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(57) **Abrégé/Abstract:**

Antibody VRC01 represents a human immunoglobulin that neutralizes ~90% of diverse HIV-1 isolates. To understand how such broadly neutralizing HIV-1 antibodies develop and recognize the viral envelope, we used X-ray crystallography and 454 pyrosequencing to characterize additional antibodies from HIV-1-infected individuals. Crystal structures revealed a convergent mode of binding of different antibodies to the same CD4-binding-site epitope. Antibody recognition was achieved through the evolution of complementary contact domains that were generated in diverse ways. Phylogenetic analysis of expressed heavy and light chains determined by deep sequencing revealed a common pathway of antibody heavy chain maturation confined to IGHV1-2*02 lineage that could pair with different light chains. The maturation pathway inferred by antibodyomics reveals that diverse antibodies evolve to a highly affinity-matured state to recognize an invariant viral structure, providing insight into the development and evolution of broadly neutralizing HIV-1 immunity.



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(57) Abstract: Antibody VRC01 represents a human immunoglobulin that neutralizes ~90% of diverse HIV-1 isolates. To understand how such broadly neutralizing HIV-1 antibodies develop and recognize the viral envelope, we used X-ray crystallography and 454 pyrosequencing to characterize additional antibodies from HIV-1-infected individuals. Crystal structures revealed a convergent mode of binding of different antibodies to the same CD4-binding-site epitope. Antibody recognition was achieved through the evolution of complementary contact domains that were generated in diverse ways. Phylogenetic analysis of expressed heavy and light chains determined by deep sequencing revealed a common pathway of antibody heavy chain maturation confined to IGHV1-2*02 lineage that could pair with different light chains. The maturation pathway inferred by antibodyomics reveals that diverse antibodies evolve to a highly affinity-matured state to recognize an invariant viral structure, providing insight into the development and evolution of broadly neutralizing HIV-1 immunity.



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FOCUSED EVOLUTION OF HIV-1 NEUTRALIZING ANTIBODIES REVEALED BY CRYSTAL STRUCTURES AND DEEP SEQUENCING

This application claims priority from U.S. Provisional application No. 61/484,184, filed May 9, 2011, the entire content of which is incorporated herein by reference.

BACKGROUND

Antibody VRC01 represents a human immunoglobulin that neutralizes ~90% of diverse HIV-1 isolates. To understand how such broadly neutralizing HIV-1 antibodies develop and recognize the viral envelope, we used X-ray crystallography and 454 pyrosequencing to characterize additional antibodies from HIV-1-infected individuals. Crystal structures revealed a convergent mode of binding of different antibodies to the same CD4-binding-site epitope. Antibody recognition was achieved through the evolution of complementary contact domains that were generated in diverse ways. Phylogenetic analysis of expressed heavy and light chains determined by deep sequencing revealed a common pathway of antibody heavy chain maturation confined to IGHV1-2*02 lineage that could pair with different light chains. The maturation pathway inferred by antibodyomics reveals that diverse antibodies evolve to a highly affinity-matured state to recognize an invariant viral structure, providing insight into the development and evolution of broadly neutralizing HIV-1 immunity.

DETAILED DESCRIPTION

The present invention relates to HIV-1 neutralizing antibodies and to methods of using same therapeutically or prophylactically in a subject (e.g., a human subject). The invention results, at least in part, from the identification of broadly neutralizing antibodies against the CD4 binding site of HIV-1 (see Example that follows). Figure 1C includes heavy and light chain amino acid sequences of VRC-CH30, VRC-CH31 and VRC-CH-31. Figure 1G includes heavy and light chain gene sequences that include sequences encoding the amino acid sequences shown in Figure 1C. These antibodies have the characteristics of being heavily somatically mutated, short HCDR3 regions, and are derived from VH1-2 heavy chain family. They all broadly neutralize HIV-1. (See also Wu X et al, Science 329:856-61 (2010)).

The invention relates to antibodies that comprise a heavy and/or light chain as set forth in Figure 1C, or at least one or more CDR's of such chains. The invention also includes antibodies having the binding specificity of VRC-CH30, VRC-CH31 and VRC-CH-32. The invention further includes nucleic acid sequences encoding such amino acid sequences/antibodies. The invention also relates to prophylactic and therapeutic uses of such antibodies.

Antibodies that are suitable for use in the prophylactic/therapeutic methods of the invention include dimeric, trimeric and multimeric antibodies, bispecific antibodies, chimeric antibodies, human and humanized antibodies, recombinant and engineered antibodies, and antigen-binding fragments thereof (e.g., Fab', F(ab')₂ fragments). Also suitable are single domain antibodies, Fv, single chain Fv, linear antibodies, diabodies, etc. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art (see, for example, Kohler and Milstein, Nature 256:495 (1975), Kosbor et al, Immunol. Today 4:72 (1983), Cote et al, PNAS 80:2026 (1983), Morrison et al, PNAS 81:6851 (1984), Neuberger et al, Nature 312:604 (1984), Takeda et al, Nature 314:452 (1985), USP 4,946,778, EP 404,097, WO93/11161, Zapata et al, Prot. Eng. 8:1057 (1995) and Liao et al, J. Virol. Methods 158(1-2):171-179 (2009)).

Antibodies of the invention can be expressed in a system that produces them as IgG1 antibodies, the dominant type present in human plasma (Liao et al, J. Virol. Methods 158(1-2):171-179 (2009) and Smith et al, Nature Protocols 4(3)(Jan. 1):372-384 (2009)). IgG1 antibodies can be passed through the placenta to infants prior to birth and can also become available at mucosal surfaces active or passive transport. In addition to the IgG1 expression system, antibodies of the invention can be expressed as other isotypes, in particular, as an IgA1 or IgA2 antibody (Carayannopoulos et al, Proc. Natl. Sci. USA 91(8) (Aug 30):8348-8352 (1994)). Such antibodies can provide additional protection at mucosal surfaces.

