



(86) Date de dépôt PCT/PCT Filing Date: 2015/03/19
(87) Date publication PCT/PCT Publication Date: 2015/09/24
(85) Entrée phase nationale/National Entry: 2017/06/29
(86) N° demande PCT/PCT Application No.: US 2015/021528
(87) N° publication PCT/PCT Publication No.: 2015/143193
(30) Priorité/Priority: 2014/03/19 (US61/955,402)

(51) Cl.Int./Int.Cl. *C12N 15/46* (2006.01),
A61K 39/21 (2006.01), *C07K 14/155* (2006.01),
C12N 15/85 (2006.01)

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(54) Titre : IMMUNISATION D'ESSAIM AVEC DES ENVELOPPES DE CH505
(54) Title: SWARM IMMUNIZATION WITH ENVELOPES FROM CH505

(57) Abrégé/Abstract:

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



(51) International Patent Classification:
C12N 15/46 (2006.01) C12N 15/85 (2006.01)
C07K 14/155 (2006.01) A61K 39/21 (2006.01)

(21) International Application Number:
PCT/US2015/021528

(22) International Filing Date:
19 March 2015 (19.03.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/955,402 19 March 2014 (19.03.2014) US

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

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

Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

— with international search report (Art. 21(3))

— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: SWARM IMMUNIZATION WITH ENVELOPES FROM CH505

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

WO 2015/143193 A1

SWARM IMMUNIZATION WITH ENVELOPES FROM CH505

[0001] This application claims the benefit of U.S. Application Serial No. 61/955,402, filed March 19, 2014, the content of which application is herein 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.

TECHNICAL FIELD

[0003] 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

[0004] 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

[0005] 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.

[0006] 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 boost as described in Selections as described herein.

[0007] 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.

[0008] In one aspect the invention provides a composition comprising any one of the envelopes described herein, or any combination thereof (Tables 13, and 14, selections A-L). 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. 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 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.

[0009] 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 mutant in Figure 30, e.g., M14 and/or M24. A composition comprising nucleic acids encoding HIV-1 envelope w000.T/F (or w004.03), M11, w014.32, and w014.12. A composition comprising nucleic acids encoding HIV-1 envelope T/F (or w004.03), M11, w030.28, w053.16, w053.31, w078.7, w078.15, w078.33, w100.A4, and w100.B6.

[0010] In one aspect the invention provides a composition comprising nucleic acids encoding HIV-1 envelope w000.T/F (or w004.03), M11, w014.32, w014.12, w030.28, w053.16, w053.31, w078.7, w078.15, w078.33, w100.A4, and w100.B6.

[0011] 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 M9. A composition comprising nucleic acids encoding HIV-1 envelope w000.TF, w004.03, w004.26, M10, M11, M19, M20, M21, M5, M6, M7, M8, and M9. A composition comprising nucleic acids encoding HIV-1 envelope 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, and w020.26. A composition comprising nucleic acids encoding HIV-1 envelope 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, and w030.36. A composition comprising nucleic acids encoding HIV-1 envelope 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, and w078.38. A composition comprising nucleic acids encoding

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, and w160.T4.

[0012] 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), 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), M11, w014.32, and w014.12 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.

[0013] In certain embodiments the methods further comprise administering a composition comprising any one of HIV-1 envelope w030.28, w053.16, w053.31, w078.7, w078.15, w078.33, w100.A4, or w100.B6, or any combination thereof as a boost, wherein the envelope is administered as a polypeptide or a nucleic acid encoding the same.

[0014] In certain embodiments the methods further comprise administering a composition comprising any one of HIV-1 envelope w014.32, w014.12, w030.28, w053.16, w053.31, w078.7, w078.15, w078.33, w100.A4, or w100.B6, or any combination thereof as a boost, wherein the envelope is administered as a polypeptide or a nucleic acid encoding the same.

[0015] In another aspect the invention provides a method of inducing an immune response in a subject comprising administering a composition comprising HIV-1 envelope w000.TF, w004.03, w004.26, M10, M11, M19, M20, M21, M5, M6, M7, M8, and M9 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.

[0016] In certain embodiments the methods further comprise administering a composition comprising any one of HIV-1 envelope 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, or w020.26, or any combination thereof as a boost, wherein the envelope is administered as a polypeptide or a nucleic acid encoding the same.

[0017] In certain embodiments the methods further comprise administering a composition comprising any one of HIV-1 envelope 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, or w030.36, or any combination thereof as a boost, 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 any one of HIV-1 envelope 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, or w078.38, or any combination thereof as a boost, wherein the envelope is administered as a polypeptide or a nucleic acid encoding the same.

[0019] In certain embodiments the methods further comprise administering a composition comprising any one of HIV-1 envelope 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, or w160.T4, or any combination thereof as a boost, wherein the envelope is administered as a polypeptide or a nucleic acid encoding the same.

[0020] 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).

[0021] 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.

[0022] 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.

[0023] 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.

[0024] 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.

[0025] In certain aspects the invention provides a composition comprising at least one nucleic acid encoding HIV-1 envelope T/F, w004.03, M11, w030.28, w053.16, w053.31, w078.7, w078.15, w078.33, w100.A4, and w100.B6 or any combination thereof.

[0026] In certain aspects the invention provides a composition comprising at least one nucleic acid encoding HIV-1 envelope T/F, w004.03, M11, w014.32, w014.12, w030.28, w053.16, w053.31, w078.7, w078.15, w078.33, w100.A4, and w100.B6, or any combination thereof.

[0027] In certain aspects the invention provides a composition comprising at least one nucleic acid encoding 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, and w160.T4, or any combination thereof.

[0028] 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.

[0029] 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.

[0030] 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] Figure 1 shows CH505 Env polymorphisms, neutralization, vaccine regimes, and phylogeny.

[0032] Figure 2 shows swarm vaccine variant frequencies in concatenated Env “hot-spot” sites, numbered as in Table 1. 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” (Table 1). Rather than eliminate sites found by multiple methods, duplicate sites are included multiple times, for emphasis.

[0033] Figure 3 shows one embodiment of alignment columns in Env “hot-spot” summaries, concatenated to comprise concatamers.

[0034] Figure 4 shows another embodiment of alignment columns in Env “hot-spot” concatamer summaries.

[0035] Figure 5 shows one embodiment of selected Envs as concatenated sites.

[0036] Figure 6 shows another embodiment of selected Envs as concatenated sites.

[0037] Figure 7 shows one embodiment of a proposed swarm of CH505 envelopes.

[0038] Figure 8 shows another embodiment of a proposed swarm of CH505 envelopes.

[0039] Figure 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.

[0040] Figure 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.

[0041] Figure 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.

[0042] Figure 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 Figures 9-11 but shows when and where variation is most active, clustering together on the vertical axis sites that share variability (entropy) profiles.

[0043] Figure 13 is an enlarged version of Figure 1. Figure 1 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 IC₅₀ (50% inhibitory concentrations, in $\mu\text{g/ml}$) 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. $<0.2 \mu\text{g/ml}$; 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.

[0044] Figure 14A shows nucleic acid sequence of T/F virus from individual CH505. Figure 14B shows CH505 HIV-1 gene sequences.

[0045] Figure 15 shows nucleic acid sequences (gp160) of CH505 envelopes.

[0046] Figure 16 shows nucleic acid sequences encoding gp120D CH505 envelopes.

[0047] Figure 17 shows amino acid sequences (gp160) of CH505 envelopes. “Z” at the end of the sequence indicates a stop codon.

[0048] Figure 18 shows amino acid sequences (gp120D8) of CH505 envelopes.

[0049] Figure 19A shows one embodiment of a swarm of CH505 envelopes. Figure 19B shows the complete sequences of the envelopes of Figure 19A. Figure 19C shows one embodiment of a swarm of CH505 envelopes.

[0050] Figure 20 shows amino acid sequences (gp145) of CH505 envelopes.

[0051] Figure 21 shows nucleic acid sequences encoding gp145 CH505 envelopes

[0052] Figure 22 shows “The HIV-1 Arms Race” as a graphical representation of mapping the Virus and Antibody from the Time of Transmission.

[0053] Figure 23 shows isolation of broad neutralizing antibodies from chronically Infected Individual CH0505 Followed From Time of Transmission

[0054] Figure 24 shows tempo and site of accumulation of mutations at the contact sites between virus and CH103 mAb.

[0055] Figure 25 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).

[0056] Figure 26 shows 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%).

[0057] Figure 27 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.

[0058] Figure 28 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.

[0059] Figure 29 is a heatmap showing neutralization potency of antibodies in the CH103 lineage against early CH505 mutations, evaluated by Feng Gao. M11 shows enhanced sensitivity relative to the TF, so might serve as a good trigger of the CH103 like lineage.

[0060] Figure 30 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.

[0061] Figure 31 shows sequences of trivalent envelope mosaics.

DETAILED DESCRIPTION OF THE INVENTION

[0062] 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.

[0063] 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.

[0064] 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).

[0065] 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.

[0066] Two other considerations are important. The first is that for the CH103 bnAb CD4 binding site lineage, the VH4-59 and V λ 3-1 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.

[0067] 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.

[0068] 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.

[0069] 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 (Figure 22-23).

[0070] 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.

[0071] 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.

[0072] 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.

[0073] 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.

[0074] 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.

[0075] 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.

[0076] 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. In 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.

[0077] Sequences/Clones

[0078] Described herein are nucleic and amino acids sequences of HIV-1 envelopes. 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 gp145s, gp140s, both cleaved and uncleaved, 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.

[0079] 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:

MRVMGIQRNYPQWWIWSMLGFWMLMICNGMWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQE...(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.

[0080] The general strategy of deletion of N-terminal amino acids of envelopes results in proteins, for example gpl20s, 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.

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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.

[0088] 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).

[0089] 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 (µg) or milligram of a single immunogenic nucleic acid. Recombinant protein dose can range from a few µg micrograms to a few hundred micrograms, or milligrams of a single immunogenic polypeptide.

[0090] 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.

[0091] 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, 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.

[0092] 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; Gleevac, anti-CD25 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.

[0093] 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.

[0094] The invention is described in the following non-limiting examples.

Table 13. 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

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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

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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

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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

identified both the nucleic acid (Figure 16, and 21) and amino acid sequences (Figures 18 20).

Table 14 shows a summary of sequence names and sequence identifiers.

	Name	Gp160 aa SEQ ID NO (Fig. 17)	Gp160 nt SEQ ID NO (Fig. 15)	Gp145 aa SEQ ID NO (Fig. 20)	Gp145 nt SEQ ID NO (coding/ upper case from Fig. 21)	Gp145 nt SEQ ID NO (Fig 21) (entire)	Gp120 aa D8 SEQ ID NO Fig. 18	Gp120D nt SEQ ID NO (coding/ upper case from Fig. 16)	Gp120D nt SEQ ID NO (Fig. 16 entire)
1.	CH505.M5	1	105	209	313	417	521	625	729
2.	CH505.M6	2	106	210	314	418	522	626	730
3.	CH505.M7	3	107	211	315	419	523	627	731
4.	CH505.M8	4	108	212	316	420	524	628	732
5.	CH505.M9	5	109	213	317	421	525	629	733
6.	CH505.M10	6	110	214	318	422	526	630	734
7.	CH505.M11	7	111	215	319	423	527	631	735
8.	CH505.M19	8	112	216	320	424	528	632	736
9.	CH505.M20	9	113	217	321	425	529	633	737
10.	CH505.M21	19	114	218	322	426	530	634	738
11.	CH505TF	11	115	219	323	427	531 *	635 *	739
12.	CH505w004.03	12	116	220	324	428	532 *	636 *	740
13.	CH505w004.10	13	117	221	325	429	533	637	741
14.	CH505w014.2	14	118	222	326	430	534	638	742
15.	CH505w014.3	15	119	223	327	431	535	639	743
16.	CH505w014.8	16	120	224	328	432	536	640	744
17.	CH505w014.10	17	121	225	329	433	537	641	745
18.	CH505w014.21	18	122	226	330	434	538	642	746
19.	CH505w014.32	19	123	227	331	435	539	643	747
20.	CH505w020.3	20	124	228	332	436	540	644	748
21.	CH505w020.4	21	125	229	333	437	541	645	749
22.	CH505w020.7	22	126	230	334	438	542	646	750
23.	CH505w020.8	23	127	231	335	439	543	647	751
24.	CH505w020.9	24	128	232	336	440	544	648	752
25.	CH505w020.11	25	129	233	337	441	545	649	753
26.	CH505w020.13	26	130	234	338	442	546	650	754
27.	CH505w020.14	27	131	235	339	443	547	651	755
28.	CH505w020.15	28	132	236	340	444	548	652	756
29.	CH505w020.19	29	133	237	341	445	549	653	757
30.	CH505w020.22	30	134	238	342	446	550	654	758
31.	CH505w020.23	31	135	239	343	447	551	655	759
32.	CH505w020.24	32	136	240	344	448	552	656	760
33.	CH505w020.26	33	137	241	345	449	553	657	761
34.	CH505w030.5	34	138	242	346	450	554	658	762
35.	CH505w030.6	35	139	243	347	451	555	659	763
36.	CH505w030.9	36	140	244	348	452	556	660	764
37.	CH505w030.10	37	141	245	349	453	557	661	765
38.	CH505w030.11	38	142	246	350	454	558	662	766

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39.	CH505w030.13	39	143	247	351	455	559	663	767
40.	CH505w030.15	40	144	248	352	456	560	664	768
41.	CH505w030.17	41	145	249	353	457	561	665	769
42.	CH505w030.18	42	146	250	354	458	562	666	770
43.	CH505w030.19	43	147	251	355	459	563	667	771
44.	CH505w030.20	44	148	252	356	460	564	668	772
45.	CH505w030.21	45	149	253	357	461	565	669	773
46.	CH505w030.23	46	150	254	358	462	566	670	774
47.	CH505w030.25	47	151	255	359	463	567	671	775
48.	CH505w030.27	48	152	256	360	464	568	672	776
49.	CH505w030.28	49	153	257	361	465	569	673	777
50.	CH505w030.36	50	154	258	362	466	570	674	778
51.	CH505w053.3	51	155	259	363	467	571	675	779
52.	CH505w053.6	52	156	260	364	468	572	676	780
53.	CH505w053.13	53	157	261	365	469	573	677	781
54.	CH505w053.16	54	158	262	366	470	574	678	782
55.	CH505w053.25	55	159	263	367	471	575	679	783
56.	CH505w053.29	56	160	264	368	472	576	680	784
57.	CH505w053.31	57	161	265	369	473	577	681	785
58.	CH505w078.1	58	162	266	370	474	578	682	786
59.	CH505w078.6	59	163	267	371	475	579	683	787
60.	CH505w078.7	60	164	268	372	476	580	684	788
61.	CH505w078.9	61	165	269	373	477	581	685	789
62.	CH505w078.10	62	166	270	374	478	582	686	790
63.	CH505w078.15	63	167	271	375	479	583	687	791
64.	CH505w078.17	64	168	272	376	480	584	688	792
65.	CH505w078.25	65	169	273	377	481	585	689	793
66.	CH505w078.33	66	170	274	378	482	586	690	794
67.	CH505w078.38	67	171	275	379	483	587	691	795
68.	CH505w100.A3	68	172	276	380	484	588	692	796
69.	CH505w100.A4	69	173	277	381	485	589	693	797
70.	CH505w100.A6	70	174	278	382	486	590	694	798
71.	CH505w100.A10	71	175	279	383	487	591	695	799
72.	CH505w100.A12	72	176	280	384	488	592	696	800
73.	CH505w100.A13	73	177	281	385	489	593	697	801
74.	CH505w100.B2	74	178	282	386	490	594	698	802
75.	CH505w100.B4	75	179	283	387	491	595	699	803
76.	CH505w100.B6	76	180	284	388	492	596	700	804
77.	CH505w100.B7	77	181	285	389	493	597	701	805
78.	CH505w100.C7	78	182	286	390	494	598	702	806
79.	CH505w136.B2	79	183	287	391	495	599	703	807
80.	CH505w136.B3	80	184	288	392	496	600	704	808
81.	CH505w136.B4	81	185	289	393	497	601	705	809
82.	CH505w136.B5	82	186	290	394	498	602	706	810
83.	CH505w136.B8	83	187	291	395	499	603	707	811
84.	CH505w136.B10	84	188	292	396	500	604	708	812
85.	CH505w136.B12	85	189	293	397	501	605	709	813
86.	CH505w136.B18	86	190	294	398	502	606	710	814
87.	CH505w136.B20	87	191	295	399	503	607	711	815
88.	CH505w136.B27	88	192	296	400	504	608	712	816

89.	CH505w136.B29	89	193	297	401	505	609	713	817
90.	CH505w136.B36	90	194	298	402	506	610	714	818
91.	CH505w160.A1	91	195	299	403	507	611	715	819
92.	CH505w160.C2	92	196	300	404	508	612	716	820
93.	CH505w160.C4	93	197	301	405	509	613	717	821
94.	CH505w160.C11	94	198	302	406	510	614	718	822
95.	CH505w160.C12	95	199	303	407	511	615	719	823
96.	CH505w160.C14	96	200	304	408	512	616	720	824
97.	CH505w160.D1	97	201	305	409	513	617	721	825
98.	CH505w160.D5	98	202	306	410	514	618	722	826
99.	CH505w160.T2	99	203	307	411	515	619	723	827
100.	CH505w160.T4	100	204	308	412	516	620	724	828
101.	CH505.w4.26	101	205	309	413	517	621	725	829
102.	CH505.w30.12	102	206	310	414	518	622	726	830
103.	CH505.w53.19	103	207	311	415	519	623	727	831
104.	CH05w020.2	104	208	312	416	520	624	728	832
	Other sequences								
	CH505 virus Figure 14A	833							
	CH505 viral genes Figure 14B: Gag, Pol, Vif, Vpr, Tat, Rev, VPU, Env, Nef	834; 835; 836; 837; 838; 839; 840; 841; 842							
	Fig 19A (13 aa sequences aligned)	843-855							
	Fig 19B the individual sequence of Fig 19A	856-868							
	Fig 19C (12 aa sequences aligned)	869-880							
	Fig. 30 (M14, M15, M16, M17, M18, M22, M23, M24, M25, M26)	881-890							
	Fig. 31	891-893							

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

EXAMPLES

Example 1

[0095] 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.

[0096] Recombinant HIV-1 proteins

[0097] 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.

[0098] ELISA

[0099] 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 $100\text{ }\mu\text{g ml}^{-1}$ to $0.000\text{ }\mu\text{g ml}^{-1}$ 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 (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.

[0100] **Surface plasmon resonance affinity and kinetics measurements**

[0101] 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 $50\text{--}400 \mu\text{g ml}^{-1}$ for UCAs and early intermediates IA8 and IA4, $10\text{--}100 \mu\text{g ml}^{-1}$ for intermediate IA3, and $1\text{--}25 \mu\text{g ml}^{-1}$ 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).

[0102] Neutralization assays

[0103] 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 heterologous 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 IC₅₀ in either reciprocal dilution for plasma samples or in micrograms per microlitre for monoclonal antibodies.

[0104] The GenBank accession numbers for 292 CH505 Env proteins are KC247375–KC247667, and accessions for 459 V_HDJ_H and 174 V_LJ_L sequences of antibody members in the CH103 clonal lineage are KC575845–KC576303 and KC576304–KC576477, respectively..

Example 2

[0105] Combinations of antigens derived from CH505 envelope sequences for swarm immunizations

[0106] 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.

[0107] 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 (for e.g. Table 5). 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.

[0108] 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.

[0109] 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].

[0110] **HIV-1 Envelope selection A (four envelopes):** w004.03 (T/F or w004.03), w053.16, w078.33, and w100.B6.

1: Prime: w004.03 (T/F or w004.03)

2: Boost: w053.16,

3: Boost: w078.33.

4: Boost: w100.B6.

[0111] **HIV-1 Envelope selection B (ten envelopes):** w004.03 (T/F or w004.03), M11, w030.28, w053.16, w053.31, w078.7, w078.15, w078.33, w100.A4, w100.B6.

1: Prime: w004.03 (T/F or w004.03), M11.

2: Boost: w030.28.

3: Boost: w053.16, w053.31, w078.7, w078.15, w078.33.

4: Boost with: w100.A4, w100.B6.

[0112] **HIV-1 Envelope selection C (twelve envelopes):** w004.03 (T/F or w004.03), M11, w014.32, w014.12, w030.28, w053.16, w053.31, w078.7, w078.15, w078.33, w100.A4, w100.B6.

1: Prime: w004.03 (T/F or w004.03), M11.

2: Boost: w014.32, w014.12

3: Boost: w030.28.

4: Boost: w053.16, w053.31, w078.7, w078.15, w078.33.

5: Boost with: w100.A4, w100.B6.

