic acid encoding an HIV-1 envelope or an HIV-1 envelope polypeptide, or any combination thereof, in an amount sufficient to induce an immune response.

However, these shared technical features are previously disclosed by WO 2016/014721 A2 to Duke University (hereinafter 'Duke').

Duke discloses a composition comprising: (a) a nucleic acid encoding a HIV-1 envelope (a composition comprising: (a) a nucleic acid encoding a HIV-1 envelope; paragraphs [0005], [0006]); and/or (b) an HIV-1 envelope polypeptide (an HIV-1 envelope polypeptide; paragraphs [0005], [0006]); and a method of inducing an immune response in a subject comprising administering a composition (a method of inducing an immune response in a subject comprising administering a composition; paragraph [0008]) comprising a nucleic acid encoding an HIV-1 envelope or an HIV-1 envelope polypeptide in an amount sufficient to induce an immune response (comprising a nucleic acid encoding an HIV-1 envelope or an HIV-1 envelope polypeptide in an amount sufficient to induce an immune response; paragraphs [0005], [0006], [0008]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Duke reference, unity of invention is lacking.