The antibodies of the invention can be used, for example, in humans, in a variety of prophylactic/therapeutic regimens. For example, the antibodies can be used for pre exposure prophylaxis, post exposure prophylaxis (i.e., exposure following sex or, in babies, following nursing), and for the treatment of HIV-1 infected individuals. The antibodies can be used in passive immunotherapy strategies to prevent or treat HIV-1 during pregnancy. The antibodies can also be used to prevent or treat perinatally acquired / congenital HIV-1 in infants.

Antibodies of the invention also find use as adjunctive therapeutics in combination with other anti-HIV-1 therapies.

The antibodies, or antibody fragments, of the invention can be formulated using standard techniques. Advantageously, the antibody/fragment is present in a composition, for example, a sterile composition suitable for injection (e.g., subcutaneously or intramuscularly) or intravenous infusion, or by other parenteral means. The composition can also take the form of a cream or ointment suitable for administration to skin or a mucosal surface (e.g., in the context of a microbicide for the prevention of HIV-1 infection in a susceptible population). The optimum amount and route of administration can vary with the antibody/fragment, the patient and the effect sought. Optimum dosing strategies can be readily established by one skilled in the art.

The invention includes nucleic acid sequences encoding the antibodies and antibody fragments disclosed herein and vectors (for example, viral vectors such as adeno associated viral vectors) comprising same. Such nucleic acid constructs can be used to

express the antibodies against the CD4 binding site (e.g., VRC-CH30, VRC-CH31 and VRC-CH-32), e.g., in a subject. (See Johnson et al, Nature Medicine 15:901-6 (2009)).

* * *

All references and other information sources cited herein are incorporated in their entirety by reference.

EXAMPLE 1

HIV-1 exhibits extraordinary genetic diversity and has evolved multiple mechanisms of resistance to evade the humoral immune response (1-3). Despite these obstacles, 10-25% of HIV-1-infected individuals develop cross-reactive neutralizing antibodies after several years of infection (4-9). Elicitation of such antibodies could form the basis for an effective HIV-1 vaccine, and intense effort has focused on identifying responsible antibodies and delineating their characteristics. A variety of monoclonal antibodies (mAbs) have been isolated that recognize a range of epitopes on the functional HIV-1 viral spike, which is composed of three highly glycosylated gp120 exterior envelope glycoproteins and three transmembrane gp41 molecules. Some broadly neutralizing antibodies are directed against the membrane-proximal external region of gp41 (10, 11), but the majority recognize gp120. These include the quaternary structure-preferring antibodies PG9, PG16, and CH01-04 (12, 13), the glycan-reactive antibodies 2G12 and PGT121-144 (14, 15), and antibodies b12, HJ16 and VRC01-03, which are directed against the region of HIV-1 gp120 involved in initial contact with the CD4 receptor (16-19).

One unusual characteristic of all these gp120-reactive broadly neutralizing antibodies is a high level of somatic mutation. Antibodies typically accumulate 5-10% changes in variable domain-amino acid sequence during the affinity maturation process (20), but for these gp120-reactive antibodies, the degree of somatic mutation is markedly increased, ranging from ~15-20% for the quaternary structure-preferring antibodies (12) and antibody HG16 (17), to ~25% for antibody 2G12 (21, 22) and to ~30% for the CD4-binding-site antibodies, VRC01, VRC02, and VRC03 (18).

In the case of VRC01, the mature antibody accumulates almost 70 total changes in amino acid sequence during the maturation process. The mature VRC01 can neutralize ~90% of HIV-1 isolates at a geometric mean IC₅₀ of 0.3 µg/ml (18), and structural studies show that it achieves

this neutralization by precisely recognizing the initial site of CD4 attachment on HIV-1 gp120 (19). By contrast, the predicted unmutated germline ancestor of VRC01 has weak affinity for typical strains of gp120 (~mM) (19). Moreover, with only two unique VRC01-like antibodies identified in a single individual (donor 45), it has been unclear whether the VRC01 mode of recognition, genetic origin, and pathway of affinity maturation represent general features of the B-cell response to HIV-1. Here we isolate VRC01-like antibodies from two additional HIV-1-infected donors, determine their liganded-crystal structures with gp120, examine cross-donor complementation of heavy and light chain function, and use deep sequencing to analyze the repertoire, lineage, and maturation pathways of related antibody sequences in each of two donors. The analysis presented here focuses primarily on the heavy chain, although some analysis of the light chain is also undertaken. Definition of the structural convergence and maturation pathways by which VRC01-like antibodies achieve broad neutralization of HIV-1 provides a framework for understanding the development of these antibodies and for efforts to guide their induction.

Isolation of neutralizing antibodies from donors 74 and 0219 with a CD4-binding-site

probe. We previously used structure-guided resurfacing to alter the antigenic surfaces on HIV-1 gp120 while preserving the initial site of attachment to the CD4 receptor (18). With the resurfaced stabilized core 3 probe (RSC3), over 30% of the surface residues of core gp120 were altered and the conformation stabilized by the addition of interdomain-disulfide bonds and cavity-filling point mutations (18). We used RSC3 and a mutant version containing a single amino acid deletion in the CD4-binding loop (Δ RSC3) to interrogate a panel of 12 broadly neutralizing sera derived from the IAVI protocol G cohort of HIV-1 infected individuals (6, 23)

(Fig. 1A). A substantial fraction of neutralization of three sera was specifically blocked by RSC3 compared with Δ RSC3, indicating the presence of CD4-binding-site-directed neutralizing antibodies. RSC3-neutralization competition assays also confirmed the presence of CD4-binding-site antibodies in the previously characterized sera 0219, identified in the CHAVI 001 cohort (8) (Fig. 1A).