[0113] **HIV-1 Envelope selection D (twelve envelopes):** w004.03 (T/F or w004.03), M11, w014.32, w014.12, w030.28, w053.16, w053.31, w078.7, w078.15, w078.33, w100.A4, w100.B6.

1: Prime: w004.03 (T/F or w004.03), M11; w014.32, w014.12

2: Boost: w030.28.

3: Boost: w053.16, w053.31, w078.7, w078.15, w078.33.

4: Boost with: w100.A4, w100.B6.

[0114] **HIV-1 Envelope selection E (excludes # viruses from selections in Table 3 and 3A):**

1: Prime: w000.TF, w004.03, M10, M11, M19, M20, M21, M5, M7, M8, M9.

2: Boost with: 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,

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

3: Boost with: 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: Boost with: 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,

w078.33, w078.38

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

w100.B2, w100.B6, w100.B7,

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.

[0115] **HIV-1 Envelope selection F (one hundred envelopes) (Table 3):**

1: Prime: w000.TF, w004.03, M10, M11, M19, M20, M21, M5, M6, M7, M8, M9.

2: Boost: 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: Boost: 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: Boost: 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: Boost: 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.

[0116] HIV-1 Envelope selection G (101 envelopes) (Table 3A):

1. Prime: w000.TF, w004.03, w004.26, M10, M11, M19, M20, M21, M5, M6, M7, M8, M9

2. Boost: 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. Boost: 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. Boost: 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. Boost: 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.

[0117] HIV-1 envelope selection **H** (eight envelopes) M11, w004.03, w030.28, w053.16, w053.31, w078.15, w078.33, w100.B6.

[0118] HIV-1 envelope selection **I** (ten envelopes) M11, M14, M24, w004.03, w030.28, w053.16, w053.31, w078.15, w078.33, w100.B6. In some embodiments M11+ w004.03, then wk030.28 + wk 078.15 then wk 053.31+ wk 078.7, then w100 B6 + w 100. A4 week 53.16, week 78.33 ; Alternatively they can be administered all together as a swarm in 4 or 5 prime and boosts with or without DNA accompanyments.

[0119] HIV-1 envelope selection **J** (twelve envelopes) M11, M14, M24, w004.03, w030.28, w053.16, w053.31, w078.07, w078.15, w078.33, w100.B6, w100.A4.

[0120] HIV-1 envelope selection **K** (ten envelopes) M11, w004.03, w030.28, w053.16, w053.31, w078.07, w078.15, w078.33, w100.B6, w100.A4).

[0121] HIV-1 envelope selection **L** (104 envelopes as gp160s, gp145s, gp140s; 103 envelopes as gp120D8s; See Tables 13 and 14): CH505.M5; CH505.M6; CH505.M7; CH505.M8; CH505.M9; CH505.M10; CH505.M11; CH505.M19; CH505.M20; CH505.M21; CH505TF; CH505w004.03; CH505w004.10; CH505w014.2; CH505w014.3; CH505w014.8; CH505w014.10; CH505w014.21; CH505w014.32; CH505w020.3; CH505w020.4; CH505w020.7; CH505w020.8; CH505w020.9; CH505w020.11; CH505w020.13; CH505w020.14; CH505w020.15; CH505w020.19; CH505w020.22; CH505w020.23; CH505w020.24; CH505w020.26; CH505w030.5; CH505w030.6; CH505w030.9; CH505w030.10; CH505w030.11; CH505w030.13; CH505w030.15; CH505w030.17;

CH505w030.18; CH505w030.19; CH505w030.20; CH505w030.21; CH505w030.23;
 CH505w030.25; CH505w030.27; CH505w030.28; CH505w030.36; CH505w053.3;
 CH505w053.6; CH505w053.13; CH505w053.16; CH505w053.25; CH505w053.29;
 CH505w053.31; CH505w078.1; CH505w078.6; CH505w078.7; CH505w078.9;
 CH505w078.10; CH505w078.15; CH505w078.17; CH505w078.25; CH505w078.33;
 CH505w078.38; CH505w100.A3; CH505w100.A4; CH505w100.A6; CH505w100.A10;
 CH505w100.A12; CH505w100.A13; CH505w100.B2; CH505w100.B4; CH505w100.B6;
 CH505w100.B7; CH505w100.C7; CH505w136.B2; CH505w136.B3; CH505w136.B4;
 CH505w136.B5; CH505w136.B8; CH505w136.B10; CH505w136.B12; CH505w136.B18;
 CH505w136.B20; CH505w136.B27; CH505w136.B29; CH505w136.B36; CH505w160.A1;
 CH505w160.C2; CH505w160.C4; CH505w160.C11; CH505w160.C12; CH505w160.C14;
 CH505w160.D1; CH505w160.D5; CH505w160.T2; CH505w160.T4; CH505.w4.26 ;
 CH505.w30.12 ; CH505.w53.19 ; CH05w020.2.

[0122] 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, eight 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.

[0123] 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, 29-30). From this large dataset, in one embodiment, eight 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, eight 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.

[0124] 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. In certain embodiments, vaccination regimens include a total of eight sequences (Selection H) that capture the antigenic diversity of CH505. In another embodiment, the two sequences that introduce the population signatures are added (Selection I), to enable the induction of antibodies by vaccination that may have even greater breadth than those antibodies isolated from CH505.

[0125] The eight CH505 sequences that represent the accumulation of viral sequence and antigenic diversity in the CD4bs epitope of CH103 in subject CH505: M11 (TF with N279D + V281G), w004.03, w030.28, w053.16, w053.31, w078.15, w078.33, w100.B6.

[0126] 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 29). These were early escape mutations for another CD4bs autologous neutralizing antibody lineage, but might have served to promote early expansion of the CH103 lineage.

[0127] 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. 30). 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.

[0128] In certain embodiments, two additional CH505 variants, w078.7 & w100.A4, are added to the selections to extend to further extend the sampling of the antigenic profile.

Example 3

[0129] Immunization protocols in subjects with swarms of HIV-1 envelopes.

[0130] 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.

[0131] 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.

[0132] **Table 4** shows a non-limiting example of an immunization protocol using a swarm of four HIV-1 envelopes

Envelope	Prime	Boost(s)	Boost(s)	Boost(s)
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w004.03	w004.03 as a nucleic acid e.g. DNA/MVA vector and/or protein			
w053.16		w053.16 as a nucleic acid e.g. DNA/MVA and/or protein		
w078.33			w078.33 as nucleic acid e.g. DNA/MVA and/or protein	
W100.B6				W100.B6 as nucleic acid e.g. DNA/MVA and/or protein

[0133] **Table 5** shows a non-limiting example of an immunization protocol using a swarm of four HIV-1 envelopes

Envelope	Prime	Boost(s)	Boost(s)	Boost(s)
w004.03	w004.03 as a nucleic acid e.g. DNA/MVA vector and/or protein	w004.03 as a nucleic acid e.g. DNA/MVA and/or protein	w004.03 as nucleic acid e.g. DNA/MVA and/or protein	w004.03 as nucleic acid e.g. DNA/MVA and/or protein
w053.16		w053.16 as nucleic acid e.g. DNA/MVA and/or protein	w053.16 as nucleic acid e.g. DNA/MVA and/or protein	w053.16 as nucleic acid e.g. DNA/MVA and/or protein
w078.33			w078.33 as nucleic acid eg. DNA/MVA and/or protein	w078.33 as nucleic acid eg. DNA/MVA and/or protein
W100.B6				W100.B6 as nucleic acid e.g. DNA/MVA and/or protein

[0134] **Table 6** shows a non-limiting example of immunization protocol using a swarm of ten HIV-1 envelopes

Envelope	Prime	Boost(s)	Boost(s)	Boost(s)
T/F or w004.03, and M11	T/F or w004.03, and M11 as nucleic acids and/or protein			
w030.28		w030.28 as nucleic acid and/or protein		
w053.16, w053.31, w078.7, w078.15, and w078.33			w053.16, w053.31, w078.7, w078.15, and w078.33 as nucleic acids and/or protein	
W100.A4, and W100.B6				W100.A4, and W100.B6 nucleic acids and/or protein

[0135] **Table 7** shows a non-limiting example of immunization protocol using a swarm of ten HIV-1 envelopes

Envelope	Prime	Boost(s)	Boost(s)	Boost(s)
T/F or w004.03, and M11	T/F or w004.03, and M11 as nucleic acids and/or protein	T/F or w004.03, and M11 as nucleic acids and/or protein	T/F or w004.03, and M11 as nucleic acids and/or protein	T/F or w004.03, and M11 as nucleic acids and/or protein
w030.28		w030.28 as nucleic acid and/or protein	w030.28 as nucleic acid and/or protein	w030.28 as nucleic acid and/or protein
w053.16, w053.31, w078.7, w078.15, and w078.33			w053.16, w053.31, w078.7, w078.15, and w078.33 as nucleic acids and/or protein	w053.16, w053.31, w078.7, w078.15, and w078.33 as nucleic acids and/or protein
W100.A4, and				W100.A4, and

W100.B6				W100.B6 nucleic acids and/or protein
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[0136] **Table 8** shows a non-limiting example of immunization protocol with a swarm of twelve HIV-1 envelopes

Envelope	Prime	Boost(s)	Boost(s)	Boost(s)	Boost(s)
T/F or w004.03, and M11	T/F or w004.03, and M11 as nucleic acids and/or protein				
w014.12, w014.32		w014.12, w014.32 as nucleic acid and/or protein			
w030.28			w030.28 as nucleic acid and/or protein		
w053.16, w053.31, w078.7, w078.15, and w078.33				w053.16, w053.31, w078.7, w078.15, and w078.33 as nucleic acids and/or protein	
W100.A4, and W100.B6					W100.A4, and W100.B6 nucleic acids and/or protein

[0137] **Table 9** shows a non-limiting example of immunization protocol with a swarm of twelve HIV-1 envelopes

Envelope	Prime	Boost(s)	Boost(s)	Boost(s)	Boost(s)
T/F or w004.03, and M11	T/F or w004.03, and M11 as nucleic acids	T/F or w004.03, and M11 as nucleic	T/F or w004.03, and M11 as nucleic acids	T/F or w004.03, and M11 as nucleic acids	T/F or w004.03, and M11 as nucleic acids

	and/or protein	acids and/or protein	and/or protein	and/or protein	and/or protein
w014.12, w014.32	w014.12, w014.32 as nucleic acids and/or protein	w014.12, w014.32 as nucleic acids and/or protein	w014.12, w014.32 as nucleic acids and/or protein	w014.12, w014.32 as nucleic acids and/or protein	w014.12, w014.32 as nucleic acids and/or protein
w030.28			w030.28 as nucleic acid and/or protein	w030.28 as nucleic acid and/or protein	w030.28 as nucleic acid and/or protein
w053.16, w053.31, w078.7, w078.15, and w078.33				w053.16, w053.31, w078.7, w078.15, and w078.33 as nucleic acids and/or protein	w053.31, w078.7, w078.15, and w078.33 as nucleic acids and/or protein
W100.A4, and W100.B6					W100.A4, and W100.B6 nucleic acids and/or protein

[0138] **Table 10** shows a non-limiting example of an immunization protocol with HIV-1 envelopes.

Envelope	Prime	Boost(s)	Boost(s)	Boost(s)	Boost(s)
w000.TF, w004.03, (optionally 004.26) M10, M11, M19, M20, M21, M5, M6, M7, M8, M9.	As nucleic acids and/or proteins				
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		As nucleic acids and/or proteins			
w030.5, w030.6, w030.9,			As nucleic		

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			acids and/or proteins		
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				As nucleic acids and/or proteins	
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					As nucleic acids and/or proteins

[0139] **Table 11** shows a non-limiting example of immunization protocol using a swarm of HIV-1 envelopes. Optionally in certain embodiments the boosts include M14, and M24 as nucleic acids and/or protein.

Envelope	Prime	Boost(s)	Boost(s)	Boost(s)
T/F or w004.03, and M11 (M14 and M24 optional)	T/F or w004.03, and M11, (optionally in certain			

	embodiments M14, and M24) as nucleic acids and/or protein			
w030.28		w030.28 as nucleic acid and/or protein		
w053.16, w053.31, w078.7, w078.15, and w078.33			w053.16, w053.31, (w078.7 optional in certain embodiments), w078.15, and w078.33 as nucleic acids and/or protein	
W100.A4 (optional), and W100.B6				(W100.A4 optional in certain embodiments), and W100.B6 nucleic acids and/or protein

[0140] **Table 12** shows a non-limiting example of immunization protocol using a swarm of HIV-1 envelopes. Optionally in certain embodiments the boosts include M14, and M24 as nucleic acids and/or protein.

Envelope	Prime	Boost(s)	Boost(s)	Boost(s)
T/F or w004.03, and M11 (M14 and M24 optional)	T/F or w004.03, and M11, (optionally in certain embodiments M14, and M24) as nucleic acids and/or protein	T/F or w004.03, and M11, (optionally in certain embodiments M14, and M24) as nucleic acids and/or protein	T/F or w004.03, and M11, (optionally in certain embodiments M14, and M24) as nucleic acids and/or protein	T/F or w004.03, and M11, (optionally in certain embodiments M14, and M24) as nucleic acids and/or protein
w030.28		w030.28 as nucleic acid and/or protein	w030.28 as nucleic acid and/or protein	w030.28 as nucleic acid and/or protein
w053.16,			w053.16,	w053.16,

w053.31, w078.7, w078.15, and w078.33			w053.31, (w078.7 optional in certain embodiments), w078.15, and w078.33 as nucleic acids and/or protein	w053.31, (w078.7 optional in certain embodiments), w078.15, and w078.33 as nucleic acids and/or protein
W100.A4 (optional), and W100.B6				(W100.A4 optional in certain embodiments), and W100.B6 nucleic acids and/or protein

[0141] In certain embodiments an immunization protocol could include the following: Prime with a bivalent or trivalent Gag mosaic (Gag1 and Gag 2, Gag 1, Gag 2 and Gag3) in a suitable vector, plus CH505 Transmitted/Founder Env gp120 or gp160 plus T/F Env protein. Boost #1 could be: Gag1 and Gag-2 in a suitable vector, plus CH505 Transmitted/Founder Env gp120 or gp160, plus Env week 53 in a suitable vector, plus T/F and week 53 Env proteins. Boost #2 could be: Gag1 and Gag-2 in a suitable vector, plus CH505 week 78 , plus week 100 Env gp120 or gp160, plus week 78 + week 100 Env proteins.

Example 4

[0142] **Env mixtures of the CH505 virus induce the beginning of CD4 binding site BnAb lineages**

[0143] Groups of Rhesus Macaques are immunized with CH505 gp120 variants as recombinant gp120 proteins: T/F, w053.16, w078.33, and w100.B6. Group 1: CH505 T/F env gp120; Group 2: w053.16, Group 3: w078.33; Group 4: Sequential of 4 Env immunization: T/F, w053.16,

w078.33, and w100.B6; Group 5: Additive T/F, T/F+ w053.16, T/F+ w053.16 +w078.33, T/F+ w053.16+w078.33+w100.B6.

[0144] Immunizations are ongoing, with only three immunizations thus far with recombinant gp120 proteins. Figure 26 shows interim results of one monkey from Group 1. Figures 26 and 27 show that three immunizations with CH505 T/F envelope stimulate reactive memory B cells which are RSC3 positive (bind the gp120 CH505 T/F envelope) and do not bind RSCD371 (indicative of CD4Binding Site bnAb antibodies).

[0145] Previous studies have shown evolution of BnAbs through autologous Nabs. For example, it was reported evolution of V3 glycan (PGT-like) antibodies by induction of autologous NAbs that drove T/F virus escape with appearance of N332 in escape mutants, that could drive N332-dependent BnAbs (Nature Med. 18: 1688, 2012). Liao et al. reported evolution of the CH103 lineage through autologous NAbs in the CH103 lineage (Nature 496: 469, 2013).

[0146] Two virus types were isolated from CH505 BnAb individual four weeks after transmission: the Transmitted /Founder virus and a variant termed week 004.3 (4.3). Transmitted/founder virus was the predominant virus quasi-species at week 4 (tier 2). One variant virus termed 4.3 is identical to the T/F virus except it has a mutation in the gp41 MPER of W680G, and it is more neutralization sensitive to the entire CH103 clonal lineage including being neutralized by the CH103 UCA (Tier 1b).

[0147] Figure 28 shows induction of autologous neutralization of both the transmitted/founder CH505 Env and neutralization sensitive CH505 Env variant w004.3 in NHPs immunized with recombinant gp120 forms of either the transmitted/founder Env, week 53.16, week 78.33 or week 100.B6 in either group 1, T/F alone X3 or in sequence (Group 4) or additive sequence

(Group 5) as in line 117 above. Shown is week 14 neutralization data after three immunizations recombinant gp120 proteins as describe above.

[0148] Following virus and antibody evolution is providing important insight into the sequence of virus Envs that induce broadly neutralizing antibodies. B cell lineage vaccine design is a strategy to target the unmutated common ancestors and their intermediates for selecting otherwise subdominant and unfavored lineages. Lineage design coupled with structural analysis of envelope-antibody co-crystals is providing a rational design of immunogens for pre-clinical immunization studies. This example demonstrated induction of autologous neutralizing antibodies of the CH103 lineage. Next steps are immunization of germline KI mouse models (CH103 GL on the way) and humans with the same immunogens.

Example 5:

[0149] One of the major obstacles to developing an efficacious preventive HIV-1 vaccine is the challenge of inducing broadly neutralizing antibodies (bnAbs) against the virus. There are several reasons why eliciting bnAbs has been challenging and these include the conformational structure of the viral envelope, molecular mimicry of host antigens by conserved epitopes which may lead to the suppression of potentially useful antibody responses, and the high level of somatic mutations in the variable domains and the requirement for complex maturation pathways [1-3]. It has been shown that up to 25% of HIV-1– infected individuals develop bnAbs that are detected 2-4 years after infection. To date, all bnAbs have one or more of these unusual antibody traits: high levels of somatic mutation, autoreactivity with host antigens, and long heavy chain third complementarity determining regions (HCDR3s)—all traits that are controlled or modified by host immunoregulatory mechanisms. Thus, the hypothesis has been put forth that typical vaccinations of single primes and boosts will not suffice to be able to induce bnAbs; rather, it

will take sequential immunizations with Env immunogens, perhaps over a prolonged period of time, to mimic bnAb induction in chronically infected individuals [4].

[0150] A process to circumvent host immunoregulatory mechanisms involved in control of bnAbs is termed B cell lineage immunogen design, wherein sequential Env immunogens are chosen that have high affinities for the B cell receptors of the unmutated common ancestor (UCA) or germline gene of the bnAb clonal lineage [4]. Envs for immunization can either be picked randomly for binding or selected, as described herein, from the evolutionary pathways of Envs that actually give rise to bnAbs *in vivo*. Liao and colleagues recently described the co-evolution of HIV-1 and a CD4 binding site bnAb from the time of seroconversion to the development of plasma bnAb induction, thereby presenting an opportunity to map out the pathways that lead to generation of this type of CD4 binding site bnAb [5]. They showed that the single transmitted/founder virus was able to bind to the bnAb UCA, and identified a series of evolved envelope proteins of the founder virus that were likely stimulators of the bnAb lineage. Thus, this work presents the HVTN with an opportunity to vaccinate with naturally-derived viral envelopes that could drive the desired B-cell responses and induce the development of broad and potent neutralizing antibodies. While the human antibody repertoire is diverse, it has been found that only a few types of B cell lineages can lead to bnAb development, and that these lineages are similar across a number of individuals [6,7]. Thus, it is feasible that use of Envs from one individual will generalize to others.

[0151] The approach in this concept sheet to address the challenge of eliciting broadly neutralizing antibodies in vaccinees involves selecting the Env immunogens, among multitude of diverse viruses that induced a CD4 binding site bnAb clonal lineage in an HIV-infected individual, by making sequential recombinant Envs from that individual and using these Envs for

vaccination. The B-cell lineage vaccine strategy thus includes designing immunogens based on unmutated ancestors as well as intermediate ancestors of known bnAb lineages. A candidate vaccine could use transmitted/founder virus envelopes to, at first, stimulate the beginning stages of a bnAb lineage, and subsequently boost with evolved Env variants to recapitulate the high level of somatic mutation needed for affinity maturation and bnAb activity. The goal of such a strategy is to selectively drive desired bnAb pathways.

[0152] Liao et al demonstrated that in the CHAVI CH505 bnAb individual, the CH103 CD4 binding site bnAb lineage started with the lineage members first developing autologous neutralizing antibody activity, and then as the CH505 Env mutated, it developed bnAb activity. Thus, the first step of bnAb development is the development of the ability to neutralize the transmitted/founder virus.