Peripheral blood mononuclear cells (PBMCs) from protocol G donor 74 (infected with A/D recombinant) and from CHAVI donor 0219 (infected with clade A) were used for antigen-specific B-cell sorting and antibody isolation. PBMCs were incubated with both RSC3 and Δ RSC3, each conjugated to a different fluorochrome, and flow cytometric analysis was used to identify and to sort individual IgG⁺ B cells reactive with RSC3 and not Δ RSC3. For donor 74 and 0219, respectively, a total of 0.13% and 0.15% of IgG⁺ B cells were identified (Figs. 1B and S1). The heavy and light chain immunoglobulin genes from individual B-cells were amplified and cloned into IgG1 expression vectors that reconstituted the full IgG (18, 24). From donor 74, two somatically related antibodies named VRC-PG04 and VRC-PG04b demonstrated strong binding to several versions of gp120 and to RSC3 but ~100-fold less binding to Δ RSC3 (Fig. S2 and Table S1). From donor 0219, three somatically related antibodies named VRC-CH30, 31, and 32 displayed a similar pattern of RSC3/ Δ RSC3 reactivity (Fig. S2 and Table S1). Sequence analysis of these two sets of unique antibodies (Fig. 1C and Table S2) revealed that they originated from the same inferred immunoglobulin heavy chain variable (IGHV) precursor gene allele IGHV1-2*02. Despite this similarity in heavy chain V- gene origin, the two unique antibody clones originated from different heavy chain J segment genes and contained different light chains. The light chains of the VRC-PG04 and 04b somatic variants originated from an IGKV3 allele while the VRC-CH30, 31 and 32 somatic variants derived from an IGKV1 allele.

Of note, all five antibodies contained unusually high mutation frequencies: VRC-PG04 and 04b displayed a VH gene mutation frequency of 30% relative to the germline IGHV1-2*02 allele, a level of affinity maturation similar to that previously observed with VRC01-03; the VRC-CH30, 31 and 32 antibodies were also highly affinity matured, with VH mutation frequency of 23-24%.

To define the reactivities of these new antibodies on gp120, we performed competition ELISAs with a panel of well-characterized mAbs. Binding by each of the new antibodies was competed by VRC01-03, by other CD4-binding-site antibodies and by CD4-Ig, but not by antibodies known to bind gp120 at other sites (Figs. 1D and S3). Despite similarities in gp120 reactivity and VH-genomic origin, sequence similarities of heavy and light chain gene regions did not readily account for their common mode of gp120 recognition (Fig. 1E). Finally, assessment of VRC-PG04 and VRC-CH31 neutralization on a panel of Env-pseudoviruses revealed their ability to potently neutralize a majority of diverse HIV-1 isolates (Fig. 1F and Table S3).

Structural definition of gp120 recognition by RSC3-identified antibodies from different donors: A remarkable convergence. To define the mode of gp120 recognition employed by donor 74-derived VRC-PG04, we crystallized its antigen-binding fragment (Fab) in complex with a gp120 core from the clade A/E recombinant 93TH057 that was previously crystallized with VRC01 (19). Diffraction data to 2.1 Å resolution were collected from orthorhombic crystals, and the structure solved by molecular replacement and refined to a crystallographic R-value of 19.8% (Fig. 2A and Tables S4 and S5). The structure of VRC-PG04 in complex with HIV-1 gp120 showed striking similarity with the previously determined complex with VRC01, despite different donor origins and only 50% amino acid identity in the heavy chain-variable

region (Fig. 2). When gp120s were superimposed, the resultant heavy chain positions of VRC-PG04 and VRC01 differed by a root-mean-square deviation (rmsd) of 2.1 Å in Cα-atoms, with even more precise alignment of the heavy chain second complementary determining (CDR H2) region (1.5 Å rmsd). Critical interactions such as the Asp368_{gp120} salt bridge to Arg71_{VRC01} were maintained in VRC-PG04 (Fig. 2B).

We also crystallized the gp120-Fab complex of donor 45-derived VRC03. VRC03 and VRC-PG04 share only 51% heavy chain-variable protein sequence identity, and the heavy chain of VRC03 contains an unusual insertion in the framework 3 region (18). Diffraction data to 1.9 Å resolution were collected from orthorhombic crystals, and the structure solved by molecular replacement and refined to a crystallographic R-value of 18.8% (Fig. 2 and Tables S4 and S6). VRC03 also showed recognition of gp120 that was strikingly similar to that of VRC-PG04 and VRC01, with pairwise rmsds in Cα-atoms of 2.4 Å and 1.9 Å. In particular, CDR H2 and CDR L3 regions showed similar recognition (pairwise Cα-rmsds ranged from 0.7 – 1.6 Å) (Fig. S4).

In general, the repertoire of possible immunoglobulin products is very large and highly similar modes of antibody recognition are expected to occur infrequently (25). We analyzed other families of HIV-1 specific antibodies that share a common IGVH-gene origin (26-29), including the CD4-induced antibodies, which often derive from a common VH1-69 allele. Analysis of the recognition of gp120 by these antibodies indicated substantial variation in their recognition, with angular difference in heavy chain recognition of over 90° (Table S7). We also analyzed other CD4-binding site antibodies that are also recognized well by the RSC3 probe, such as antibodies b12 and b13 (16, 30); these other RSC3-reactive antibodies also showed dramatic differences in heavy chain orientation (Table S8).

The remarkable convergence in recognition observed with VRC01, VRC03, and VRC-PG04 suggested a common mode of HIV-1 gp120 recognition, conserved between donors infected with a clade B (donor 45) and clade A/D (donor 74) strain of HIV-1. The precision required for this mode of recognition likely arises as a consequence of the multiple mechanisms of immune evasion that protect the site of CD4 attachment on HIV-1 gp120 (30). We analyzed paratope surface properties and found that the average energy of antibody hydrophobic interactions (Δ^iG) correlated with the convergence in antibody recognition ($P=0.0427$) (Fig. 3A) (31). Thus while precise H-bonding is required for this mode of recognition (Fig. 2C), the convergence in structure appears to optimize regions with hydrophobic interactions. Another important feature of this mode of recognition is its ability to focus precisely on the initial site of CD4 receptor attachment (19, 32). Indeed, the breadth of HIV-1 neutralization among CD4-binding-site ligands correlated with targeting onto this site ($P=0.0405$) (Fig. 3B).

This convergence in epitope recognition is accompanied by a divergence in antibody sequence identity (Figs. 1C, 1E and 3C). All eight antibodies isolated by RSC3 binding utilize the germline IGHV1-2*02 and accrue 70-90 nucleotide changes. Despite the similarity in mature antibody recognition, only 2 residues from the germline IGHV1-2*02 allele change to the same amino acids (Fig. 1C). Both of these changes occur at a hydrophobic contact in the critical CDR H2 region (Gly₅₆Thr $\rightarrow$ Ala₅₆Val). The light chains for donors 45 and 74 antibodies arise from either IGV κ 3-11*01 or IGV κ 3-20*01, while the light chains of donor 0219 antibodies are derived from IGV κ 1-33*01. For these light chains, no maturational changes are identical. Despite this diversity in maturation, comparison of the VRC01, VRC03, and VRC-PG04 paratopes shows that many of these changes are of conserved chemical character (Fig. 3C); a hydrophobic patch in the CDR L3, for example, is preserved. These observations suggest that

divergent amino acid changes among VRC01-like antibodies nevertheless afford convergent recognition when guided by affinity maturation.

Functional complementation of heavy and light chains among VRC01-like antibodies.

While the identification and sorting of antigen-specific B cells with resurfaced probes has resulted in the isolation of several broadly neutralizing antibodies, genomic analysis of B-cell cDNA libraries provide substantially greater sequence complexity. These sequences specify the functional antibodyome, the repertoire of expressed antibody heavy and light chain sequences in each individual. High-throughput sequencing methods provide heavy chain and light chain sequences, but do not retain information about their pairings. For VRC01-like antibodies, the structural convergence revealed by the crystallographic analysis indicated a potential solution: different heavy and light chains might achieve functional complementation within this antibody family.

Heavy and light chain chimeras of VRC01, VRC03, VRC-PG04 and VRC-CH31 were produced by transient transfection (Table S9) and tested for HIV-1 neutralization (Table S10). VRC01 (donor 45) and VRC-PG04 (donor 74) light chains were functionally compatible with VRC01, VRC03 and VRC-PG04 heavy chains, though the VRC03 light chain was compatible only with the VRC03 heavy chain (Fig. 4A and Table S10). Similarly, despite ~50% differences in sequence identity (Fig. 1E), the VRC-CH31 (donor 0219) heavy and light chains were able to functionally complement most of the other antibodies (Fig. 4A and Table S10).

Identification of VRC01-like antibodies by deep sequencing of donors 45 and 74. To study the antibody repertoire in these individuals, we performed deep sequencing of cDNA from donor

45 PBMC (33). Because the variable regions of heavy and light chains are roughly 400 nucleotides in length, 454 pyrosequencing methods, which allow read lengths of 500 nucleotides, were used for deep sequencing. We first assessed heavy chain sequences from a 2008 PBMC sample from donor 45, the same time point from which antibodies VRC01, VRC02, and VRC03 were isolated by RSC3-probing of the memory B-cell population (18). mRNA from 5 million PBMC was used as the template for PCR to preferentially amplify the IgG and IgM genes from the IGHV1 family. 454 pyrosequencing provided 221,104 sequences of which 33,386 encoded heavy chain variable domains that encompassed the entire V(D)J region (Appendix 1).

To categorize the donor 45-heavy chain sequence information, we chose characteristics particular to the heavy chains of VRC01 and VRC03 as filters: (i) sequence identity, (ii) IGHV gene allele origin, and (iii) sequence divergence from the germline IGHV-gene as a result of affinity maturation (Fig. 4B). Specifically, we divided sequences into IGHV1-2*02 allelic origin (4597 sequences) and non-IGHV1-2*02 origin (28,789 sequences), and analyzed divergence from inferred germline genes, and sequence identity to the template antibodies VRC01 and VRC03 (Fig. 4B). Interestingly, no sequence of higher than 75% identity to the VRC01 or VRC02 heavy chain was found, although 109 sequences of greater than 90% sequence identity to VRC03 were found and all were of IGHV1-2*02 origin (Figs. 4B and S6). These sequences formed a well segregated cluster on a contour plot. To assess biological function, chimeric antibodies were made by pairing each of the two heavy chain sequences from the 454 sequence set with the VRC03 light chain. In both cases, potent neutralization was observed, with neutralization similar to the original VRC03 antibody (Fig. 4E and Table S15) (34).

A similar heavy chain-deep sequencing analysis was performed with donor 74 PBMC from the same 2008 time point from which VRC-PG04 and VRC-PG04b were isolated. In the

initial analysis, despite obtaining 263,764 sequences of which 85,851 encompassed the full V(D)J regions of the heavy chain, no sequences of greater than 75% identity to VRC-PG04 were found (Fig. S8 and Appendix 4). Because the number of unique heavy chain mRNAs present in the PBMC sample was likely much larger than the number of unique sequences obtained in the initial analysis, we repeated the deep sequencing of this sample with an increased number of 454 pyrosequencing reads and with protocols that optimized read length. In this analysis, 110,386 sequences of IGHV1-2*02 origin and 606,047 sequences of non-IGHV1-2*02-origin were found to encompassed the V(D)J region of the heavy chain, a 10-fold increase in sequencing depth. Among these sequences, 4920 displayed greater than 75% identity to VRC-PG04 (Fig. 4B and Appendix 2). Heavy chain sequences of the IGHV1-2*02 allelic origin segregated into several clusters, one at ~25% divergence and ~85% identity to the VRC-PG04 heavy chain, and several at 25-35% divergence and 65%, 85%, and 95% identity to VRC-PG04 (Fig. 4B).