[0153] The CH505 transmitted/founder (T/F) virus that we propose to use in Trial 1 in the concept has been tested in rhesus macaques; after 3 immunizations it induced plasma antibodies that neutralized the T/F virus and an early (week 4) T/F variant with only one mutation. In addition, flow phenotypic analysis of memory B cells in CH505 T/F Env-immunized rhesus macaques has demonstrated the presence of antigen-specific memory B cells that bind the Env protein RSC3 but not the RSC3 371 mutant protein [8], strongly indicating B cells that have begun a CD4 binding site bnAb lineage.

[0154] In certain embodiments, the CH505 virus used in Trial# 1 and Trial #2 is w004.03 instead of CH505 T/F.

[0155] Broadly neutralizing antibodies likely will not be induced by a single Env, and even a mixture of polyvalent random Envs (e.g. HVTN 505) is unlikely to induce bnAbs. Rather, immunogens must be designed to trigger the UCAs of bnAb lineages to undergo initial bnAb

lineage maturation, and then use sequential immunogens to fully expand the desired lineages.

The proposed trial will represent the first of many experimental clinical trials testing this concept in order to develop the optimal set of immunogens to drive multiple specificities of bnAbs. The HVTN will be at the cutting edge of this effort.

[0156] The concept is applicable to driving CD4 binding site lineage in multiple individuals due to the convergence of a few bnAb motifs among individuals. The adjuvant will be the GSK AS01E adjuvant containing MPL and QS21. Other suitable adjuvants can be used. This adjuvant has been shown by GSK to be as potent as the similar adjuvant AS01B but to be less reactogenic using HBsAg as vaccine antigen [Leroux-Roels et al., IABS Conference, April 2013,9].

[0157] Trial #1 will involve 5 immunizations IM with the CH505 transmitted/founder (T/F) Env gp120 at months 0, 1, 3, 6 and 12 and evaluating different doses of protein. The follow up Trial #2 will have combinations of the T/F Env and week-53, week-78 and week-100 Env mutants. Because it takes over a year to develop bnAbs, the second trial will include the possibility of a month 18 boost as well.

[0158] This study aims to be the first of several iterative experimental phase I trials to test the ability of these Envs to initiate bnAb lineages, and to use the isolated B cells from the vaccinees to identify the lineages induced.

[0159] Hypotheses: The T/F vaccine strategy will be safe and well tolerated among HIV-uninfected individuals. The vaccine strategy will elicit HIV Env-specific binding antibodies in a dose-dependent manner. The vaccine will elicit autologous neutralizing antibodies to transmitted/founder viruses. The vaccine will induce CD4+T cell responses. The vaccine will initiate CD4 binding site-specific-antibody lineages.

[0160] Proposed study

[0161] Schema Trial #1 (Dose finding): T/F = transmitted/founder protein

Study arm	N	Dose	Month 0 (Day 0)	Month 1 (Day 28)	Month 3 (Day 84)	Month 6 (Day 168)	Month 12 (Day 364)
Group 1	12	10 mcg	T/F	T/F	T/F	T/F	T/F
Group 2	12	20 mcg	T/F	T/F	T/F	T/F	T/F
Group 3	12	100 mcg	T/F	T/F	T/F	T/F	T/F
Group 4	6		placebo	placebo	placebo	placebo	placebo
Total	42 (36/6)						

[0162] Products: CH505TF: HIV gp120 transmitted/founder with AS01E; Placebo for CH505TF: sodium chloride for injection

[0163] Participants: 42 healthy, HIV-1-uninfected volunteers aged 18 to 50 years

[0164] Number of participants: Total 42: 36 vaccine, 6 placebo

[0165] Study duration: 18 months per participant [HVTN standard is 6 months after last vaccination.]

[0166] Objectives and endpoints

[0167] Primary objective 1: To evaluate the safety and tolerability of different doses of a prime-boost regimen of CH505TF vaccine in HIV-uninfected healthy adults

[0168] Primary endpoint 1: Local and systemic reactogenicity signs and symptoms, laboratory measures of safety, and AEs and SAEs

[0169] Primary objective 2: To evaluate binding antibody responses elicited by different doses of the CH505TF vaccine

[0170] Primary endpoint 2: HIV-specific binding Ab responses as assessed by binding Ab multiplex assay two weeks after the fourth vaccination

[0171] Secondary objective 1: To evaluate the ability of the regimen to elicit HIV-specific nAbs

[0172] Secondary endpoint 1: nAb magnitude and breadth against autologous viral isolates as assessed by area under the magnitude-breadth curves two weeks after the fourth vaccination

[0173] Secondary objective 2: To evaluate HIV-specific T-cell responses induced by different doses of the CH505TF vaccine

[0174] Secondary endpoint 2: Response rate and magnitude of CD4+ T-cell responses as assessed by intracellular cytokine staining assays (ICS) two weeks after the fourth vaccination

[0175] Exploratory objectives:

[0176] To further evaluate the immunogenicity of the vaccine regimen at different timepoints

[0177] To isolate single B cells with desired specificities and determine lineage characteristics

[0178] To determine the B cell repertoire of HIV-specific B cells

[0179] To assess vaccine-induced follicular helper T cell (Tfh) responses

[0180] Study design considerations

[0181] Trial #1 is a dose finding trial to evaluate the safety and immunogenicity of the transmitted/founder gp120 protein, CH505TF. The first protocol will be used in establishing an IND. CH505TF will be available for clinical use approximately 6-7 months before additional three gp120 proteins, representing variants from later timepoints in infection, are available.

Assuming an acceptable safety and immunogenicity profile, trial #2 would follow with combinations of the T/F Env and week 53, 78 and 100 Env mutants. The doses for trial #2 will be informed by data from Trial #1. Because it takes over a year to develop bnAbs, the second trial will include the possibility of a month 18 boost as well. Combined, these studies will test the ability of these Envs to initiate bnAb lineages and to use the isolated B cells from the vaccinees to identify the lineages induced.

[0182] Schema Trial #2 (Sequential doses)

Study arm	N	Month 0 (Day 0)	Month 1 (Day 28)	Month 3 (Day 84)	Month 6 (Day 168)	Month 12 (Day 364)	Month 18 (Day 546)
Group 1	12	100mcg T/F	100mcg 53	100mcg 78	100mcg 100	100mcg Swarm	100mcg Swarm
Group 2	12	100mcg T/F + 4mg DNA mosaic	100mcg 53 + 4mg DNA mosaic	100mcg 78 + 4mg DNA mosaic	100mcg 100 + 4mg DNA mosaic	100mcg Swarm	100mcg Swarm
Group 3	12	100mcg T/F	50mcg T/F +50mcg 53	33mcg T/F +33mcg 53 +33mcg 78	100mcg Swarm	100mcg Swarm	100mcg Swarm
Group 4	12	100mcg Swarm	100mcg Swarm	100mcg Swarm	100mcg Swarm	100mcg Swarm	100mcg Swarm
Group 5	12	placebo	placebo	placebo	placebo	placebo	placebo
Total	60						

(48/12)

[0183] Notes: T/F = transmitted/founder protein; Swarm = mixture of T/F, 53, 78, and 100;

Example protein doses included, total actual dose to be informed by Trial #1

[0184] Products: CH505TF: transmitted/founder HIV gp120 with AS01E; CH505w53.1: week 53 HIV gp120 with AS01E; CH505w78.33: week 78 HIV gp120 with AS01E; CH505w100.6: week 100 HIV gp120 with AS01E

[0185] DNA Mosaic env: trivalent vaccine composed of mosaic HV13284, HV13285 and HV13286 that optimizes global coverage. All express gp160 Env protein. In certain embodiments, bivalent mosaic envelopes can be used. Placebo: sodium chloride for injection.

[0186] Statistical considerations

[0187] Accrual and sample size calculations: Recruitment into trial #1 will target 42 healthy, HIV-uninfected adults aged 18 to 50 years old at low risk of HIV infection in regions where clade B is the predominant clade. Enrollment will be concurrent with receiving the first study vaccination, thus all participants will provide some safety data. For immunogenicity analyses, however, it is possible that data may be missing for various reasons such as participants terminating from the study early, problems in shipping specimens, or low cell viability of processed peripheral blood mononuclear cells (PBMCs). Immunogenicity data from 11 phase 1 and 1 phase 2a HVTN trials, which began enrolling after June 2005 (data as of June 2011), indicate that 10% is a reasonable estimate for the rate of missing data. For this reason, the sample size calculations below account for 10% of enrolled participants having missing data for the primary immunogenicity endpoint.

[0188] Sample size calculations for safety: The ability of the study to identify SAEs can be expressed by the true event rate above which at least 1 event would likely be observed and the true event rate below which no events would likely be observed. Specifically, in each vaccine

arm of the study ($n = 12$), there is a 90% chance of observing at least 1 event if the true rate of such an event is 17.5% or more; and there is a 90% chance of observing no events if the true rate is 0.8% or less. In all vaccine arms of the study combined ($n = 36$), there is a 90% chance of observing at least 1 event if the true rate of such an event is 6.2% or more; and there is a 90% chance of observing no events if the true rate is 0.2% or less.

[0189] Sample size calculations for immunogenicity: To address antibody endpoints, the analysis will descriptively summarize binding response positivity call rates and test superiority of the magnitude and breadth of the IgG binding Ab response to a panel of gp120 proteins for each of two comparisons (Group 1 vs 2, Group 2 vs 3), using a two- sided Wilcoxon rank sum test with 2.5% type-I error rate per comparison. The sample size of 12 vaccinees per group will give 80% power to detect a true difference of 1.82 standard deviations (SDs) between the mean non-zero responses and 90% power to detect a true difference of 2.04 SDs. These calculations assume a 10% loss-to-follow-up rate and the (94%) response rate observed in the HVTN 088 vaccine recipients. The same approach will be used to test superiority of the magnitude of the IgG binding Ab response to each individual gp120 antigen in the panel.

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Example 6

[0200] DNA and mRNA vaccination for mimicking HIV envelope evolution during broad neutralizing antibody induction

[0201] 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.

[0202] 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.

[0203] The example will use the new CH505 set of T/F and sequential evolved Env envelopes (in certain embodiments the set includes 103/104 envelopes—Table 14) that gave rise to the CH103 bNAbs lineage to generated broadly neutralizing CD4 binding site (bs) bNAbs responses. In certain embodiment, w004.03 envelope is used instead of CH505 T/F envelope. In certain embodiments, D-loop mutants are optionally included. The CH505 set of Envs is derived from the CHAVI bNAbs individual, CH505 who is one of the CHAVI001 cohort of Africans who were followed from the time of acute HIV infection to the development of high titers of bNAbs. CH505 plasma neutralizing activity and resulting CH103 lineage bNAbs are targeted to the CD4 binding site (Nature 496: 469, 2013). 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.

[0204] 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®.

[0205] Stage 1. Comparison of the immunogenicity of DNAs vs. mRNAs expressed in Nanotaxi® formulation. For this comparison, we will make 4 sequential CH505 Envs as DNA vs mRNA gp120s and gp160s in the presence or in the absence of an immunostimulatory sequence (IS) linked to the CH505 env genes and then formulate them with Nanotaxi® and test their immunogenicity in C57BL6 mice for their ability to induce anti-Env antibodies. Each of the 4 Envs will be tested both alone as a prime-boost injection model, and with recombinant protein administered either as a boost or co-administered with the DNA or mRNA. In certain embodiments gp145 is used instead of gp160.

[0206] Below is a list of groups to be tested:

[0207] **Groups 1, 2, 3 and 4-** Immunization with each gp120 DNA formulated with Nanotaxi® X4.

[0208] **Groups 5, 6, 7 and 8-** Immunization with each gp120-IS DNA formulated with Nanotaxi® X4.

[0209] **Groups 9, 10, 11 and 12-** Immunization with each gp160 DNA formulated with Nanotaxi® X4.

[0210] **Groups 13, 14, 15 and 16-** Immunization with each gp160-IS formulated with DNA Nanotaxi® X4.

[0211] **Groups 17, 18, 19 and 20-** Immunization with each gp120 mRNA formulated with Nanotaxi® X4.

[0212] **Groups 21, 22, 23, and 24-** Immunization with each gp120-IS mRNA formulated with Nanotaxi® X4.

[0213] **Groups 25, 26, 27 and 28-** Immunization with each gp160 mRNA formulated with Nanotaxi® X4.

[0214] **Groups 29, 30, 31 and 32-** Immunization with each gp160-IS mRNA formulated with Nanotaxi® X4.

[0215] **Groups 33, 34, 35 and 36-** Immunization with each Env gp120 protein alone X4

[0216] **Groups 37, 38, 39 and 40-** Immunization with each Env gp140 protein alone X4

[0217] **Groups 41, 42, 43 and 44-** Immunization with each Env as optimal genetic form from studies above (mRNA vs. DNA with or without the immunostimulatory sequence (IS); gp120 vs. gp160) in sequential format.

[0218] **Groups 45, 46, 47 and 48-** Immunization with each Env as optimal genetic form from studies above (mRNA vs. DNA with or without the immunostimulatory sequence (IS); gp120 vs. gp160) in sequential format, combined with four homologous Envs as proteins—delivered simultaneously with mRNA or DNAs.

[0219] All immunizations will be performed intramuscularly (IM) with 6 C57BL6 mice per group. Mouse immunizations will be followed for induction of titers of CH505 Env antibodies by ELISA. Other suitable non-human animal models can be used.

[0220] Once comparisons are made per above, a series of comparisons will be made of the optimal genetic immunizations given IM alone vs IM with Nanotaxi® as follows to ensure that Nanotaxi® is the optimal administration mode.

[0221] **Group 49-** Immunization with DNA or mRNA alone IM X4

[0222] **Group 50-** Immunization with DNA or mRNA formulated with Nanotaxi® IM X4.

[0223] **Stage 2.** Study of the immunogenicity of the optimized DNA or mRNA CH505 gp160 or gp120 Envs formulated with Nanotaxi® in CD4 binding site CH103 germline knockin mice and rhesus macaques. Here we will take the most immunogenic form of genetic immunization from Stage 1 and formulate 100 sequential evolved Envs for administration in the following manner:

[0224] For **CH103 germline knockin mice**:

[0225] **Group 1** Immunization with DNA or mRNA formulated with Nanotaxi® with CH505 transmitted/founder (T/F) Env first, followed by a mixture of the next Envs, followed by a mixture of the next 33 Envs, followed by a mixture of the final Envs. Loop D mutants could be included in either prime and/or boost.

[0226] **Group 2** Immunization with DNA or mRNA formulated with Nanotaxi® with CH505 transmitted/founder (T/F) Env first, followed by a mixture of the next 32 Envs, followed by a mixture of the next 33 Envs, followed by a mixture of the final 33 Envs. Here the genetic immunization will be the same as in group 1 except each immunization will be accompanied by 4 (T/F, week 53, week 78, week 100) CH505 Env protein as gp120s.

[0227] For **Rhesus Macaques** (NHP study #79)

[0228] Key is to determine how long it is necessary to immunize and what a precise regimen might be regarding sequential, additive or swarm types if immunizations. NHP study #79 is already ongoing and can inform the work by determining how long immunizations are needed and also by providing a protein only control set of experiments.

[0229] NHP #79 is divided into 6 groups (4 animals per group) as follows:

[0230] **Group 1.** Immunization with the Transmitted/Founder Env gp120 alone X5; last immunization finished 11/21/13; induced autologous (CH505 T/F) neutralizing antibodies (slides 1 and 2 above) ; animals now being studied for VH and VL lineages induced. Once autologous Nabs isolated, plans are next to boost with “swarm” of all 4 Envs.

[0231] **Group 2.** Immunization with second Env (week 53) only X5.

[0232] **Group 3.** Immunization with third Env (week 78) only X5.

[0233] **Group 4.** Immunization with sequential T/F, then week 53, then week 78, then week 100 Env, then a swarm of all 4 was completed; induced autologous Neutralizing antibodies of CH505 founder virus (Figures 26-29); animals now being studied for VH and VL lineage induced.

[0234] **Group 5.** Immunization with additive Envs (T/F first then T/F + 53; then T/F + 53 + 78; then T/F + 53 + 78 + 100) then swarm of all 4 ; last immunization finished 11/21/13; induced autologous neutralizing antibodies; animals now being studied for VH and VL lineages induced.

[0235] **Group 6.** Immunization with fourth Env (week 100) only X5.

[0236] It took ~90 weeks for heterologous nAbs to appear in the CH505 plasma, and it took ~136 weeks for full bnAb activity to appear. Thus, a major way the NHP #79 study can inform the future studies to project how long and how many immunizations will be needed using genetic immunization.

[0237] Secondly, a key protocol to evaluate is the contribution of protein to genetic immunization when proteins are added to mRNA or DNA immunizations. We believe that that the most effective way to immunize will likely be the simultaneous combination of nucleotides in Nanotaxi® plus proteins. Thus, the NHP #79 studies probe the route and use of proteins alone.

[0238] NHP Study for testing of genetic immunization of swarms of Envs in rhesus macaques.

[0239] **Group 1** Immunization with DNA or mRNA formulated with Nanotaxi® with CH505 transmitted/founder (T/F) Env first, followed by a mixture of the next Envs, followed by a mixture of the next Envs, followed by a mixture of the final Envs.

[0240] **Group 2** Immunization with DNA or mRNA formulated with Nanotaxi® with CH505 transmitted/founder (T/F) Env first, followed by a mixture of the next Envs, followed by a mixture of the next Envs, followed by a mixture of the final Envs. Here the genetic

immunization will be the same as in group 1 except each immunization will be accompanied by 4 (T/F, week 53, week 78, week 100) CH505 Env protein as gp120s.

[0241] All immunizations will be performed IM with 6 rhesus macaques per group.

Immunizations will continue for 2.5 years in the rhesus macaques. NHP immunizations will be followed for 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.

[0242] In addition, we will genetic immunizations in two types of humanized mice: the KYMAB® lambda mice (CH103 utilizes V λ 3-1) and our CH103 knockin mice that only express the germline VH4-59 and V λ 3-1 genes of CH103 lineage. The latter mice will test the integrity of the Env immunogens for triggering of the CH103 lineage in the absence of germinal center competition for space by other clones, and the KYMAB® lambda mice will test the immunogens in a wildtype repertoire system much as in the rhesus macaques.

[0243] For both mouse lines, we will test 12 mice per group and the mode of monitoring the response will be identical to that in rhesus macaques.

[0244] Each of the models above has their advantages and disadvantages.

[0245] The CH103 GL mouse has the advantage of being able to see exactly what the CH505 immunogens can do for the CH103 lineage. The disadvantage is that the T cells are mouse and the Ig repertoire is human.

[0246] The KYMAB lambda mouse has the advantage of having the entire VH and Vlambda human repertoire and has the disadvantage of having mouse T helper cells and TFH.

[0247] The rhesus has the advantage of being primate and being most similar to human in repertoire and TFH cells with the disadvantage of cost and not being human. Nonetheless, the rhesus macaque for these immunogenicity studies is most like human of all the models and if our strategy works in rhesus macaques, we believe this is the best indicator that it will work in humans.

[0248] **Stage 3.** GMP Production of the 100 “Swarm” of CH505 Evolved Envs As Either DNAs or mRNAs (Downselected from Stage 1 above).

[0249] This stage of the project will consist in producing by subcontracting with a GMP manufacturer of plasmid DNA or mRNA molecules depending on the selected format.

Subcontractors have already been identified by the members of the present proposal. Discussion with manufacturers will aim to define the timelines and the cost for the production of the 100 “swarm” of CH505 Evolved Env as DNAs or mRNAs. For proteins, the CH505 T/F, week 53, week 78 and week 100 gp120 Envs are already produced in bulk under GMP conditions for use in Phase I clinical trials, and these Envs will be available to for use with genetic immunization in Phase 1 trials should the genetic immunizations as “swarms” be successful in CH103 knockin mice and/or rhesus macaques.

[0250] Milestones

[0251] Stage 1. Decide on mRNA vs DNA regarding optimal immunogenicity in C57BL/6 mice. Criteria for deciding will be based on titers of CH505 Env antibodies in mouse plasma.

[0252] Stage 2. Criteria for proceeding to GMP DNA or mRNA production with Nanotaxi® formulation will be the demonstration in the CH103 bnAb germline knockin mice of the DNA or

mRNA/NanoTaxi® formulation to induce clonal lineages of VH4-59—the VH of the CH103 lineage, or Vλ3-1, or induce any clonal lineages with binding to RSC3 protein but not to the RSC3 protein with an isoleucine deleted at position 371 (signifying a CD4 binding site BnAb lineage).

[0253] Similarly, criteria for proceeding to GMP DNA or mRNA production with Nanotaxi® formulation in KYMAB lambda mice and rhesus macaques will be for the DNA or mRNA/NanoTaxi® formulation to induce clonal lineages of the orthologues of VH4-59—the VH of the CH103 lineage, or orthologues of Vλ3-1, or induce any clonal lineages with binding to RSC3 protein but not to the RSC3 protein with an isoleucine deleted at position 371 (signifying a CD4 binding site BnAb lineage).