To assess the biological function of these numerous 454-identified heavy chain sequences, we selected representative sequences from the quadrant defined by high divergence (16-38%) and high sequence similarity (60-100%) to VRC-PG04 (Fig. S9). A total of 63 sequences were synthesized and expressed with the VRC-PG04 light chain (Table S14). Remarkably, many of these antibodies displayed potent HIV-1 neutralization (35), confirming that these were functional VRC-PG04-like heavy chains (Fig. 4E and Table S15).

We next performed a similar analysis of the antibody light chain. Because VRC01-03 and VRC-PG04 derive from IGκV3 alleles, we used primers designed to amplify the IGκV3 gene family. We chose a donor 45 2001 time point to maximize the likelihood of obtaining light chain sequences capable of functional complementation (36). A total of 305,475 sequences were determined of which 87,658 sequences encompassed the V-J region of the light chain (Appendix

3). To classify the donor 45-light chain sequences into useful subsets, we again chose biologically specific characteristics: A distinctive 2-amino acid deletion in the first complementary-determining region and high affinity maturation (17% and 19% for VRC01 and VRC-PG04, respectively). Two such sequences with ~90% sequence identity to their VRC01 and VRC03 light chains, respectively, were identified (Fig. 4C). We assessed their biological function after synthesis in combination with the VRC01, VRC03, and VRC-PG04 heavy chains (Table S16). When paired with their respective matching wild type heavy chain to produce a full IgG, both chimeric antibodies displayed neutralization similar to the wild type antibody (Fig. 4D and Table S15).

Maturation similarities of VRC01-like antibodies in different donors revealed by phylogenetic analysis. The structural convergence in gp120 recognition and the functional complementation between VRC01-like antibodies from different donors suggested similarities in their maturation processes. We therefore performed phylogenetic analysis to assess the evolutionary relationship among sequences derived from the same precursor germline gene. We hypothesized that if known VRC01-like sequences from one donor were added to the analysis of sequences of another donor, a genomic-rooted phylogenetic tree might reveal similarities in antibody maturation pathways. Specifically, with such an analysis, the exogenous sequences would be expected to interpose between branches in the dendrogram containing VRC01-like antibodies and branches containing non-VRC01-like antibodies from the original donor's antibodyome. We performed this analysis with heavy chains, as all of the probe-identified VRC01-like antibodies derived from the same heavy chain IGHV1-2*02 allele. We added the donor 74-derived VRC-PG04 and 4b and donor 0219-derived VRC-CH30, 31 and 32 heavy

chain sequences to the donor 45 antibodyome sequences of IGHV1-2*02 genomic origin and constructed a phylogenetic tree rooted by the predicted VRC01 unmutated germline ancestor (18). This analysis revealed that sequences of high identity to VRC03 clustered as a subtree of a common node that was also the parent to donor 74 and 0219 VRC01-like heavy chain sequences (Fig. 5A, left). When donor 45 neutralization was assessed according to this phylogenetic segregation, a *P*-value less than 0.0001 was observed indicating significant correlation between the phylogenetic segregation and predicted neutralization (Fig. S7).

We also assessed the donor 74-derived IGHV1-2*02 heavy chain sequences by including probe-identified VRC01-like antibodies from donor 45 and donor 0219 in the phylogenetic analysis. In the tree rooted by the predicted VRC-PG04 unmutated germline ancestor, 5047 sequences segregated within the donor 45 and 0219-identified subtree (Fig. 5A, right). This subtree included the actual VRC-PG04 and 04b heavy chain sequences, 4693 sequences of >85% identity to VRC-PG04, and several hundred sequences with identities as low as 68% to VRC-PG04. To test the functional activity of heavy chain sequences identified by this phylogenetic analysis, we first assessed the phylogenetic location of the 63 heavy chain sequences that were identified and expressed from the previously described identity/divergence grid (Fig. S9). To these 63 sequences, we added 7 additional sequences from the donor 74 phylogenetic tree to enhance coverage of the phylogenetically segregated sequences (Fig. S10). These sequences were also synthesized and expressed with the VRC-PG04 light chain (Table S14). Among these 70 synthesized heavy chain sequences, 27 did not express. Of the remaining 43 reconstituted antibodies, 22 were able to neutralize HIV-1 (Table S15). Remarkably, all of the neutralizing sequences segregated into the subtree identified by the exogenously added donor 45 and 0219 VRC01-like antibodies (*P*-value=0.0085) (Fig. 6D).

We also applied this phylogenetic-segregation method to the light chains antibodyome of donor 45. The light chains from donor 74 and 0219 did not segregate with known VRC01-like light chains from donor 45 (Fig. S11), likely because these three light chains do not arise from the same inferred germline sequences. This difference may also reflect the dissimilarities in focused maturation of the two chains (see Fig. 3A): in the heavy chain, focused maturation occurs in the CDR H2 region (encompassed solely within the 2*02 VH gene from which all VRC01-like heavy chains derive) and, in the light chain, selection pressures occur in the CDR L3 region (which is a product of different types of V-J recombination).

CDR H3-lineage analysis. The 35 heavy chain sequences that both segregated into the VRC01-neutralizing subtree and expressed when reconstituted with the VRC-PG04 light chain could be clustered into 9 CDR H3 classes (Fig. 6B), with sequences in each class containing no more than 5 nucleotide differences in CDR H3 from other sequences in the same class (Fig. S12). A detailed junction analysis of the V(D)J recombination origins of these classes suggested that 8 of the 9 classes arose by separate recombination events (Fig. S13); two of the classes (7 and 8) differed primarily by a single three residues insert/deletion, Arg-Tyr-Ser, and may have arisen from a single V(D)J recombination event (Fig. S13b). Three of these classes (CDR H3-1, 2, and 9) were represented only by non-neutralizing antibodies, three by a single neutralizing antibody (CDR H3-4, 5 and 6), and three by a mixtures of neutralizing and non-neutralizing antibodies (CDR H3-3, 7 and 8). While it was not clear if the non-neutralizing heavy chain sequences truly lacked neutralization function or if this phenotype was due to incompatibilities in light chain pairing, we chose to analyze CDR H3 classes only for those in which neutralization had been confirmed.

We further analyzed donor 74 IGHV1-2*02 heavy chain sequences to identify those with CDR H3 sequences identical to the CDR H3s in each of the neutralizing classes (Fig. 7). This analysis identified four clonal lineages (CDR H3-classes 3, 6, 7 and 8), with sequences that extended to 15% or less affinity maturation. CDR H3 class 7 included the probe-identified antibodies, VRC-PG04 and 04b. In each case, a steady accumulation of changes lead to increased neutralization activity, and changes at positions 48, 52, 58, 69, 74, 82 and 94 in the V gene, among others, appeared to be selected in several lineages (Fig. 7). Overall, more than 2000 unique sequences could be classified into these four CDR H3 lineages (Fig. 7). Although these CDR H3 lineages were inferred from a single timepoint they likely provide insight into the specific maturation pathway by which the heavy chain of a VRC01-like antibody evolves from an initial recombinant to a broadly neutralizing antibody.

J chain analysis and maturation complexities. In the heavy chains of VRC01-like sequences identified by phylogenetic analysis, a significant skewing of J chain usage was observed (Fig. 5A): in donor 45, over 87% of the phylogenetic-segregated sequences utilize the IGHJ1*01 allele, and in donor 74, 99% of the segregated sequences utilize the IGHJ2*01 allele. This preferential J chain usage does not appear to be a requirement for binding specificity; indeed, the use of the J1 allele in VRC01, the J2 allele in VRC-PG04, and the J4 allele in VRC-CH31 provide examples for the functional compatibility of at least three different IGHJ alleles in VRC01-like antibodies. In addition to preferential J chain usage, other complexities in the maturation process could be inferred from similarities in mature heavy chain genes and differences in CDR H3 sequence. In the absence of information on the natural pairing of heavy and light chains, the antibody maturation processes underlying these complexities is difficult to

infer. Nevertheless, the deep sequencing data, with thousands of CDR H3-defined maturation intermediates (Fig. 7), provide sufficient information to suggest that the maturation may involve heavy chain revision or other mechanisms of B cell diversification (37, 38).

Antibody genomics, HIV-1 immunity, and vaccine implications. Affinity maturation that focuses a developing antibody onto a conserved site of HIV-1 vulnerability provides a mechanism to achieve broad recognition of HIV-1 gp120. Such focused evolution may be common to broadly neutralizing antibodies that succeed in overcoming the immune evasion that protect HIV-1 gp120 from humoral recognition; the multiple layers of evasion may constrain or focus the development of nascent antibodies to particular pathways during maturation.

The structure-based genomics approach described here provides tools for understanding antibody maturation. We show how deep sequencing can be utilized to determine the repertoire of sequences that compose the light chain and heavy chain antibodyomes in HIV-1 infected individuals. These antibodyomes can then be interrogated for unusual properties in sequence, or in maturation, to identify antibodies for functional characterization. We demonstrate three means of sieving a large database of antibody sequences: 1) by identity to a known mAb sequence and by divergence from putative germline (identity/divergence-grid analysis), 2) by cross-donor phylogenetic analysis of maturation pathway relationships, and 3) by CDR H3-lineage analysis. An important aspect of our analyses was the functional characterization of selected sequences achieved through expression of and reconstitution with known VRC01-like heavy or light chains, although other means of pairing such as by frequency analysis (39) are possible. While neutralization has been assessed on less than 100 of the antibodyomics-derived heavy-light reconstituted antibodies, the thousands of identified sequences provide a large dataset for

analysis, which should enhance our understanding of the critical features of VRC01-like antibodies. For example, the correlation of sequence variation at particular positions with neutralization should provide insight into the allowed diversity and required elements of neutralization by this family of antibodies (Fig. S15).

The deep sequencing and structural bioinformatics methodologies presented here facilitate analysis of the human antibodyome (Fig. S16). This genomics technology allows interrogation of the antibody responses from infected donors, uninfected individuals or even vaccine recipients and has several implications. For example, a genomic rooted phylogenetic analysis of the VRC01 antibodyome may reveal a general maturation pathway for the production of VRC01-like antibodies. Indeed, cross-donor phylogenetic analysis (Fig. 5B) suggests that common maturation intermediates with 20-30 affinity maturation changes from the IGHV1-2*02 genomic precursor are found in different individuals. These intermediates give rise to mature, broadly neutralizing VRC01-like antibodies, which have about 70-90 changes from the IGHV1-2*02 precursor (Fig. 5). If modified gp120s with affinity to the maturation intermediates represented by the nodes of the phylogenetic tree were to stimulate the elicitation of these intermediates, then the analysis presented here can help guide the vaccine-induced elicitation of VRC01-like antibodies. Deep sequencing not only provides a means to identify such intermediates, but also a means to facilitate their detection. Overall, the application of genomic technologies to analysis of antibodies facilitates both highly sensitive feedback and an unprecedented opportunity to understand the response of the antibodyome to infection and vaccination.

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34. We also assessed 454-derived sequences for structural compatibility with the VRC01, VRC03, and VRC-PG04 gp120-complex crystal structures using a threading algorithm

which assessed structural compatibility using the DFIRE statistical potential (40). None of the ten sequences with optimal DFIRE scores (Table S11), nor those with high germline divergence of non-IGHV1-2*02 genomic origin (Table S12) gave neutralization when reconstituted with the VRC01 light chain (Figs. 4E and S7 and Table S13). Thus, sequence similarity, IGHV1-2*02 origin, and divergence all correlate with neutralization potential, but other factors such as predicted structural compatibility failed to identify VRC01-like antibodies.