[0254] Thus, if either the right lineages are induced in the CH103 germline knockin mouse model or in KYMAB lambda mice or in rhesus macaques, we will move forward to GMP production. We will have this very high bar as a go-no go decision, since moving to stage 3 will be relatively expensive. That is to say, we will need to know our immunogens are inducing the correct lineages prior to moving to Stage 3.

[0255] Stage 3. Criteria for moving to a Phase I clinical trial will be the above immunogenicity in rhesus macaques, and the ability to scale up and produce the 100 Envs as DNAs or RNAs with NanoTaxi®.

What is claimed is:

1. A composition comprising a nucleic acid encoding HIV-1 envelope from Table 14 or any combination thereof.
2. A composition comprising an HIV-1 envelope polypeptide from Table 14 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 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. A method of inducing an immune response in a subject comprising administering a composition comprising a nucleic acid encoding HIV-1 envelope from Table 14 or an HIV-1 polypeptide of Table 14, or any combination thereof, in an amount sufficient to induce an immune response.
9. The method of claim 8, wherein the composition is administered as a prime.
10. The method of claim 8, further comprising administering an adjuvant.
11. The method of claim 8, further comprising administering an agent which modulates host immune tolerance.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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JUMBO APPLICATIONS/PATENTS

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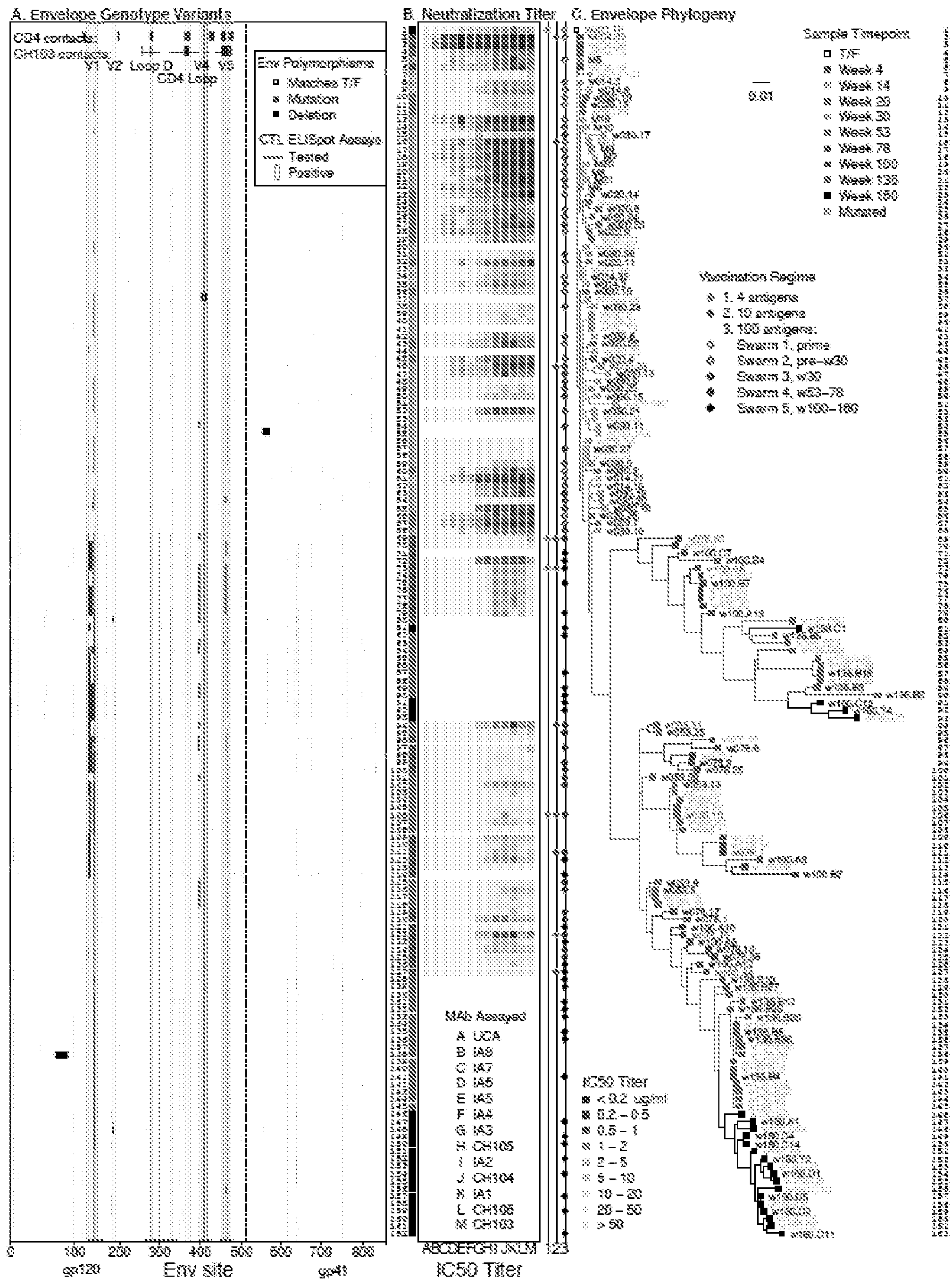


Fig. 1

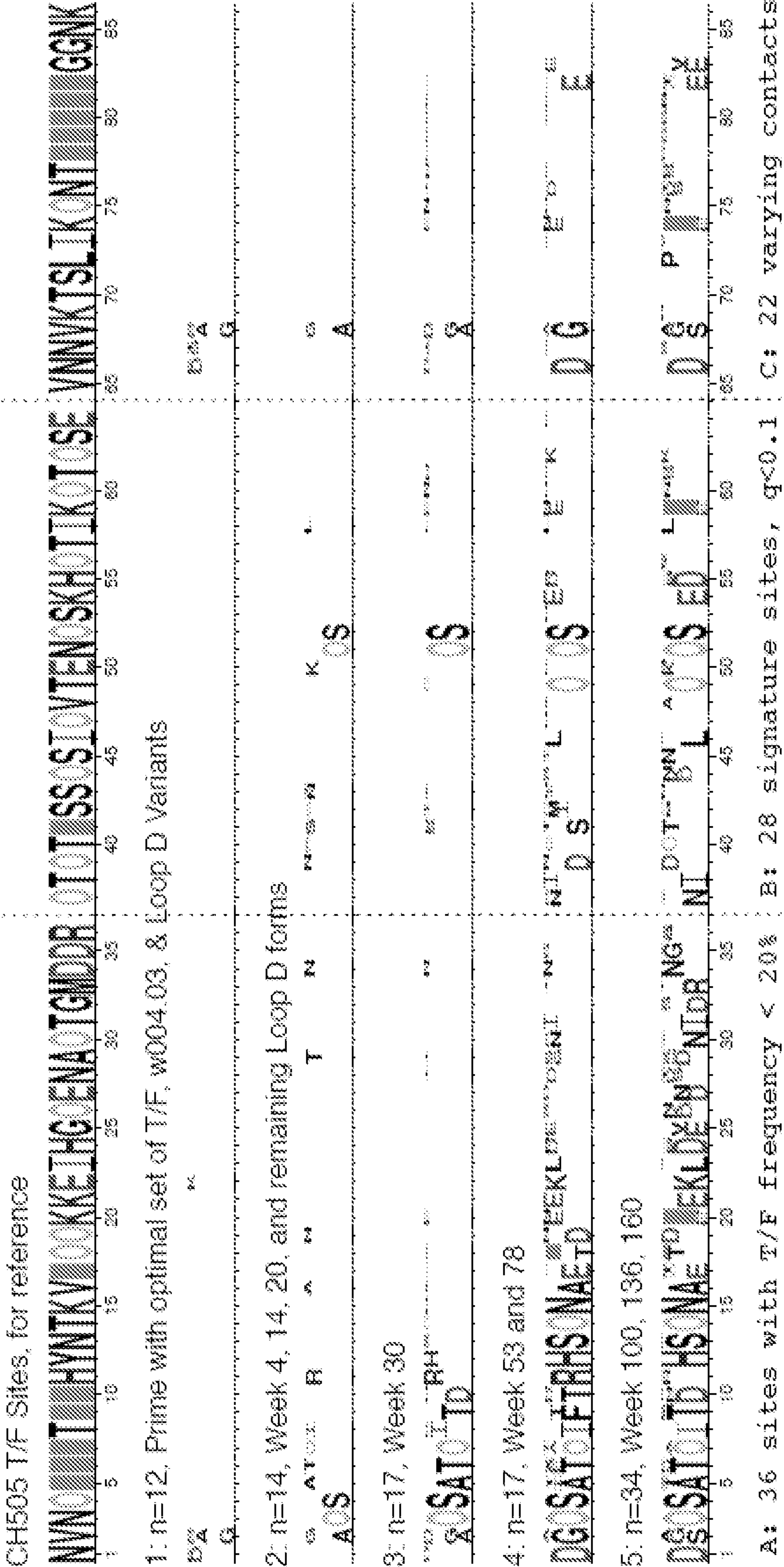


Fig. 2

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Table 1. Alignment columns in Env "hot-spot" concatamer summaries.

Col	Aln	HXB2	AA	CH505	Feature	Col	Aln	HXB2	AA	CH505	Feature
A: 36 sites with T/F < 20%						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	C: 22 varying contacts					
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: 28 signature sites, q<0.1						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

Fig. 3

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Table 1A. Alignment columns in Env "hot-spot" concatamer summaries.

Col	Aln	HXB2	AA	CH505	Feature	Col	Aln	HXB2	AA	CH505	Feature
A: 36 sites with T/F < 20%						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	C: 22 varying contacts					
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	368	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: 28 signature sites, q<0.1						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

Fig. 4

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Table 2. Ten selected Envs as concatenated sites. Columns follow rows of Table 1. 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**.

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				
w006.FF																
#NVNO---T--HYNTKV-GCKNEIHGOENADTSMDDR							OTOT-SSOSIGVTENOSKHOTIKOTOSS					VNNVKTSLIKONT-----GGNM				
M10																
#.G.....												.G.....				
<u>M11</u>																
DG.....												.D.G.....				
M6																
#.A.....												.A.....				
<u>w004.03</u>																
*.....																
w004.26																
#.....																
w020.14																
#.AOS.....							.N.....KOS.....L.....					.A.....				
<u>w030.28</u>																
.DOS.....RH.....							.I.....OS.....					.B.....				
<u>w053.16</u>																
*DGOSIEIIFTRHSONAETH.E.....G.....							.NO.M.....O.OS.E.....					.D.G.....E.....				
<u>w053.31</u>																
DGOSAT.I..RHSON..-DN.....							.D.S.....O.OS.....N..K..					.D.G.....N.....				
<u>w078.7</u>																
DGOSIE.IFTRHSONAE.NEENLD.....G.....G.....							.NOTM...L..O.OS.ED...E.....					.B.G.....E.....E.....				
<u>w078.15</u>																
DGOSATOI..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.....				
<u>w078.33</u>																
*.AGSATG.T..HSONA..-DN.....G.NI.T...							NIDOT.....OKOS.....LN.....					.SA.....N.O.....				
<u>w100.A4</u>																
DGOSATO.TDSHSONA....EKLDE.DD.NI.....							NI.....L..O.OS.ED.....					.D.G.....B.....E.....				
w100.B4																
#DGOSATOI..RHSON...DO.K.DEK.S..D.....							.DOT.NK...O.OS..D...OS.K..					.D.G...P.OSS.....E...				
<u>w100.B6</u>																
*DSOSATG.TN.HSONAO-DE...DEKDT....R...							.DOT..K...O.OS..D..LENR...					.D.S...P.ENTRDGGNOE...				
w100.C7																
#DGOSATOITN.HSONAO-DE.K.D.KD.....							.DOT..K...O.OS..D...EN-...					.D.G...P.EN--GGKO...				

Fig. 5

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Table 2A. Ten selected Envs as concatenated sites. Columns follow rows of Table 1. 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**.

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				
w009.TF																
#NVNO---T--HYNTXV-OCNKEIHCOENACFGMDR OTOT-SSOSIOVTEROSKNOPIKOTOSE VNNVKTSLIKONT---GENE																
M10																
#.G.....G.....																
M11																
DG.....E.G.....																
M6																
#.A.....A.....																
w004.03																
*.....																
w004.26																
#.....																
w014.12_AG																
.....A.....NA.....D...N.A.....																
w014.32_AG																
.....K.....																
w020.14																
#.AOS.....N.....KOS.....L.....A.....																
w030.28																
.DGS.....RH.....I.....OS.....D.....																
w051.15																
*DGOSIKIIFTRHSONAETN.E.....G.....NO.M.....O.OS.E.....D.G.....E.....																
w053.31																
DGOSAT.I..RHSON.-DN.....D.S.....O.OS.....N..K..D.G.....M.....																
w078.7																
DGOSIE.IFTRHSONAE.NEEKLD....G.....G...NOTN...L..O.OS.ED...E.....D.G.....E.....E.....																
w078.15																
DGOSATOI..HSONA.T..EKL.E..D.S.....S.....L..O.OS.E.....D.G.....D.....E.....																
w078.25																
#DGOSTAAIFTRHSONAE.D.EN.N.E..S.....D.SO.SI...O.OS.EN.....K..D.G.....E.....																
w078.33																
*.AOSATO.T..HSONA.-DN.....O.NI.T...NIDOT.....OKOS.....LN.....SA.....N.O.....																
w100.A4																
DGOSATO.TDHSONA....EKLSE.DD.NI.....NI.....L..G.OS.KD.....E.G.....D.....E.....																
w100.B4																
#DGOSATOI..RHSON...DG.K.DK.S...D....DOT.NK....O.OS..D...OS.K..D.G...P.OSS.....E.....																
w100.B5																
*DGOSATO.TN.HSONAC-DE...DEKDT....R....DOT..K....O.OS..D..LENK...D.S...P.ENTRUCGNOC...																
w100.C7																
#DGOSATOI.TN.HSONAC.DE.K.D.KD.....DOT..K....O.OS..D...EN-...D.G...P.EN...GONO...																

Fig. 6

Table 3. Proposed CH505 swarm.

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**Table 3A.** Proposed CH505 swarm.

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

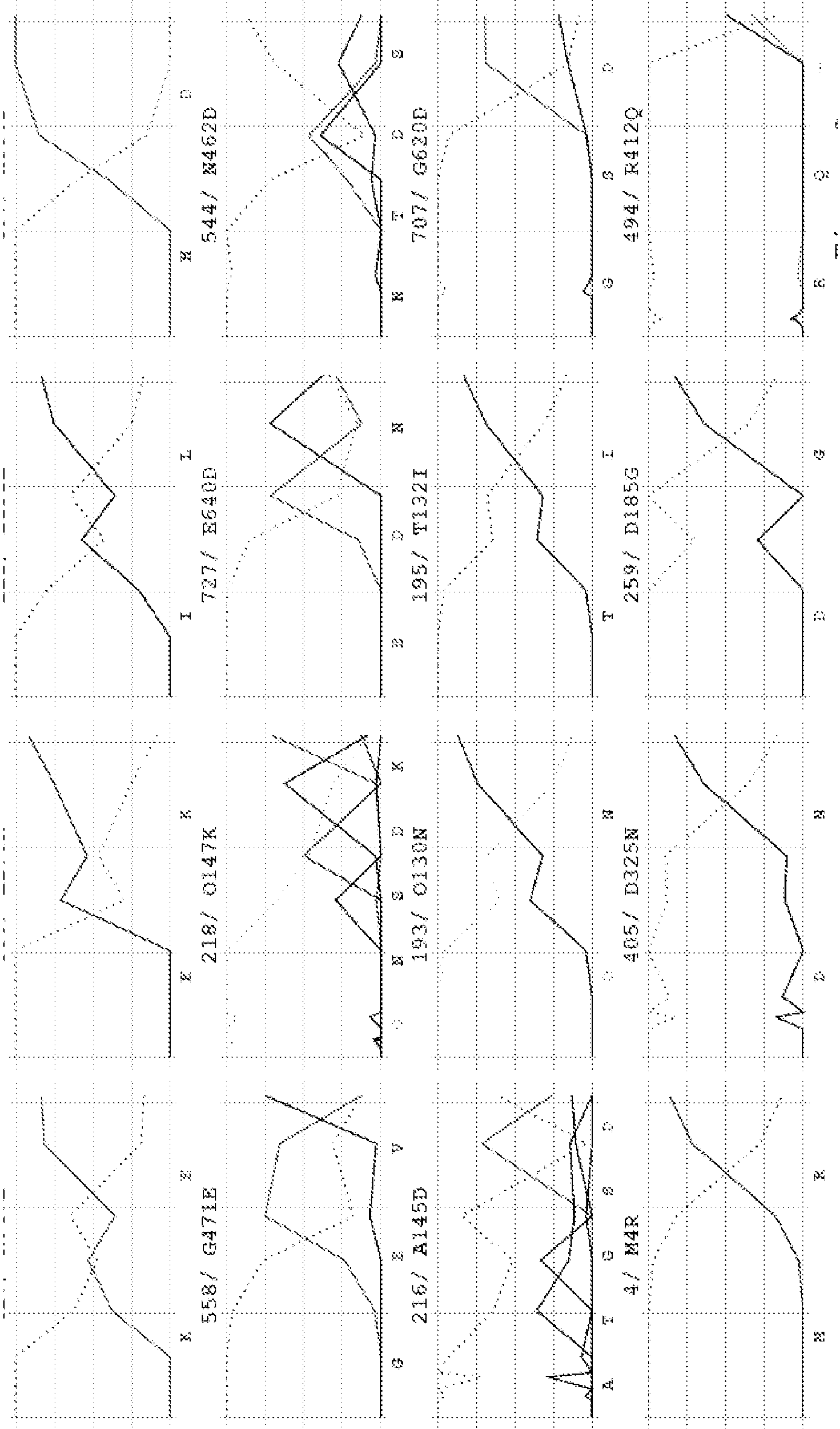


Fig. 9 (cont.)

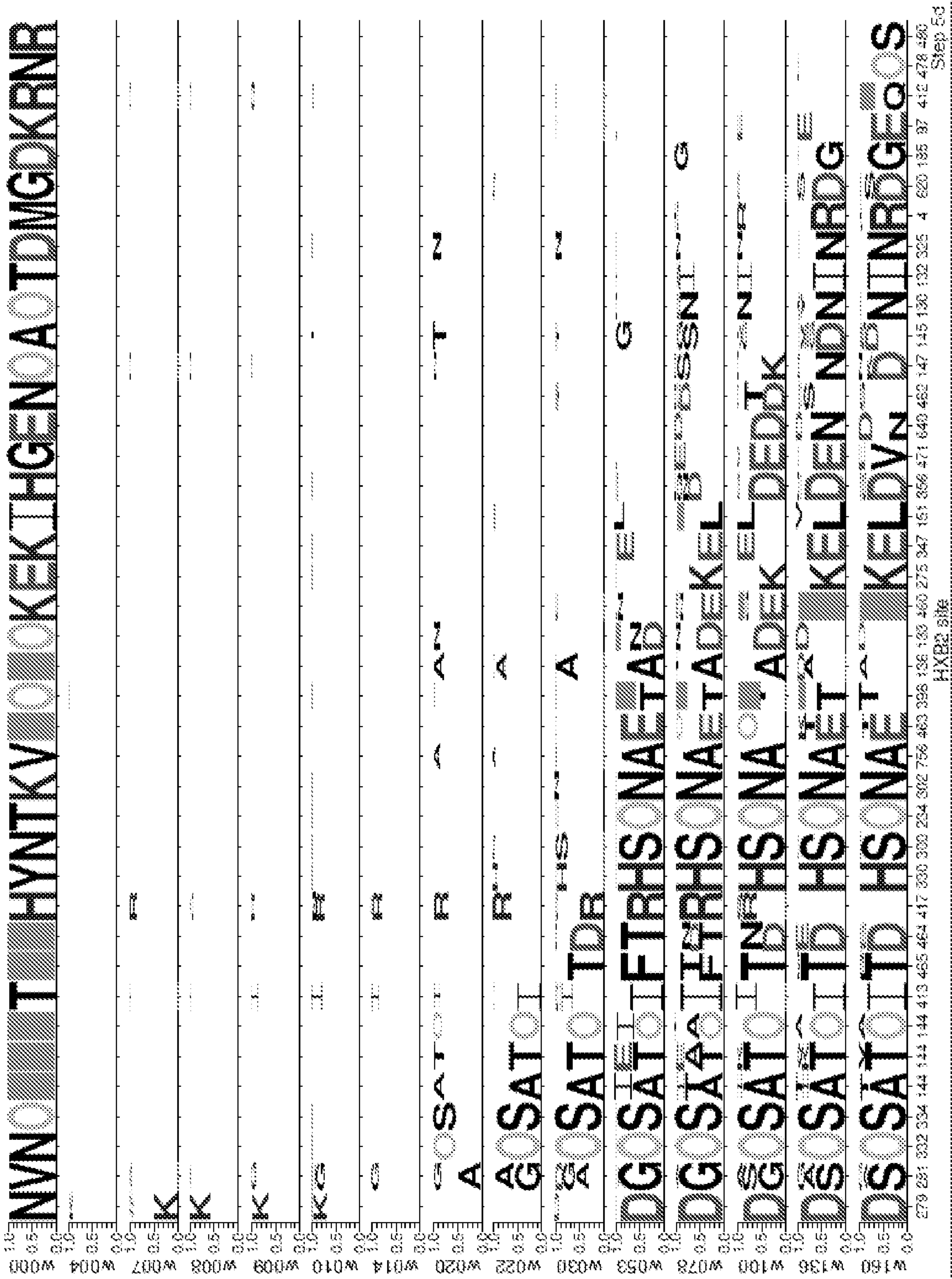


Fig. 10

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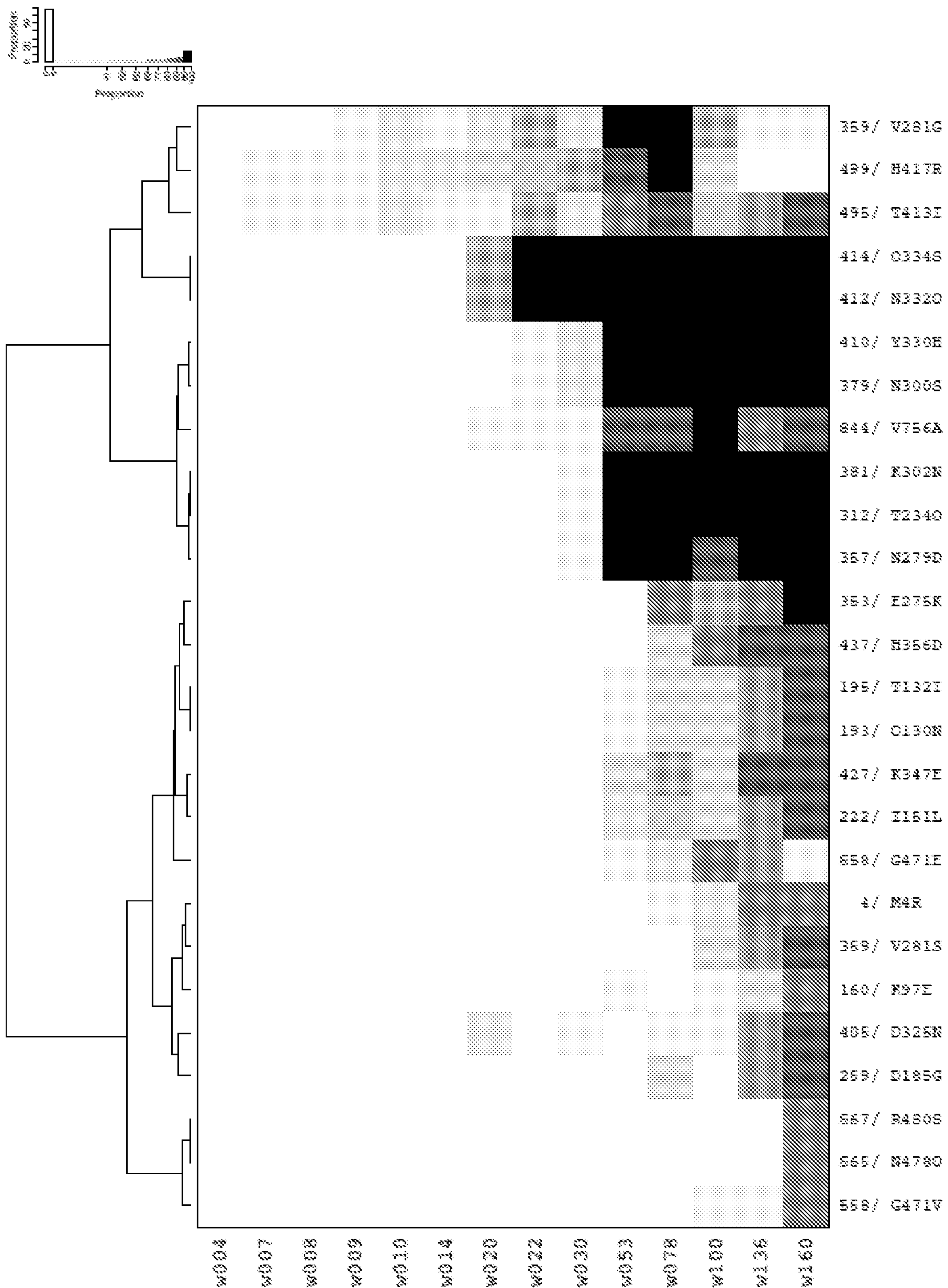


Fig. 11

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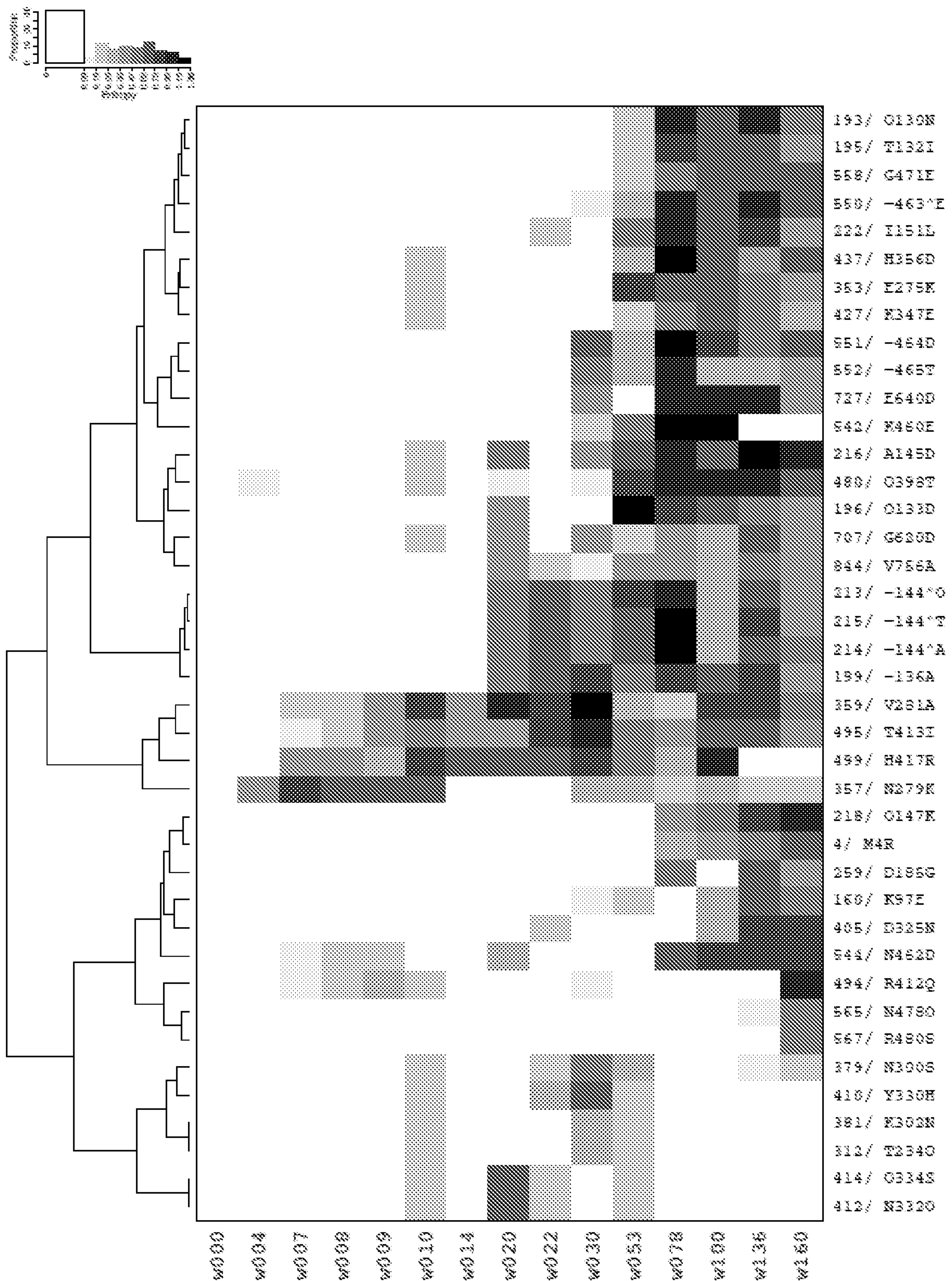


Fig. 12

SUBSTITUTE SHEET (RULE 26)

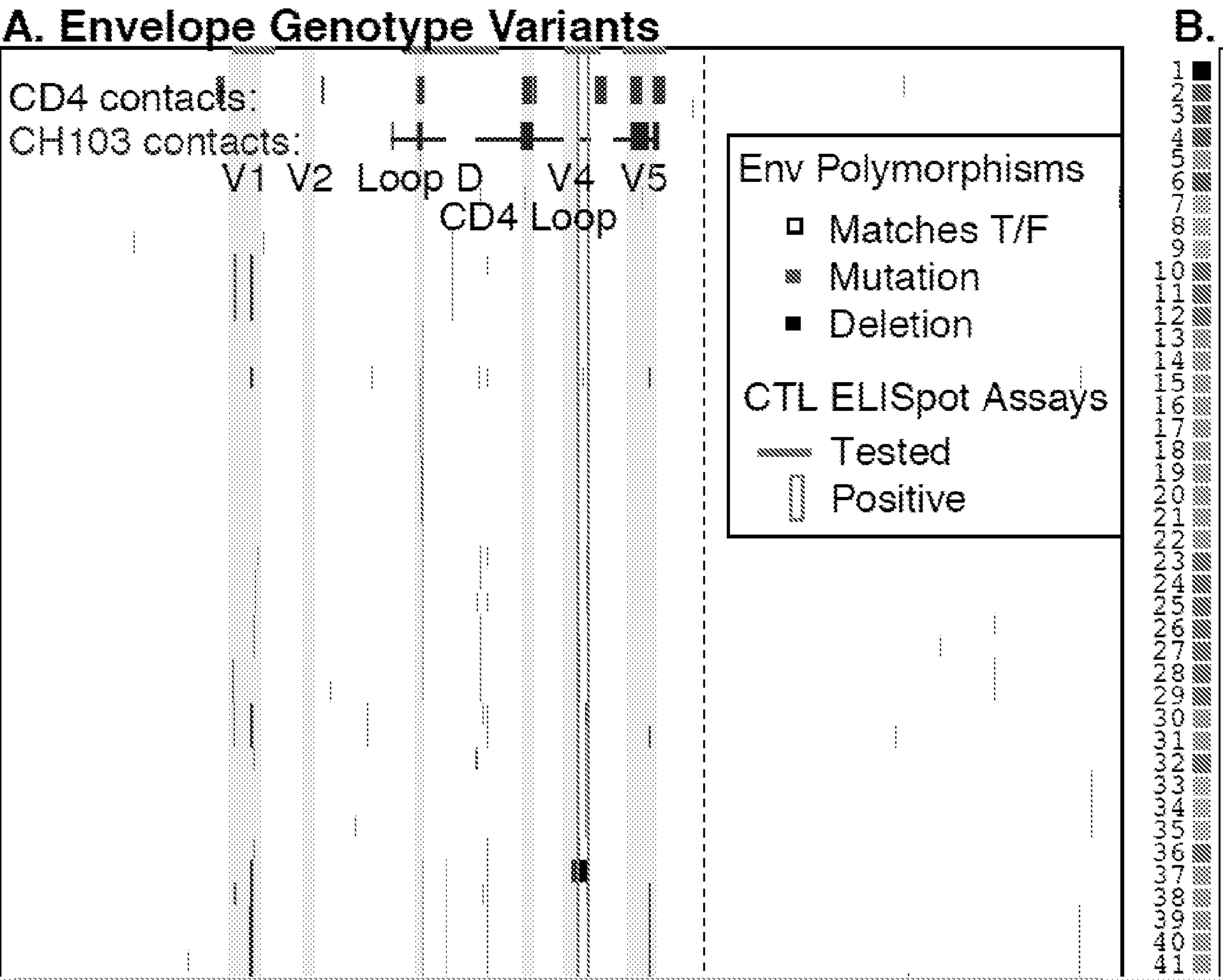


Fig. 13 A

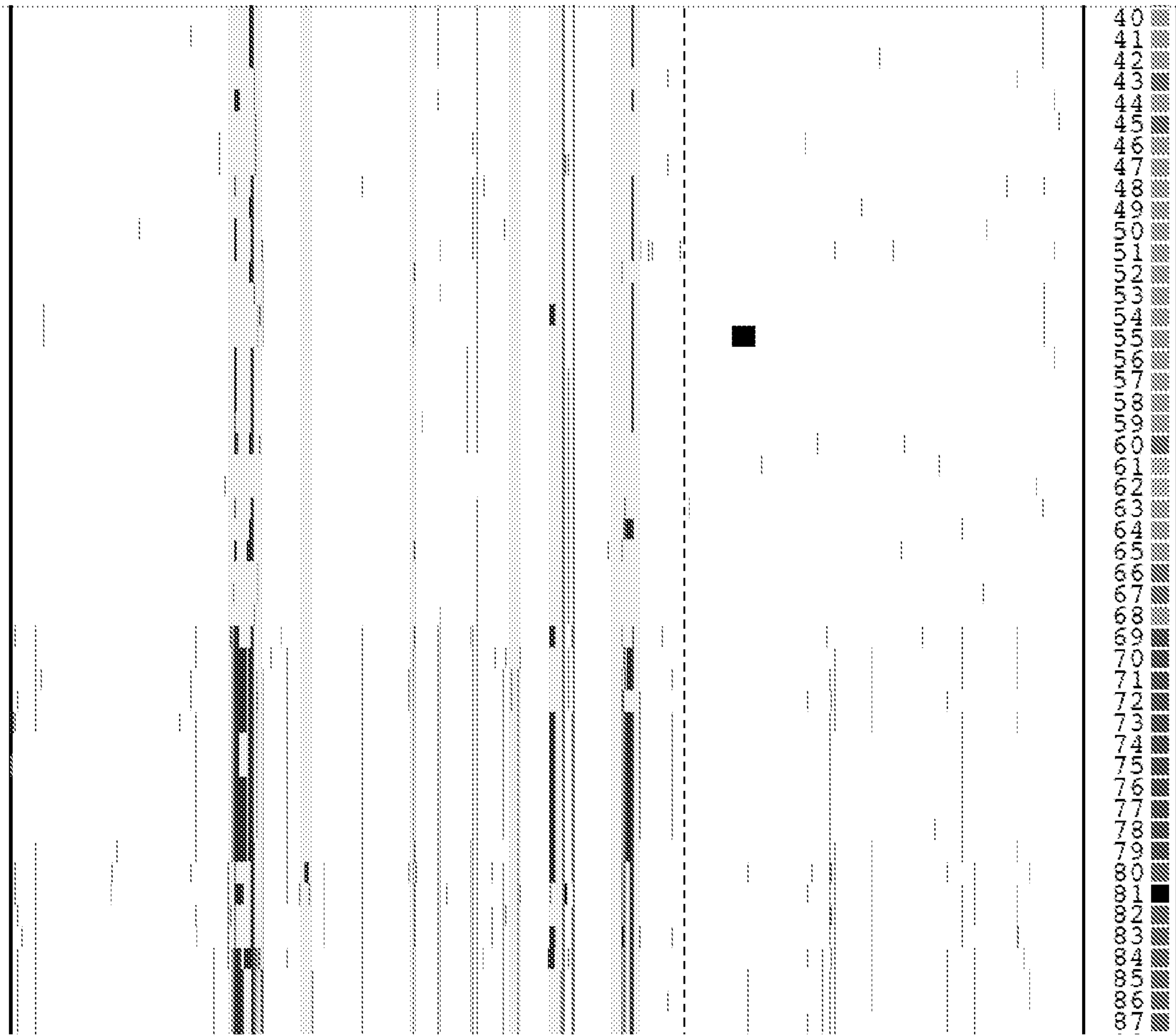


Fig. 13A (cont.)

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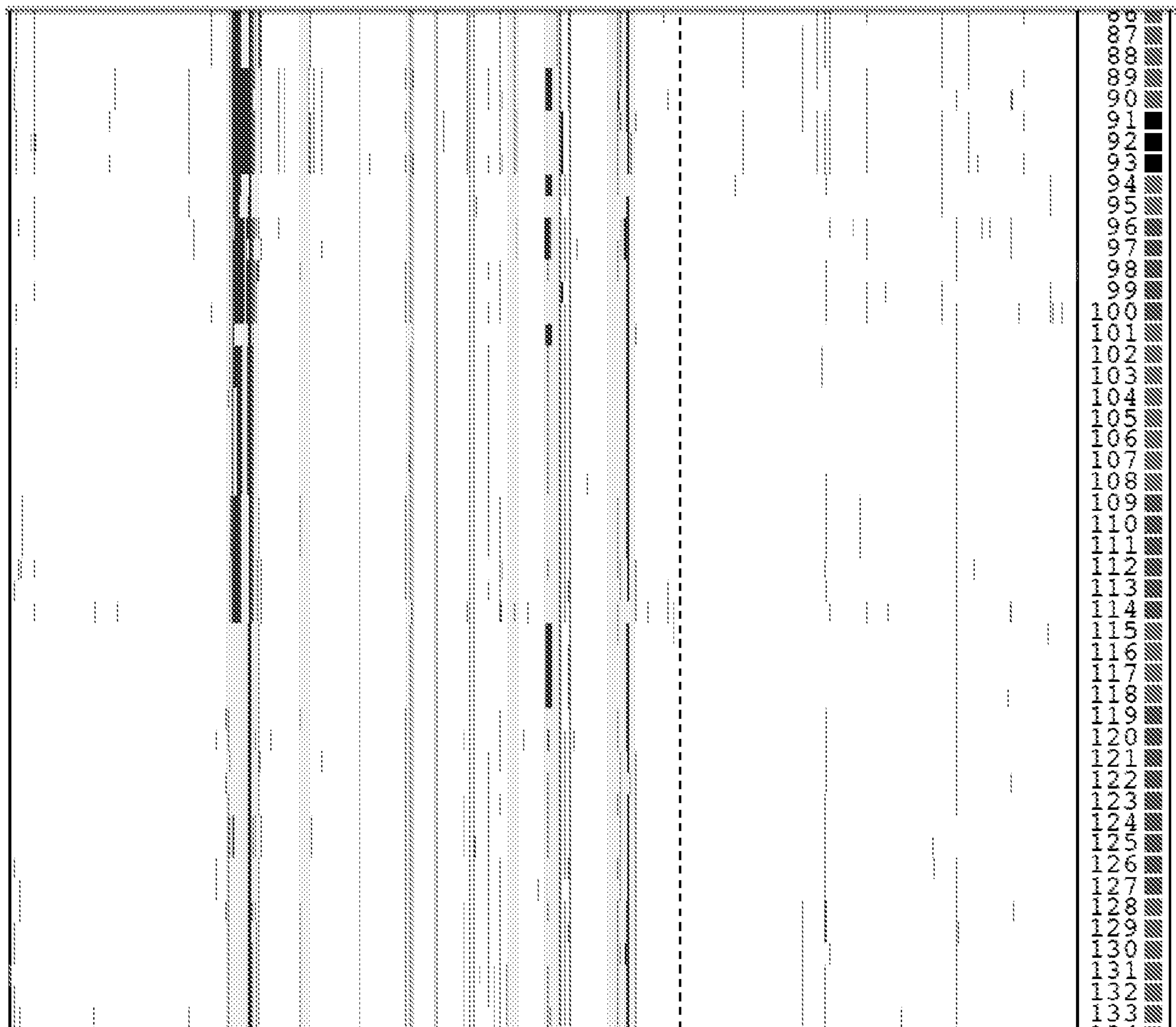


Fig. 13A (cont.)