35. Six of the reconstituted antibodies displayed a mean IC₅₀ of ~0.1 µg/ml, a level of potency similar to that observed with the original probe-identified VRC-PG04 antibodies.
36. 1. VRC03L does not complement well; 2. VRC01 and VRC02 H no longer present in 2008 plasmablasts; 3. VRC03 H is present in 2008; 4. VRC01-3 are in memory B-cell population; Results 1-4 suggests that VRC03 came after VRC01; we therefore choose a pre-2008 timepoint to maximize chances of obtaining light chains that allowed for functional complementation with known VRC01 heavy chains.
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44. X.W., T.Z., J. Z., G.J.N., M.R., L.S., P.D.K. and J.R.M. designed research; B.Z., C.W., X.C., M.L., K.M., S.O.D., S.P., S.D.S., W.S., L.W., Y.Y., Z.Y.Y., Z.Y., NISC and J.M. performed experiments, X.W. isolated and characterized VRC01-like antibodies by RSC3 probe, devised and prepared samples for 454 pyrosequencing and assisted with functional characterization, T.Z. determined and analyzed structures of VRC-PG04 and VRC03 with gp120 and assisted with functional characterization, J.Z. devised and carried out computational bioinformatics on the antibodyome, M.B., J.A.C, S.H.K, S.E.N., B.F.H. contributed donor 0219 materials, M.S., D.R.B., and W.C.K contributed PG materials including donor 74, and N.D.R. and M.C. contributed donor 45 materials; X.W., T.Z., J.Z, I.G., N.S.L., Z.Z., L.S., P.D.K., and J.R.M. analyzed the data, L.S., P.D.K. and J.R.M. wrote the first draft of the paper, on which all authors commented. We thank J. Almeida and D. Douek for protocols of PBMC cDNA preparation and for helpful discussions, H. Coleman, M. Park, B. Schmidt, and A. Young for 454 pyrosequencing at the NIH Intramural Sequencing Center (NISC), J. Stuckey for assistance with figures, T.

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Figure Legends

Figure 1. Identification and characterization of broadly neutralizing CD4-binding-site mAbs from HIV-1-infected donors, 74 and 0219. The RSC3 probe was used to identify five broadly neutralizing mAbs, all of which were inferred to derive from the IGVH1-2*02 allele and displayed a high levels of somatic mutation. **(A)** RSC3 analysis of serum. Twelve sera from the IAVI Protocol G cohort (donors 17-74) and one serum from the CHAVI 001 cohort (donor 0219) were analyzed for RSC3 reduction in serum neutralization on HIV-1 strains JR-FL, PVO.4, YU2 and ZA12.29. Blue bars show the mean serum reduction in neutralization IC_{50} resulting from RSC3 versus Δ RSC3 competition. Sera with greatest reduction were further analyzed on HIV-1 strains Q168.a2, RW020.2, Du156.12 and ZM109.4. Red bars show the mean reduction on eight viruses. **(B)** RSC3- and Δ RSC3-binding profile of IgG⁺ B cells from donors 74 and 0219. Gating and percentage of B cells of interest (RSC3⁺ Δ RSC3⁻) are indicated, with 40 and 26 sorted single B cells from donors 74 and 0219, respectively. Additional sorting details are shown in Fig. S1. **(C)** Protein sequences of heavy and light chain variable regions of mAbs VRC-PG04 and VRC-PG04b, isolated from donor 74, and mAbs VRC-CH30, VRC-CH31 and VRC-CH32, isolated from donor 0219. Sequences are aligned to putative germline ancestral genes and to previously identified broadly neutralizing antibodies VRC01 and VRC03. Framework regions (FR) and complementary-determining regions (CDRs) are based on Kabat nomenclature (41). **(D)** Competition ELISAs. The binding to YU2 gp120 by a single concentration of biotin-labeled VRC-PG04 or VRC-CH31 was assessed against increasing concentrations of competitive ligand. CD4-Ig is a fusion protein of the N-terminal two domains of CD4 with IgG1 Fc. **(E)** Amino acid sequence identities between VRC-PG04 or VRC-CH31 and other antibodies reactive with the CD4-binding site on gp120 (CD4bs) or with the CD4-induced co-receptor-binding site (CD4i).

(F) Neutralization dendrograms. VRC-PG04 and VRC-CH31 were tested against genetically diverse Env-pseudoviruses representing the major HIV-1 clades. Neighbor-joining dendrograms display the protein distance of gp160 sequences from 179 HIV-1 isolates tested against VRC-PG04 and a subset (52 isolates) tested against VRC-CH31. A scale bar denotes the distance corresponding to a 1% change in amino acid sequence. Dendrogram branches are colored by the neutralization potencies of VRC-PG04 and VRC-CH31 against each particular virus.

Figure 2. Structure of antibodies VRC-PG04 and VRC03 in complex with HIV-1 gp120.

Despite being elicited and maturing in different individuals, broadly neutralizing antibodies VRC-PG04 and VRC03 display remarkable similarities in recognition of HIV-1. (A) Overall structures. The liganded complex for the Fab of antibody VRC-PG04 from donor 74 and the HIV-1 gp120 envelope glycoprotein from isolate 93TH057 is depicted with polypeptide backbones in ribbon representation in the left image. The complex of Fab VRC03 from donor 45 is depicted in the right image, with surfaces of all variable domain residues that differ between VRC03 and VRC-PG04 colored according to their chemical characteristics. Although VRC-PG04 and VRC03 derive from the same inferred heavy chain V-gene, roughly 40% of their variable domain residues have been altered relative to each other during the maturation process. (B and C) Interaction close-ups. Critical interactions are shown between the CD4-binding loop of gp120 (purple) and the CDR H2 region of the broadly neutralizing mAbs, VRC03 and VRC-PG04 (reported here) and VRC01 (reported previously (19)), with hydrogen bonds depicted as dotted lines. The 1.9 and 2.1 Å resolution structures of VRC03 and VRC-PG04, respectively, were sufficient to define interfacial waters shown in (C), which were unclear in the 2.9 Å

structure of VRC01. The orientation shown in (C) is $\sim 180^\circ$ rotated about the vertical axis from the orientation shown in (B).