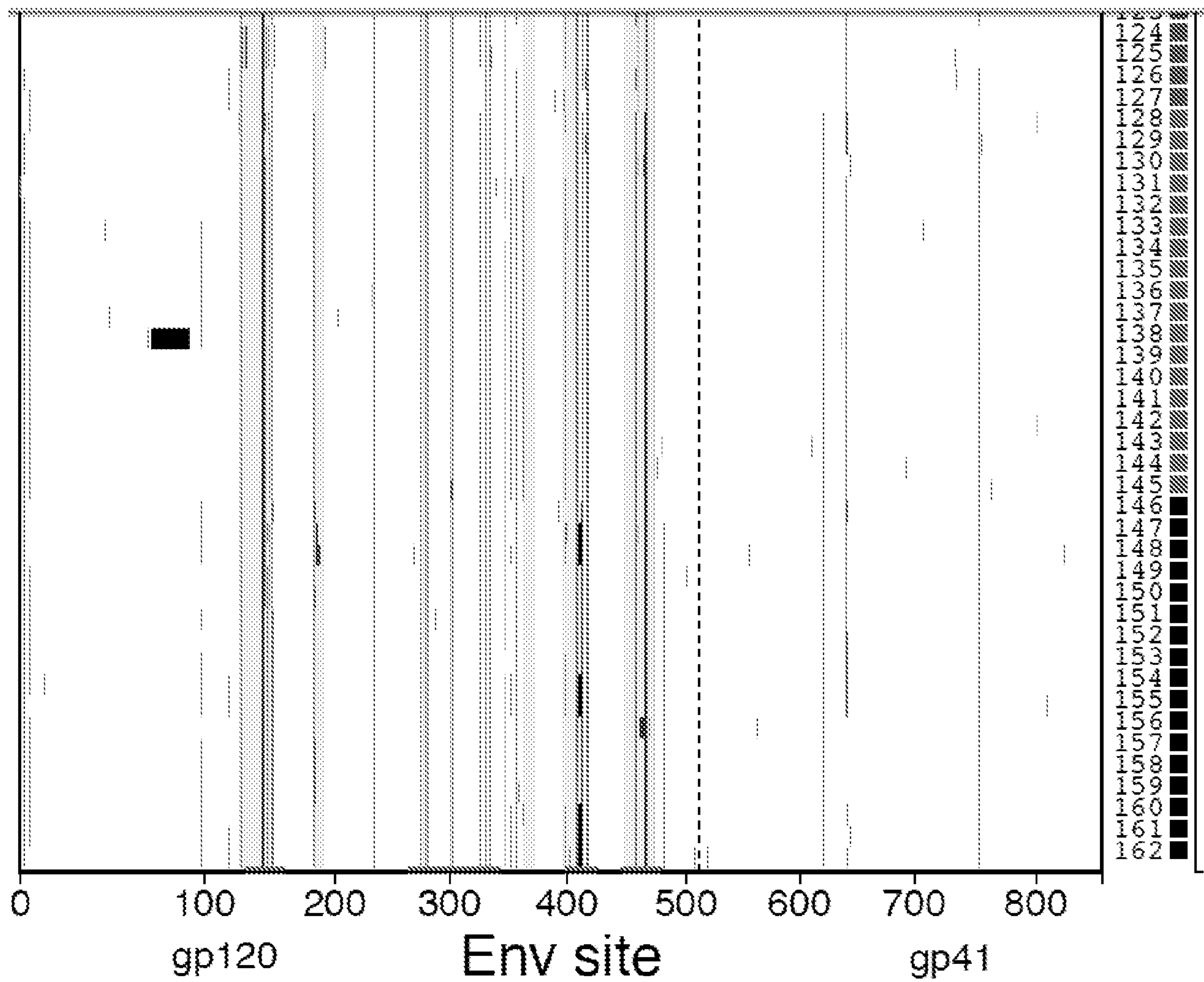


Fig. 13A (cont.)

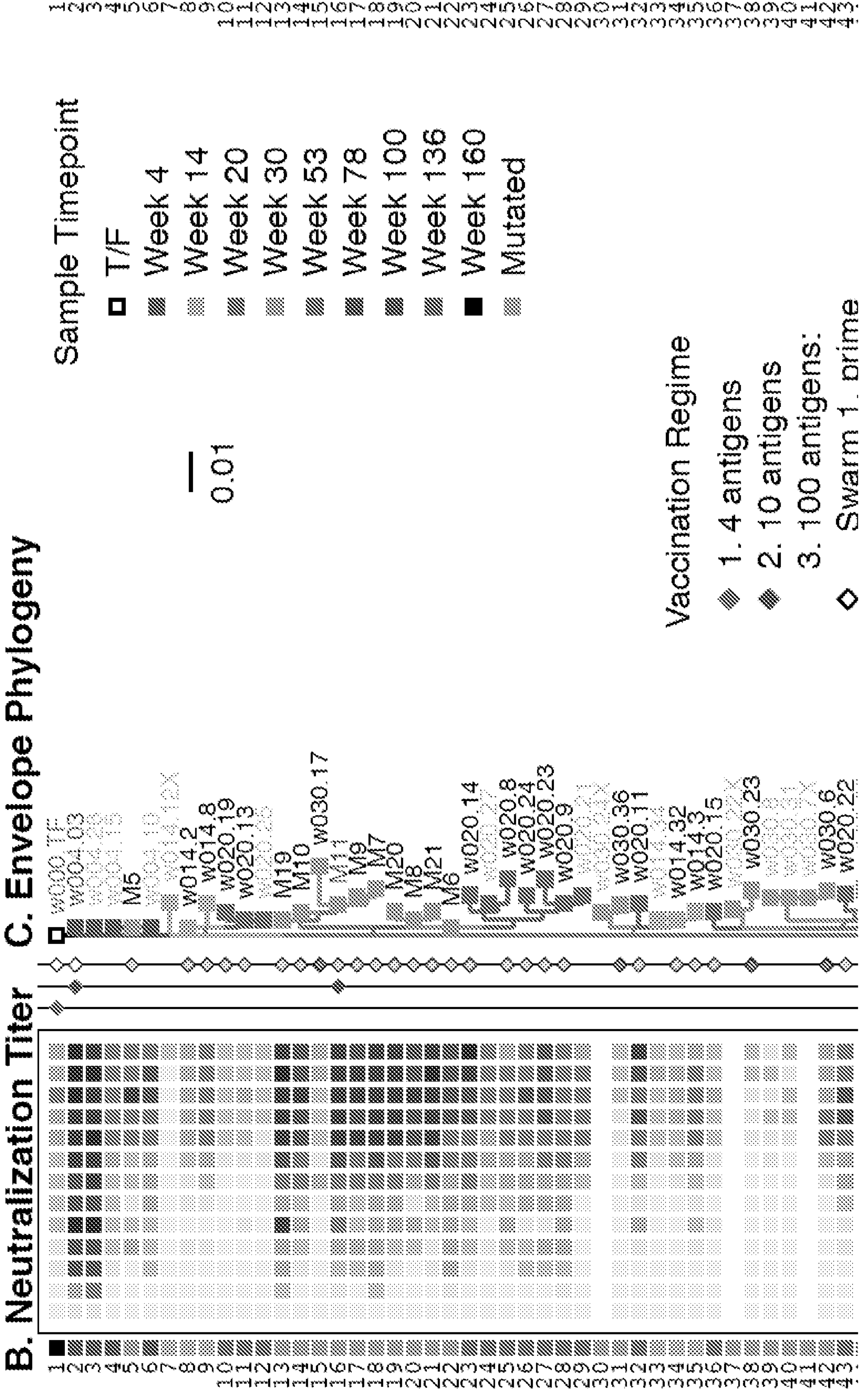


Fig. 13 B and C

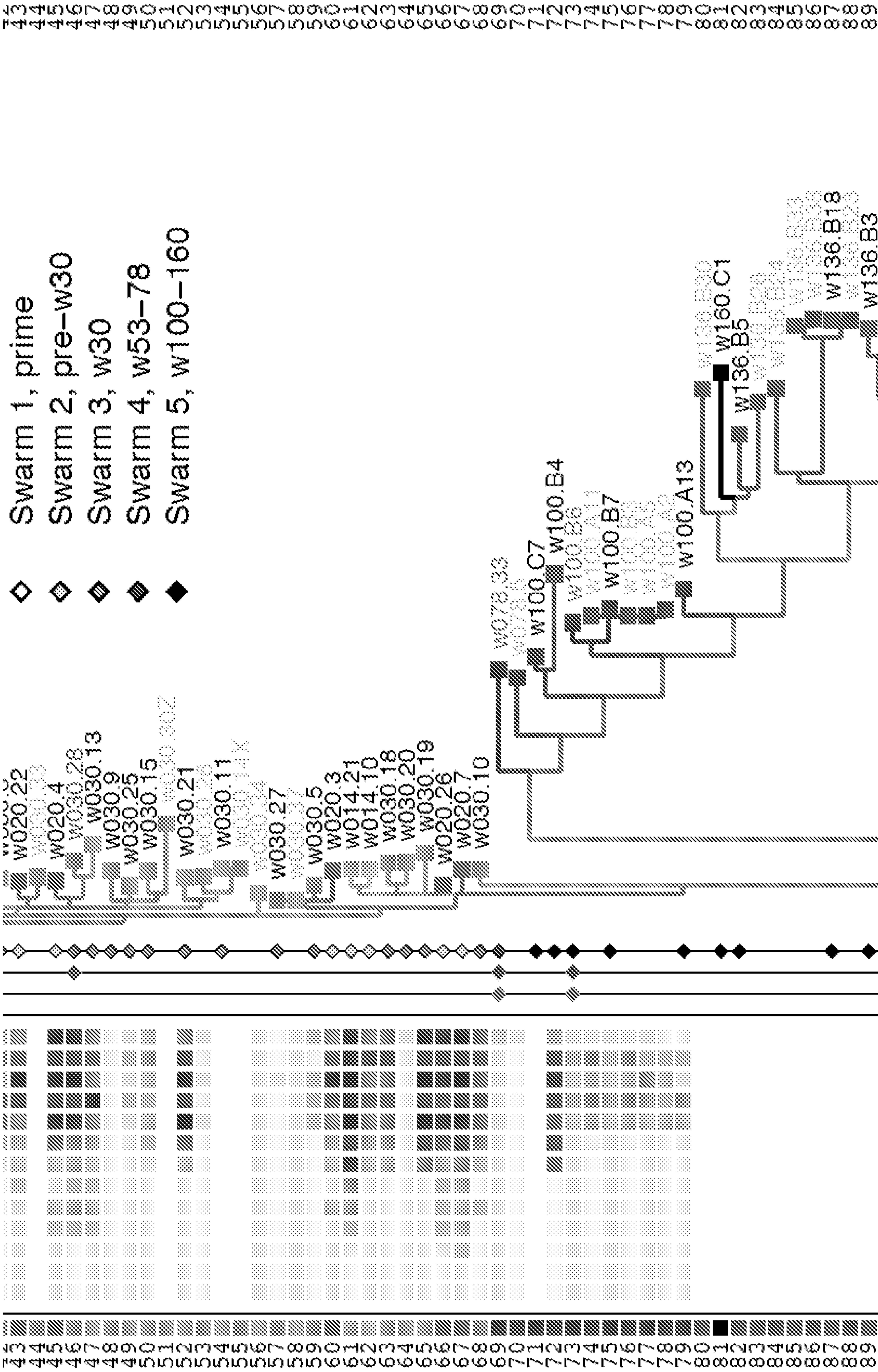


Fig. 13 B and C (cont.)

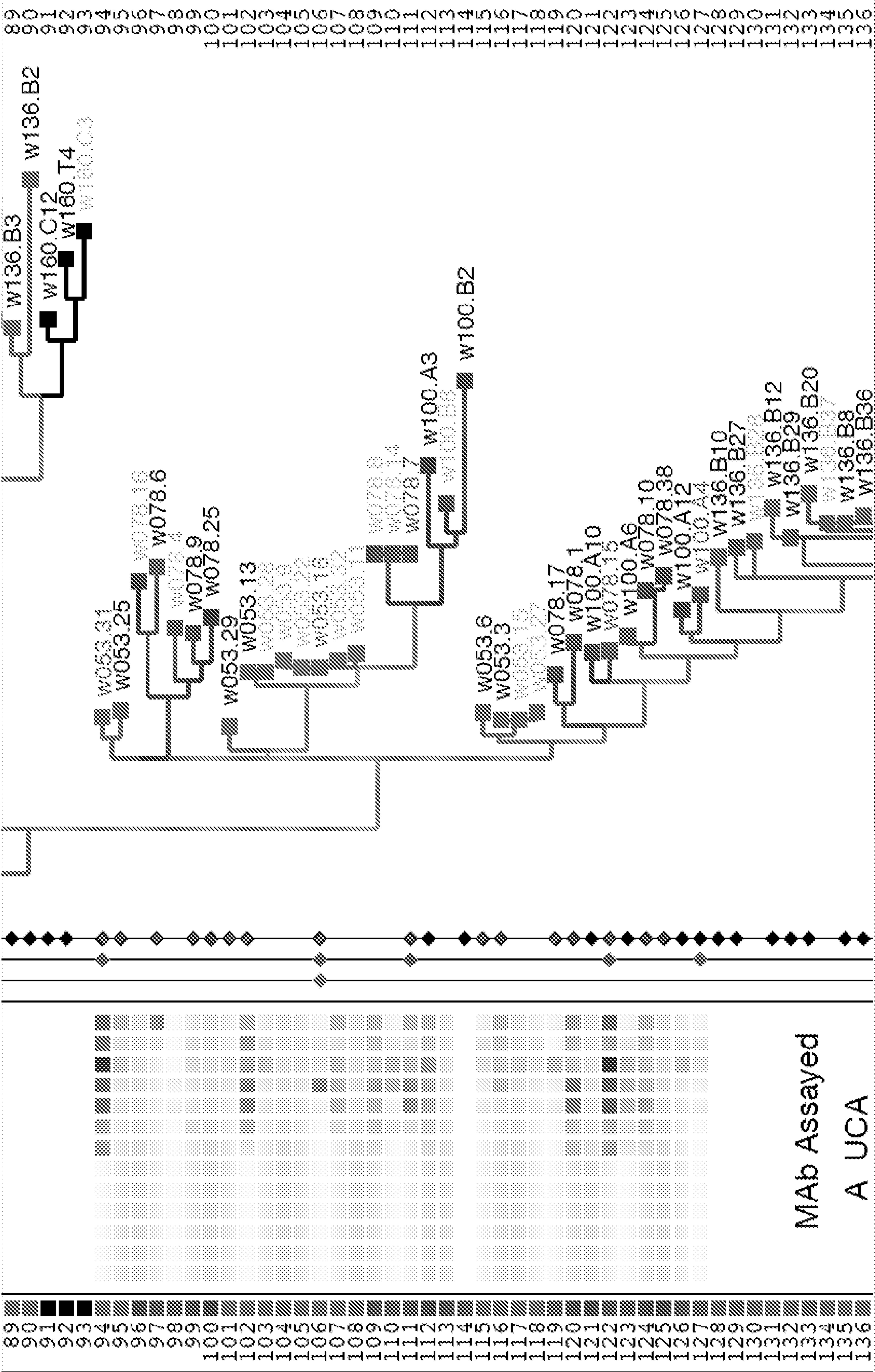


Fig. 13 B and C (cont.)

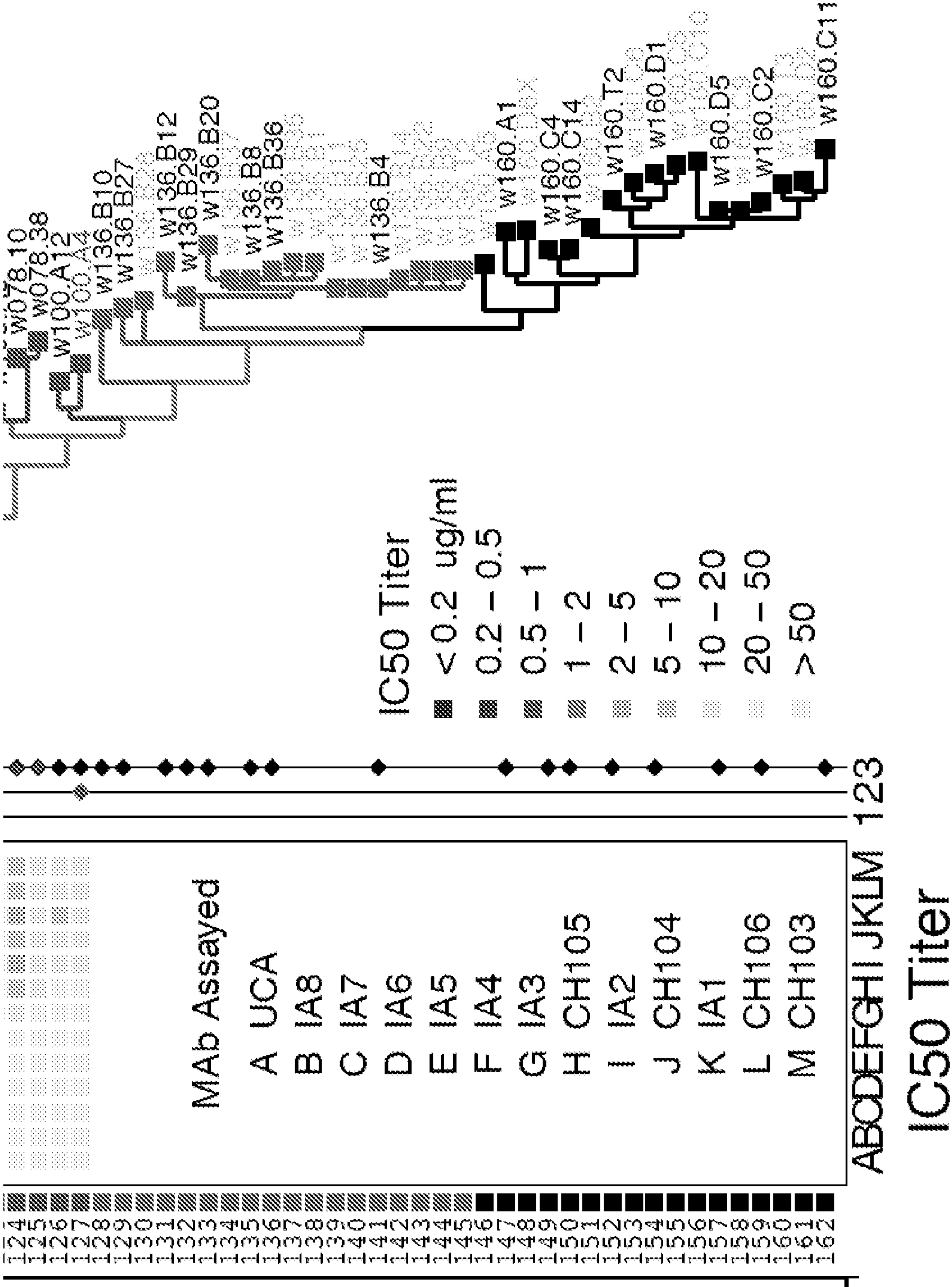


Fig. 13 B and C (cont.)

Fig. 14A (from page 21/306 to page 24/306)

>CH0505.TF

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GTCTATAACACACAAGGCTTCTTCCCTGATTGGCAAACTACACACCGGG
ACCAGGGGTCAGATATCCACTGACCTTTGGATGGTGCTTCAAGCTAGTGC
CAGTCGATCCAGAGGAAGTAGAAGAGGCCAATAAAGGAGAAAACAACTGC
TACTACACCCTATGAGCCAGCATGGAATGGATGATGAGGACAGAGAAGT
ACTAAAGTGGAAGTTTGACAGTCAGCTAGCACGCAGACACATGGCCCGCG
AGCTACATCCGGAGTGGTACAAAGACTGCTGACACAGAAGGGACTTTCCG
CTGGGACTTTCCACTGGGGCGTTCCAGGGGGAGTGGTCTGGGCGGGACTG
GGAGTGGCCAACCCTCAGATGCTGCATATAAGCAGCTGCTTTTCGCCTGT
ACTGGGTCTCTCTAGGTAGACCAGATCTGAGCCCGGGAGCTCTCTGACTA
CCTAGGGAACCCACTGCTTAAGCCTCAATAAAAGCTTGCCTTGAGTGCTC
TAAGTAGTGTGTGCCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCT
CAGACCCTTTGTGGTAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAG
GGACTTGAAAGCGAAAGTAGAACCAGAGGAGATCTCTCGACGCAGGACTC
GGCTTGCTGAAGTGCACCTCGGCAAGAGGGCGAGAGCGGCGGCTGGTGAGTA
CGCCAAATTTTATTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCG
AGAGCGTCAATATTAAGAGGGGGAAAATTAGATAAATGGGAAAGAATTAG
GTTAAGGCCAGGGGGAAAGAAATGCTATATGATAAAACACTTAGTATGGG
CAAGCAGGGAGTTGGAAAGATTTGCACTTAATCCTGGCCTCTTAGAAACA
TCAGAAGGCTGTAGACAAATAATAAAGCAGCTACAACCATCTCTTCAGAC
AGGAACAGAGGAACCTTAGATCATTATATAACACAGTAGTAACTCTCTATT
GTGTACATGAAGAGATAGAAGTACGAGACACCAAAGAAGCCTTAGACAAA
CTAGAGGAAGAACAAAACAAATGTCAGCAAAAAGCACAGCAAGCAGAGGC
GGCTGACAAAGGAAAGGTCAGCCAAAATTATCCTATAGTACAGAATCTCC
AAGGGCAAATGGTACACCAGCCCCTATCACCTAGAACTTTGAATGCATGG
GTGAAAGTAATAGAAGAGAAGGGTTTTAAACCCAGAGGTAATACCCATGTT
TTCAGCATTATCAGAAGGAGCCACCCACAAAGACTTAAACACCATGTAA
ATACAGTAGGGGGACATCAAGCAGCCATGCAAATGTTGAAAGATACCATC
AATGAGGAGGCTGCAGAATGGGATAGATTACATCCAGTCCATGCAGGGCC
TATTGCACCAGGCCAAATGAGAGAACCAAGGGGAAGTGATATAGCAGGAA
CAACTAGCAACCTTCAGGAACAAATAGCATGGATGACAGCTAACCCACCT
ATCCCAGTGGGAGAATTGTATAAAAGATGGATAATTCTGGGATTAAATAA
AATAGTAAGAATGTATAGCCCTGTCAGCATTTTGGACATAAAACAAGGGC
CAAAAGAACCCTTTAGAGACTATGTAGACCGGTTCTTTAAACTTTGAGA
GCTGAACAAGCTACACAAGATGTAAAAAATTGGATGACAGACACCTTGTT
GACCCAAAATGCGAACCCAGATTGTAAGACCATTTTAAGAGCATTAGGAC
CAGGGGCTACATTAGAAGAAATGATGACAGCATGCCAAGGAGTGGGAGGA
CCTAGCCACAAAGCAAGAGTGCTAGCTGAAGCAATGAGCCAAGTAAATCA
TCCAAACATAATGATGCAGAGAAACAATTTTAAAGGACCAAAAAGAATTG
TTAAATGCTTCAACTGTGGCAAGGAAGGGCACATAGCCAGAAATTGCAGG
GCACCTAGGAAAAGGGGCTGTTGGAAATGTGGAAAGGAAGGACACCAAAT
GAAAGACTGTACTGAAAGGCAGGCTAATTTTTTAGGGAAAATTTGGCCTT
CCCACAAGGGGAGGCCAGGGAATTTTCATCCAGAACAGGCTAGAGCCCACA
GCCCCACCAGCAGAGAGTTTCAGGTTTCGAGGAGACAACCCCCAGTCTGAA
GCAGGAGCCGAAGGAGAGGGGAACCACCCTTAACTTCCCTCAAATCACTCT
TTGGCAGCGACCCCTTGTCTCAATAAGAGTAGGGGGCCAGATAAAGGAGG
CTCTCTTAGACACAGGAGCAGATGATACAGTATTAGAAGAAATAAATTTG
CCAGGGAAATGGAAACCAAAAATGATAGGAGGAATTGGAGGCTTTATCAA
AGTAAGACAGTATGATCAAATATCTATAGAAATTTGTGGAAAAAAGGCTA
TAGGTACAGTATTAGTGGGACCTACACCTGTCAACATAATTGGAAGGAAT

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CTGTTGACTCAGCTTGGATGTACATTAAATTTTCCAATTAGTCCCATTTGA
AACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAGTTAAAC
AATGGCCATTGACAGAAGAGAAAATAAAAGCATTAAACAGCAATCTGTGAA
GACATGGAGAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATA
TAACACTCCAGTATTTGCCATAAAAAAGAAGGACAGTACAAAGTGGAGAA
AATTAGTAGATTTTCAGGGAACCTCAACAAGAGAACTCAAGACTTCTGGGAA
GTTCAACTAGGAATACCACACCCAGCAGGGTTAAAAAAGAAAAAATCAGT
GACAGTACTAGATGTGGGGGATGCATATTTTTTCAGTACCTTTAGATGAAG
GCTTCAGGAAATATACTGCGTTCACCATACTAGTATAAACAATGAAACA
CCAGGGATTAGATATCAATATAATGTGCTTCCACAGGGATGGAAAGGATC
ACCAGCAATATTCCAGAGTAGCATGACAAAAATCTTAGAGCCCTTTAGGA
TAAAAAATCCAGACATAGTTATCTATCAATATATGGATGACTTGTATGTA
GGATCTGACTTAGAATTAGGACAGCATAGAGCAAAAATAGAAGAGCTAAG
GGAACATTTATTAAAATGGGGACTTACCACACCAGACAAGAAACATCAAA
AAGAACCCCCATTTCTTTGGATGGGGTATGAACTCCATCCTGACAAATGG
ACAGTACAGCCTATAAAGCTGCCAGACAAGGAAAGCTGGACTGTCAATGA
TATACAAAAGTTAGTAGGAAAATTAAACTGGGCAAGTCAGATTTACCCAG
GGATTAAAGTAAGGCAACTCTGTAAACTCCTTAGGGGGGCCAAAGCACTA
ACAGACATAGTACCACTAACTGAAGAAGCAGAATTAGAATTGGCAGAGAA
CAGGGAAATTCTAAAAGAACCAGTACATGGAGTATATTATGACCCTGCTA
AAGAATTAATAGCTGAAATACAGAAGCAGGGGCATGACCAATGGACATAC
CAAATTTACCAAGAACCATTCAAAAATCTGAAAACAGGAAAGTATGCAAA
AATGAGGGCTGCCCATACCAATGATGTAAAGCAATTAACAGAGGCAGTGC
AAAAAATAGCTACAGAAAGCATAGTAATATGGGGAAAGACCCCTAAGTTT
AGATTACCCATCCAAAAAGAAACATGGGAGACATGGTGGACAGACTATTG
GCAGGCTACCTGGATTCCCTGAGTGGGAGTATGTTAATACTCCTCCCCTAG
TAAAATTATGGTACCAACTGGAAAAAGAACCCTAGTAGGAGTAGAACT
TTCTATGTAGATGGAGCAGCTAATAGGGAACTAAGTTAGGAAAAGCAGG
GTATGTTACTGACAGGGGAAGGCAGAAAGTCGTTTCCCTAACTGAAACAA
CAAATCAGAAGGCTGAGTTACAAGCAATTCAGTTAGCTTTGCAGGATTCA
GGATCAGAAGTAACATAGTAACAGACTCACAGTATGCATTAGGAATCAT
TCAAGCACAACCAGATAAGAGTGAATCAGGGTTAGTTAACCATAAATAG
AACAGTTAATAAACAAGGAAAGAATCTACCTGTCATGGGTACCAGCACAT
AAGGGAATTGGAGGGAATGAACAAGTGGACAAATTAGTAAGTAAGGGGAT
CAGGAAAGTGCTGTTTCTAGATGGAATAGAGAAGGCTCAAGAAGAGCATG
AAAGATATCACAACAATTGGAGAGCAATGGCTAGTGAGTTTAATCTGCCA
CCCATAGTAGCAAAAGAAATAGTAGCTAGCTGTGATAAATGTCAGTTAAA
AGGGGAAGCCATACATGGACAAGTAGACTGTAGTCCAGGGATATGGCAAT
TAGACTGCACACATTTAGAAGGAAAAACTATCTTGGTAGCAGTCCATGTA
GCCAGTGGCTACATAGAAGCAGAGGTCATTCCAGCAGAAACAGGACAAGA
AACAGCATACTATATACTAAAATTAGCAGGAAGATGGCCAGTCAAAATGA
TACATACAGACAATGGCAGTAATTTACCAGTGCTGCAGTTAAGGCAGCC
TGTTGGTGGGCGGGTATCCAACAGGAATTTGGAATTCCTTACAATCCCCA
AAGTCAGGGAGTAGTAGAATCCATGAATAAAGAATTAAAGAAAATCATAG
GGCAAGTAAGAGATCAAGCTGAGCACCTTAAGACAGCAGTACAAATGGCA
GTATTCATTCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGC
AGGGGAAAGAATAATAGACATGATAGCAACAGACATACAACTAAAGAAT
TACAAAAACAAATTATAAAAATTCAAAATTTTCGGGTTTATTACAGAGAC
AGCAGAGATCCTATTTGGAAAGGACCAGCCAACTACTCTGGAAAGGTGA
AGGGGCAGTAGTCATACAAGATAACAGTGACATAAAGGTAGTACCAAGAA
GGAAAGTAAAAATCATTAGGGACTATGGAAAACAGATGGCAGGTGCTGAT
TGTGTGGCAGGTAGACAGGATGAGGATTAGAACATGGCACAGTTTAGTAA
AGCACCACATGTATGTCTCAAGGAGAGCTAGTGGATGGTTCTACAGACCT
CATTATGCAAGTAGACATCCAAAAATAAGTTCAGAGGTACACATCCCATT

SUBSTITUTE SHEET (RULE 26)

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AGGGGAGGCTAGATTAGTAATAACAACATATTGGGGTTTGCAAACAGGAG
AAAGAGAGTGGCACTTAGGTAATGGAGCCTCCATAGAATGGAGAATGAGA
AAATATAGCACACAAATAGATCCTGGCCTGGCAGACCAGCTAATTCATAT
GCATTATTTTGATTGTTTTGCAGACTCTGCCATAAGAAAAGCCATATTAG
GACATATAGTTATCCCTAGGTGTGACTATCAAGCAGGACATAATAAGGTA
GGATCTCTTCAATACTTGGCACTGACAGCATTGATAAAACCAAAAAAGAG
AAAGCCACCTCTGCCTAGTGTTAAGAAATTAGTAGAGGATAGATGGAACA
ACCCCCAGAAGACCAGGGGCCGAGAGGGAACCATATAACGAGTGGACAC
TAGAACTCTTAGAGGAACTCAAGCAGGAAGCTGTCAGACACTTTCCTAGA
CCATGGCTCCATGCATTAGGACAACATATCTATGATACCTATGGGGATAC
TTGGACAGGAGTTGAAGCTATAATAAGAATACTTCAACAGTTACTGTTTA
CTCATTTTCAGAATTGGGTGCCAACATAGCAGAATAGGCATTCTGCGACAG
AGAAGAGCAAGAAATGGAGCCAGTAGACCCTAACCTAGAGCCCTGGAATC
ATCCAGGAAGTCAGCCCCAAACTCCTTGTAATAAGTGTTATTGTAAGCGA
TGCTGCTATCATTGTCTAGTTTGCTTTCAGACAAAAGGCTTAGGCATTTC
CCATGGCAGGAAGAAGCGGAGACAGCGACGAAGCGCTCCTCCAAGCAGTG
AGAATCATCAAAATCCTTTATCAAAGCAGTGAGTATTCAATAAGCATATG
TAATGTTTGATTTATATGCAAGAGTAGATTATAGAATAGGAGTAGGAGCA
TTGGCAATAGCACTAATCATAGCAATAATAGTGTGGACCATAGTATATAT
AGAATATAGGAAATTAGTAAGACAAAGAAAAATAGACCAGTTAATTAAAA
GAATTAGGGAAAGAGCAGAAGACAGTGGCAATGAGAGTGATGGGGATACA
GAGGAATTATCCACAATGGTGGATATGGAGCATGTTAGGCTTTTGGATGC
TAATGATTTGTAATGGGATGTGGGTACAGTCTACTATGGGGTACCTGTG
TGGAAGAAGCAAAAATACTCTATTTTGTGCATCAGATGCTAAAGCATA
TGAGAAAGAAGTGCATAATGTCTGGGCTACACATGCCTGTGTACCCACAG
ACCCAATCCACAAGAAATGGTTTTAAAAAATGTAACAGAAAATTTCAAC
ATGTGGAAAAATGACATGGTGGATCAGATGCATGAAGATGTAATTAGTTT
ATGGGATCAAAGCCTCAAGCCATGTGTAAAGTTGACCCCACTCTGTGTCA
CTCTAAACTGTACCAATGCTACTGCCAGCAATAGCAGTATAATAGAGGGA
ATGAAAAATTGCTCTTTCAATATAACCACAGAATTAAGAGATAAGAGAGA
GAAAAAGAATGCACTTTTTTTATAAACTTGATATAGTACAACCTAGATGGCA
ACTCTAGTCAGTATAGATTAATAAAATTGTAATACCTCAGTCATAACACAA
GCCTGTCCAAAGGTCTCTTTTGACCCAATTCCTATACATTATTGTGCTCC
AGCTGGTTATGCGATTCTAAAGTGTAATAATAAGACATTCACTGGAACAG
GACCGTGTAATAATGTCAGCACAGTACAATGTACACATGGAATTAAGCCA
GTGGTTTCAACTCAACTATTGTAAATGGTAGCCTAGCAGAAGGAGAGAT
AATAATTAGATCTGAAAATATAACAAACAATGTCAAAACAATAATAGTAC
ATCTCAATGAATCTGTAAAGATTGAGTGTACGAGACCCAATAATAAAACA
AGAACAAGTATAAGAATAGGACCAGGACAAGCATTTTTATGCAACAGGACA
AGTAATAGGAGACATAAGAGAAGCATATTGTAACATTAATGAAAGTAAAT
GGAATGAACTTTACAAAGGGTAAGTAAAAAATTAAAAGAATACTTCCCT
CATAAGAATATAACATTTCAACCATCCTCAGGAGGGGACCTAGAAATTAC
AACACATAGCTTTAATTGTGGAGGAGAATTTTTCTATTGCAATACATCAA
GCCTGTTTAATAGGACATATATGGCTAATAGTACAGATATGGCTAATAGT
ACAGAACTAACAGTACACGAACCATCACAATCCACTGCAGAATAAAACA
AATTATAAACATGTGGCAGGAGGTGGGACGAGCAATGTATGCCCCTCCCA
TTGCAGGAAACATAACATGTATATCAAATATCACAGGACTACTATTGACA
AGGGATGGAGGAAAAACAATACGGAGACATTCAGACCTGGAGGAGGAAA
TATGAAGGACAATTGGAGAAGTGAATTATATAAATATAAAGTGGTAGAAG
TTAAGCCATTAGGAGTAGCACCCACTAATGCAAGAAGGAGAGTGGTGGAG
AGAGAAAAAAGAGCAGTGGGAATGGGAGCTGTGTTTCTTGGGTTCTTGGG
AGCGGCAGGAAGCACTATGGGCGCAGCATCAATAACGCTGACGGTACAGG
CCAGACAATTATTGTCTGGTATAGTGCAACAGCAAAGCAATTTGCTGAAG
GCTATAGAGGCTCAACAGCATATGTTGAAACTCACGGTCTGGGGCATTA

SUBSTITUTE SHEET (RULE 26)

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ACAGCTCCAGGCAAGAGTCCTGGCCTTGGAAGATACCTAAAGGATCAAC
AGCTCCTAGGGATGTGGGGCTGCTCTGGAAAACATCTGCACCACTAAT
GTATATTGGAACCTCTAGTTGGAGTAATAAACTTATGGTGATATTTGGGA
TAACATGACCTGGATGCAGTGGGAGAGAGAAATTAGCAATTATACAGAAA
TAATATATGAATTGCTTGAAGAATCACAAAACCAGCAGGAAAAGAATGAA
CAAGATTTACTAGCATTGGACAGATGGAACAGTCTGTGGAATTGGTTTAA
CATAACAAATTGGCTGTGGTATATAAAAATATTCATAATGATAGTAGGAG
GCTTGATAGGTTTAAGAATAATTTTTGCTGTGCTTTCTTTAGTAAATAGA
GTTAGGCAGGGATACTCACCTCTGTCTGTCGTTGCAGACCCTTATCCCAAGCCC
GAGGGGACCAGACAGGCCCGGAGGAATCGAAGAAGAAGGTGGAGAGCAAG
ACAGAAACAGATCAACGCGATTAGTGAGCGGATTCTTAGCGCTTGTCTGG
GACGACCTGCGGAGCCTGTGCCTTTTCATCTACCAACCGATTGAGAGACTT
CATATTAATTGCAGCGAGAGCGGGGGAACCTTCTGGGACGCAGCAGTCTCA
AGGGACTACGGAGAGGATGGGAAGCCCTTAAGTATCTGGGAAGTCTTGTG
CAGTATTGGGGCCTGGAACATAAAAAGGAGTGCTATTAGTCTATTGGATAC
CCTAGCAATAGCAGTAGGTGAAGGAACAGATAGGATTCTAGAATTTGTAT
TAGGAATTTGTAGAGCTATCCGCAACATACCTACAAGAATAAGACAGGGC
TTTGAAACAGCTTTGCTATAAAAATGGGGGGCAAGTGGTCAAAAAGCAGTA
TAGTTGGATGGCCTGATGTAAGAGAAAGAATAAGAAGAACTGATCCAGCA
GCAGAGGGAGTAGGAGCAGCATCTCAAGACTTAGATAGACATGGGGCACT
TACAATTAACAACACAGCCGCCAATAATGCTGATTGTGCCTGGCTGGAGG
CACAAGAGGAGGAGGGAGAAGTAGGCTTTCCAGTCAGACCTCAGGTACCT
TTAAGACCAATGACTTATAAGGAAGCATTCGACCTCAGCTTCTTTTTTAA
AGAAAAGGGGGGACTGGAAGGGTTAGTTTACTCCAAGAAAAGACAAGAGA
TCCTTGATTTGTGGGTCTATAACACACAAGGCTTCTTCCCTGATTGGCAA
AACTACACACCGGGACCAGGGGTCAGATATCCACTGACCTTTGGATGGTG
CTTCAAGCTAGTGCCAGTCGATCCAGAGGAAGTAGAAGAGGCCAATAAAG
GAGAAAACAACCTGCTTACTACACCCTATGAGCCAGCATGGAATGGATGAT
GAGGACAGAGAAGTACTAAAGTGGAAGTTTGACAGTCAGCTAGCACGCAG
ACACATGGCCCGCGAGCTACATCCGGAGTGGTACAAAGACTGCTGACACA
GAAGGGACTTTCCGCTGGGACTTTCCACTGGGGCGTTCCAGGGGGAGTGG
TCTGGGCGGGACTGGGAGTGGCCAACCCTCAGATGCTGCATATAAGCAGC
TGCTTTTCGCCTGTACTGGGTCTCTCTAGGTAGACCAGATCTGAGCCCGG
GAGCTCTCTGACTACCTAGGGAACCCACTGCTTAAGCCTCAATAAAAGCT
TGCCTTGAGTGCTCTAAGTAGTGTGTGCCCCGTCTGTTGTGTGACTCTGGT
AACTAGAGATCCCTCAGACCCTTTGTGGTAGTGTGGAAAATCTCTAGCA

Fig. 14B (CH0505 gene sequences)

GAG

ATGGGTGCGAGAGCGTCAATATTAAGAGGGGGAAAATTAGATAAATGGGAAAGAATTAGGTTAAGGCCAG
GGGGAAAGAAATGCTATATGATAAAACACTTAGTATGGGCAAGCAGGGAGTTGGAAAGATTTGCACTTAA
TCCTGGCCTCTTAGAAACATCAGAAGGCTGTAGACAAATAATAAAGCAGCTACAACCATCTCTTCAGACA
GGAACAGAGGAACTTAGATCATTATATAACACAGTAGTAACTCTCTATTGTGTACATGAAGAGATAGAAG
TACGAGACACCAAAGAAGCCTTAGACAAACTAGAGGAAGAACAAAACAAATGTCAGCAAAAAGCACAGCA
AGCAGAGGCGGCTGACAAAGGAAAGGTCAGCCAAAATTATCCTATAGTACAGAATCTCCAAGGGCAAATG
GTACACCAGCCCCCTATCACCTAGAACTTTGAATGCATGGGTGAAAGTAATAGAAGAGAAGGGTTTTAACC
CAGAGGTAATACCCATGTTTTTCAGCATTATCAGAAGGAGCCACCCACAAAGACTTAAACACCATGTAAA
TACAGTAGGGGGACATCAAGCAGCCATGCAAATGTTGAAAGATACCATCAATGAGGAGGCTGCAGAATGG
GATAGATTACATCCAGTCCATGCAGGGCCTATTGCACCAGGCCAAATGAGAGAACCAAGGGGAAGTGATA
TAGCAGGAACAACCTAGCAACCTTCAGGAACAAATAGCATGGATGACAGCTAACCCACCTATCCCAGTGGG
AGAATTGTATAAAAGATGGATAATTCTGGGATTAAATAAAATAGTAAGAATGTATAGCCCTGTCAGCATT
TTGGACATAAAACAAGGGCCAAAAGAACCCTTTAGAGACTATGTAGACCGGTTCTTTAAAACTTTGAGAG
CTGAACAAGCTACACAAGATGTAAAAAATTGGATGACAGACACCTTGTTGACCCAAAATGCGAACCAG
TTGTAAGACCATTTTAAGAGCATTAGGACCAGGGGCTACATTAGAAGAAATGATGACAGCATGCCAAGGA
GTGGGAGGACCTAGCCACAAAGCAAGAGTGCTAGCTGAAGCAATGAGCCAAGTAAATCATCCAAACATAA
TGATGCAGAGAAACAATTTTAAAGGACCAAAAAGAATTGTTAAATGCTTCAACTGTGGCAAGGAAGGGCA
CATAGCCAGAAATTGCAGGGCACCTAGGAAAAGGGGCTGTTGGAAATGTGGAAAGGAAGGACACCAAATG
AAAGACTGTACTGAAAGGCAGGCTAATTTTTTTAGGGAAAATTTGGCCTTCCCACAAGGGGAGGCCAGGGA
ATTTTCATCCAGAACAGGCTAGAGCCCACAGCCCCACCAGCAGAGAGTTTCAGGTTTCGAGGAGACAACCCC
CAGTCTGAAGCAGGAGCCGAAGGAGAGGGAACCACCCTTAACCTTCCCTCAAATCACTCTTTGGCAGCGAC
CCCTTGCTCTCAATAA

Fig. 14B (cont.)

POL

TTTTTTAGGGAAAATTTGGCCTTCCCACAAGGGGAGGCCAGGGAATTTTCATCCAGAACAGGCTAGAGCCC
ACAGCCCCACCAGCAGAGAGTTTCAGGTTTCGAGGAGACAACCCCCAGTCTGAAGCAGGAGCCGAAGGAGA
GGGAACCACCCTTAACCTCCCTCAAATCACTCTTTGGCAGCGACCCCTTGTCTCAATAAGAGTAGGGGGC
CAGATAAAGGAGGCTCTCTTAGACACAGGAGCAGATGATACAGTATTAGAAGAAATAAATTTGCCAGGGA
AATGGAAACCAAAAATGATAGGAGGAATTGGAGGCTTTATCAAAGTAAGACAGTATGATCAAATATCTAT
AGAAATTTGTGGAAAAAAGGCTATAGGTACAGTATTAGTGGGACCTACACCTGTCAACATAATTGGAAGG
AATCTGTTGACTCAGCTTGGATGTACATTAAATTTTCCAATTAGTCCCATTGAAACTGTACCAGTAAAT
TAAAGCCAGGAATGGATGGCCCCAAAAGTTAAACAATGGCCATTGACAGAAGAGAAAATAAAAGCATTAAC
AGCAATCTGTGAAGACATGGAGAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATATAACACT
CCAGTATTTGCCATAAAAAAGAAGGACAGTACAAAGTGGAGAAAATTAGTAGATTTTCAGGGAACTCAACA
AGAGAACTCAAGACTTCTGGGAAGTTCAACTAGGAATACCACACCCAGCAGGGTTAAAAAAGAAAAAATC
AGTGACAGTACTAGATGTGGGGGATGCATATTTTTCAGTACCTTTAGATGAAGGCTTCAGGAAATATACT
GCGTTCACCATACCTAGTATAAACAATGAAACACCAGGGATTAGATATCAATATAATGTGCTTCCACAGG
GATGGAAAGGATCACCAGCAATATTCCAGAGTAGCATGACAAAAATCTTAGAGCCCTTTAGGATAAAAAA
TCCAGACATAGTTATCTATCAATATATGGATGACTTGTATGTAGGATCTGACTTAGAATTAGGACAGCAT
AGAGCAAAAATAGAAGAGCTAAGGGAACATTTATTAAATGGGGACTTACCACACCAGACAAGAAACATC
AAAAAGAACCCCCATTTCTTTGGATGGGGTATGAACTCCATCCTGACAAATGGACAGTACAGCCTATAAA
GCTGCCAGACAAGGAAAGCTGGACTGTCAATGATATACAAAAGTTAGTAGGAAAATTAACTGGGCAAGT
CAGATTTACCCAGGGATTAAAGTAAGGCAACTCTGTAACTCCTTAGGGGGGCCAAAGCACTAACAGACA
TAGTACCACTAACTGAAGAAGCAGAATTAGAATTGGCAGAGAACAGGGAAATTCTAAAAGAACCAGTACA
TGGAGTATATTATGACCCTGCTAAAGAATTAATAGCTGAAATACAGAAGCAGGGGCATGACCAATGGACA
TACCAAATTTACCAAGAACCATTCAAAAATCTGAAAACAGGAAAGTATGCAAAAATGAGGGCTGCCATA
CCAATGATGTAAAGCAATTAACAGAGGCAGTGCAAAAAATAGCTACAGAAAGCATAGTAATATGGGGAAA
GACCCCTAAGTTTAGATTACCCATCCAAAAAGAAACATGGGAGACATGGTGGACAGACTATTGGCAGGCT
ACCTGGATTCTGAGTGGGAGTATGTTAATACTCCTCCCCTAGTAAAATTATGGTACCAACTGGAAAAAG
AACCCATAGTAGGAGTAGAACTTTCTATGTAGATGGAGCAGCTAATAGGGAACTAAGTTAGGAAAAGC
AGGGTATGTTACTGACAGGGGAAGGCAGAAAGTCGTTTCCCTAACTGAAACAACAAATCAGAAGGCTGAG
TTACAAGCAATTCAGTTAGCTTTGTCAGGATTCAGGATCAGAAGTAAACATAGTAACAGACTCACAGTATG
CATTAGGAATCATTCAAGCACAAACCAGATAAGAGTGAATCAGGGTTAGTTAACCAAATAATAGAACAGTT
AATAACAAGGAAAGAATCTACCTGTCTATGGGTACCAGCACATAAGGGAATTGGAGGGAATGAACAAGTG
GACAAATTAGTAAGTAAGGGGATCAGGAAAGTGCTGTTTCTAGATGGAATAGAGAAGGCTCAAGAAGAGC
ATGAAAGATATCACAACAATTGGAGAGCAATGGCTAGTGAGTTTAATCTGCCACCCATAGTAGCAAAAGA
AATAGTAGCTAGCTGTGATAAATGTCAGTTAAAAGGGGAAGCCATACATGGACAAGTAGACTGTAGTCCA
GGGATATGGCAATTAGACTGCACACATTTAGAAGGAAAAACTATCTTGGTAGCAGTCCATGTAGCCAGTG
GCTACATAGAAGCAGAGGTCATTCCAGCAGAAACAGGACAAGAAACAGCATACTATATACTAAAATTAGC
AGGAAGATGGCCAGTCAAAATGATACATACAGACAATGGCAGTAATTTACCAGTGCTGCAGTTAAGGCA
GCCTGTTGGTGGGCGGGTATCCAACAGGAATTTGGAATTCCTTACAATCCCCAAAGTCAGGGAGTAGTAG
AATCCATGAATAAAGAATTAAAGAAAATCATAGGGCAAGTAAGAGATCAAGCTGAGCACCTTAAGACAGC
AGTACAAATGGCAGTATTCATTCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAA
AGAATAATAGACATGATAGCAACAGACATACAACTAAAGAATTACAAAAACAAATTATAAAAATTCAAA
ATTTTCGGGTTTATTACAGAGACAGCAGAGATCCTATTTGGAAAGGACCAGCCAACTACTCTGGAAAGG
TGAAGGGGCAGTAGTCATACAAGATAACAGTGACATAAAGGTAGTACCAAGAAGGAAAGTAAAAATCATT
AGGGACTATGGAAAACAGATGGCAGGTGCTGATTGTGTGGCAGGTAGACAGGATGAGGATTAG

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Fig. 14B (cont.)

VIF

ATGGAAAACAGATGGCAGGTGCTGATTGTGTGGCAGGTAGACAGGATGAGGATTAGAACATGGCACAGTT
TAGTAAAGCACACATGTATGTCTCAAGGAGAGCTAGTGGATGGTTCTACAGACCTCATTATGCAAGTAG
ACATCCAAAAATAAGTTCAGAGGTACACATCCCATTAGGGGAGGCTAGATTAGTAATAACAACATATTGG
GGTTTGCAAACAGGAGAAAGAGAGTGGCACTTAGGTAATGGAGCCTCCATAGAATGGAGAATGAGAAAAT
ATAGCACACAAATAGATCCTGGCCTGGCAGACCAGCTAATTCATATGCATTATTTTGATTGTTTTGCAGA
CTCTGCCATAAGAAAAGCCATATTAGGACATATAGTTATCCCTAGGTGTGACTATCAAGCAGGACATAAT
AAGGTAGGATCTCTTCAATACTTGGCACTGACAGCATTGATAAAACCAAAAAGAGAAAGCCACCTCTGC
CTAGTGTTAAGAAATTAGTAGAGGATAGATGGAACAACCCCCAGAAGACCAGGGGCCGCAGAGGGAACCA
TATAACGAGTGGACACTAG

VPR

ATGGAACAACCCCCAGAAGACCAGGGGCCGCAGAGGGAACCATATAACGAGTGGACACTAGAACTCTTAG
AGGAACTCAAGCAGGAAGCTGTCAGACACTTTCCTAGACCATGGCTCCATGCATTAGGACAACATATCTA
TGATACCTATGGGGATACTTGGACAGGAGTTGAAGCTATAATAAGAATACTTCAACAGTTACTGTTTACT
CATTTTCAGAATTGGGTGCCAACATAGCAGAATAGGCATTCTGCGACAGAGAAGAGCAAGAAATGGAGCCA
GTAGACCCTAA

TAT

ATGGAGCCAGTAGACCCTAACCTAGAGCCCTGGAATCATCCAGGAAGTCAGCCCCAAACTCCTTGTAATA
AGTGTTATTGTAAGCGATGCTGCTATCATTGTCTAGTTTGCTTTCAGACAAAAGGCTTAGGCATTTCCCA
TGGCAGGAAGAAGCGGAGACAGCGACGAAGCGCTCCTCCAAGCAGTGAGAATCATCAAATCCTTTATCA
AAGCAACCCTTATCCCAAGCCCGAGGGGACCAGACAGGCCCGGAGGAATCGAAGAAGAAGGTGGAGAGCA
AGACAGAAACAGATCAACGCGATTAG

REV

ATGGCAGGAAGAAGCGGAGACAGCGACGAAGCGCTCCTCCAAGCAGTGAGAATCATCAAATCCTTTATC
AAAGCAACCCTTATCCCAAGCCCGAGGGGACCAGACAGGCCCGGAGGAATCGAAGAAGAAGGTGGAGAGC
AAGACAGAAACAGATCAACGCGATTAGTGAGCGGATTCTTAGCGCTTGTCTGGGACGACCTGCGGAGCCT
GTGCCTTTTTCATCTACCACCGATTGAGAGACTTCATATTAATTGCAGCGAGAGCGGGGGAACCTTCTGGGA
CGCAGCAGTCTCAAGGGACTACGGAGAGGATGGGAAGCCCTTAAGTATCTGGGAAGTCTTGTGCAGTATT
GGGGCCTGGAACCTAAAAAGGAG

VPU

ATGTTTGATTTTATATGCAAGAGTAGATTATAGAATAGGAGTAGGAGCATTGGCAATAGCACTAATCATAG
CAATAATAGTGTGGACCATAGTATATATAGAATATAGGAAATTAGTAAGACAAAGAAAAATAGACCAGTT
AATTAAGAATTAGGGAAAGAGCAGAAGACAGTGGCAATGAGAGTGATGGGGATACAGAGGAATTATCC
ACAATGGTGGATATGGAGCATGTTAGGCTTTTGGATGCTAATGATTTGTAA

Fig. 14B (cont.)

ENV

ATGAGAGTGATGGGGATACAGAGGAATTATCCACAATGGTGGATATGGAGCATGTTAGGCTTTTGGATGC
TAATGATTTGTAATGGGATGTGGGTACAGTCTACTATGGGGTACCTGTGTGGAAAGAAGCAAAAACCTAC
TCTATTTTGTGCATCAGATGCTAAAGCATATGAGAAAGAAGTGCATAATGTCTGGGCTACACATGCCTGT
GTACCCACAGACCCCAATCCACAAGAAATGGTTTTTAAAAAATGTAACAGAAAATTTCAACATGTGGAAAA
ATGACATGGTGGATCAGATGCATGAAGATGTAATTAGTTTATGGGATCAAAGCCTCAAGCCATGTGTAAA
GTTGACCCCACTCTGTGTCACTCTAAACTGTACCAATGCTACTGCCAGCAATAGCAGTATAATAGAGGGA
ATGAAAAATTGCTCTTTCAATATAACCAACAGAATTAAGAGATAAGAGAGAGAAAAAGAATGCACTTTTTT
ATAAACTTGATATAGTACAACCTAGATGGCAACTCTAGTCAGTATAGATTAATAAATTGTAATACCTCAGT
CATAACACAAGCCTGTCCAAAGGTCTCTTTTGACCCAATTCCTATACATTATTGTGCTCCAGCTGGTTAT
GCGATTCTAAAGTGTAATAATAAGACATTCCTGGAACAGGACCGTGTAATAATGTCAGCACAGTACAAT
GTACACATGGAATTAAGCCAGTGGTTTTCAACTCAACTATTGTTAAATGGTAGCCTAGCAGAAGGAGAGAT
AATAATTAGATCTGAAAATATAACAAACAATGTCAAAACAATAATAGTACATCTCAATGAATCTGTAAAG
ATTGAGTGTACGAGACCCAATAATAAAACAAGAACAAGTATAAGAATAGGACCAGGACAAGCATTTTTATG
CAACAGGACAAGTAATAGGAGACATAAGAGAAGCATATTGTAACATTAATGAAAGTAAATGGAATGAAAC
TTTACAAAGGGTAAGTAAAAAATTAAAAGAATACTTCCCTCATAAGAATATAACATTTCAACCATCCTCA
GGAGGGGACCTAGAAATTACAACACATAGCTTTAATTGTGGAGGAGAATTTTTCTATTGCAATACATCAA
GCCTGTTTAATAGGACATATATGGCTAATAGTACAGATATGGCTAATAGTACAGAACTAACAGTACACG
AACCATCACAATCCACTGCAGAATAAAACAAATTATAAACATGTGGCAGGAGGTGGGACGAGCAATGTAT
GCCCCCTCCCATTCAGGAAACATAACATGTATATCAAATATCACAGGACTACTATTGACAAGGGATGGAG
GAAAAACAATACGGAGACATTCAGACCTGGAGGAGGAAATATGAAGGACAATTGGAGAAGTGAATTATA
TAAATATAAAGTGGTAGAAGTTAAGCCATTAGGAGTAGCACCCACTAATGCAAGAAGGAGAGTGGTGGAG
AGAGAAAAAAGAGCAGTGGGAATGGGAGCTGTGTTCTTGGGTTCTTGGGAGCGGCAGGAAGCACTATGG
GCGCAGCATCAATAACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAACAGCAAAGCAA
TTTGCTGAAGGCTATAGAGGCTCAACAGCATATGTTGAACTCACGGTCTGGGGCATTAACAGCTCCAG
GCAAGAGTCCTGGCCTTGGAAGATACCTAAAGGATCAACAGCTCCTAGGGATGTGGGGCTGCTCTGGAA
AACTCATCTGCACCACTAATGTATATTGGAACCTCTAGTTGGAGTAATAAACTTATGGTGATATTTGGGA
TAACATGACCTGGATGCAGTGGGAGAGAGAAATTAGCAATTATACAGAAATAATATATGAATTGCTTGAA
GAATCACAAAACCAGCAGGAAAAGAATGAACAAGATTTACTAGCATTGGACAGATGGAACAGTCTGTGGA
ATTGGTTTTAACATAACAAATTGGCTGTGGTATATAAAAATATTCATAATGATAGTAGGAGGCTTGATAGG
TTTAAGAATAATTTTTGCTGTGCTTTCTTTAGTAAATAGAGTTAGGCAGGGATACTCACCTCTGTCGTTG
CAGACCCTTATCCCAAGCCCGAGGGGACCAGACAGGCCCGGAGGAATCGAAGAAGAAGGTGGAGAGCAAG
ACAGAAACAGATCAACGCGATTAGTGAGCGGATTCTTAGCGCTTGTCTGGGACGACCTGCGGAGCCTGTG
CCTTTTCATCTACCACCGATTGAGAGACTTCATATTAATTGCAGCGAGAGCGGGGGAACCTTCTGGGACGC
AGCAGTCTCAAGGGACTACGGAGAGGATGGGAAGCCCTTAAGTATCTGGGAAGTCTTGTGCAGTATTGGG
GCCTGGAACATAAAAAGGAGTGCTATTAGTCTATTGGATACCCTAGCAATAGCAGTAGGTGAAGGAACAGA
TAGGATTCTAGAATTTGTATTAGGAATTTGTAGAGCTATCCGCAACATACCTACAAGAATAAGACAGGGC
TTTGAAACAGCTTTGCTATAA

Fig. 14B (cont.)

NEF

ATGGGGGGCAAGTGGTCAAAAAGCAGTATAGTTGGATGGCCTGATGTAAGAGAAAGAATAAGAAGAAGTGA
ATCCAGCAGCAGAGGGAGTAGGAGCAGCATCTCAAGACTTAGATAGACATGGGGCACTTACAATTAACAA
CACAGCCGCCAATAATGCTGATTGTGCCTGGCTGGAGGCACAAGAGGAGGAGGGAGAAGTAGGCTTTCCA
GTCAGACCTCAGGTACCTTTAAGACCAATGACTTATAAGGAAGCATTTCGACCTCAGCTTCTTTTTTAAAAG
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CACACAAGGCTTCTTCCCTGATTGGCAAAACTACACACCGGGACCAGGGGTCAGATATCCACTGACCTTT
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	Nucleotide position in the T/F sequence of Figure 14A
Gag	792 -> 2276
Pol	2078 -> 5080
Vif	5025->5603
Vpr	5543->5833
Tat1	5814->6028
Rev1	5953->6028
Vpu	6053->6313
Env	6231 -> 8771
Tat2	8334 -> 8424
Rev2	8334 -> 8629
Nef	8773 -> 9396

Fig. 15 (from page 30/306 to page 123/306)

(CH505 gp160 nucleic acid sequences)

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SUBSTITUTE SHEET (RULE 26)

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SUBSTITUTE SHEET (RULE 26)

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41/306

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48/306

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78/306

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SUBSTITUTE SHEET (RULE 26)

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CTATAA

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Fig. 16 (from page 124/306 to page 160/306)

(DNA sequences of 103 CH505Dgp120s)

The coding region of CH505 HIV-1 Env gene inserts are in upper case.

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SUBSTITUTE SHEET (RULE 26)

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Fig. 17 (from page 161/306 to page 194/306)

(CH505 gp160 amino acid sequences)

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163/306

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>w014.10

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164/306

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>w020.7

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>w020.8

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>w020.11

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AISLLDTLAI AVGEGTDRILEFVLGICRAIRNIPTRIRQGFETALLZ

>w020.24

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>w020.13

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168/306

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169/306

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171/306

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>w053.13

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>w078.25

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>w136.B27

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>w136.B29

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>w136.B20

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>w136.B3

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>w014.12

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 INCNTSVITQACPKVSFDPIPIHYCAPAGYAILKCNKTFGTGTCNNVSTVQCTHGIK
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LIPSPRGPDRPGGIEEEEGGEQDRNRSTRLVSGFLALVWDDLRLSLCLFIYHRLRDFILIAARAGELLGRSSLKGLRRGW
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Fig. 18 (from page 195/306 to page 213/306)

(Amino acid sequences of CH505 D8gp120 constructs)

>HV1300531_v2

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 VEREKE*

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 VEREKE*

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>HV1300536_v2

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NARERVVEREKE*

>HV1300567_v2

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NARERVVEREKE*

>HV1300568_v2

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QRVSKKLKEYFPHKNITFQPSSGGDLEITTHSFNCGGEFFYCNTSSLFNRTYMANSTDMANSTETNNTRPITIHCRIKQI
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NARERVVEREKE*

>HV1300569_v2

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TNARERVVEREKE*

>HV1300570_v2

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LLLNGSLAEGEIIIRSENITDNGKTIIVHLNESVKIECTRPNNKTRTSIRIGPGQAFYATGQVIGDIKEAYCNISESKWN
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>HV1300571_v2

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>HV1300572_v2

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>HV1300573_v2

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GVAPTNA RERVVEREKE*

>HV1300574_v2

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RERVVEREKE*

>HV1300575_v2

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NARERVVEREKE*

>HV1300577_v2

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>HV1300579_v2

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>HV1300580_v2

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NARERAVEREKE*

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>HV1300582

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>HV1300585

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>HV1300587

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NARERVVEREKE*

>HV1300588

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PLGVAPT NARERVVEREKE*

>HV1300589

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>HV1300590

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>HV1300591

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TNARERVVEREKE*

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>HV1300616

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DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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JUMBO APPLICATIONS/PATENTS

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