Figure 3. Focused evolution of VRC01-like antibodies. The maturational processes that facilitate the evolution of VRC01-like antibodies from low affinity unmutated antibodies to high affinity potent neutralizers involve divergence in antibody sequence and convergence in epitope recognition. **(A)** Antibody convergence. The gp120 portions of liganded complexes with VRC01, VRC03 and VRC-PG04 were superimposed to determine the average antibody per-residue C α deviation, and the per-residue hydrophobic interaction (Δ^iG) was calculated (42). These two quantities were found to correlate (P -value = 0.0427), with antibody residues containing strong hydrophobic interactions (e.g. at positions, 53, 55, 91 and 97) displaying high structural conservation. This correlation is visualized on VRC-PG04 in the left image, where the ribbon thickness is proportional to the corresponding per-residue C α deviation and the paratope surface is colored according to hydrophobicity, from white (low) to red (high); notably, red surface patches map to thin ribbons. **(B)** Epitope convergence. The HIV-1 gp120 surface involved with CD4 binding contains conformationally invariant regions (e.g. associated with the outer domain) and conformationally variable regions (e.g. associated with the bridging sheet). We previously hypothesized that the conformationally invariant outer domain-contact for CD4 represents a site of vulnerability (19). We analyzed the precision of CD4-binding-site ligand recognition (vertical axis) versus the IC₈₀ neutralization breadth (horizontal axis) and observed significant correlation ($R^2=0.6$, P -value=0.040). **(C)** Divergences in sequence and convergences in recognition. The development of VRC01-like antibodies involves a heavy chain derived from the IGHV1-2*02 allele and selected light chain V κ alleles. The far left image depicts ribbon representation model

of a putative germline antibody. Somatic hypermutation during the process of affinity maturation leads to a divergence in sequence, yet results in the convergent recognition of similar epitopes. Intersection of the epitope surfaces recognized by VRC01, VRC03 and VRC-PG04 (far right image), reveals a remarkable similarity to the site of vulnerability. The primary divergence of this intersection from the hypothesized site of vulnerability occurs in the region of HIV-1 gp120 recognized by the light chain of the VRC01-like antibodies. While the separate epitopes on gp120 do show differences in recognition surface, these primarily involve the bridging sheet region, which is likely to adopt a different conformation in the functional viral spike prior to engagement of CD4.

Figure 4. Deep sequencing of expressed heavy and light chains from donors 45 and 74. 454

pyrosequencing facilitates the determination of the repertoire of heavy and light chain sequences (the heavy and light chain antibodyomes). Heavy and light chain complementation, computational bioinformatics, and neutralization measurements on reconstituted chimeric antibodies provide functional assessment. (A) Heavy and light chain complementation. The neutralization profiles of VRC01 and VRC03 (donor 45), VRC-PG04 (donor 74), and VRC-CH31 (donor 0219) and their heavy and light chain chimeric swaps are depicted with 20-isolate neutralization dendrograms. Explicit neutralization IC_{50} s are provided in Table S10. (B) The repertoire of heavy chain sequences from donor 45 (2008 sample) and donor 74 (2008 sample). Heavy chain sequences are plotted as a function of sequence identity to the heavy chain of VRC01 (left), VRC03 (middle) and VRC-PG04 (right) and of sequence divergence from putative genomic VH-alleles: upper row plots show sequences of putative IGHV1-2*02 allelic origin; lower row plots show sequences from other allelic origins. Color coding indicates the number of

sequences. (C) Repertoire of expressed light chain sequences from donor 45 (2001 sample). Light chain sequences are plotted as a function of sequence identity to VRC01 (left) and VRC03 (right) light chains, and of sequence divergence from putative genomic V-gene alleles. Sequences with 2-residue deletions in the CDR L1 region (which is observed in VRC01 and VRC03) are shown as black dots. Two sequences, with 92.0% identity to VRC01 (sequence ID 181371) and with 90.3% identity to VRC03 (sequence ID 223454) are highlighted with red triangles. (D) Functional assessment of light chain sequences identified by deep sequencing. The neutralization profiles of sequence 181371 reconstituted with the VRC01 heavy chain (named gVRC-L1_{d45}) and of sequence 223454 reconstituted with the VRC03 heavy chain (named gVRC-L2_{d45}) are depicted with 20-isolate neutralization dendrograms; explicit neutralization IC₅₀s are shown provided in Table S15. (E) Functional assessment of heavy chain sequences identified by deep sequencing. Heavy chain sequences from donors 45 and 74 were synthesized and expressed with either the light chain of VRC01 or VRC03 (for donor 45) or the light chain of VRC-PG04 (for donor 74) and evaluated for neutralization. Neutralizing antibodies are shown as red stars and are labeled. Comprehensive expression and neutralization results are presented in Tables S14 and S15 (43). gVRC-H(n) refers to the heavy chains with confirmed neutralization when reconstituted with the light chain of VRC-PG04 (Tables S14 and S15).

Figure 5. Maturation similarities of VRC01-like antibodies in different donors revealed by phylogenetic analysis. The structural convergence in maturation of VRC01-like antibodies suggested similarities of their maturation processes; phylogenetic analysis revealed such similarities and allowed maturation intermediates to be inferred. (A) Neighbor-joining phylogenetic trees of heavy chain sequences from donor 45 (left) and donor 74 (right). The donor

45 tree is rooted by the putative reverted unmutated ancestor of the heavy chain of VRC01, and also includes specific neutralizing sequences from donor 74 and 0219 (shown in red). Similarly the donor 74 tree is rooted in the putative reverted unmutated ancestor of the heavy chain of VRC-PG04, and sequences donor 45 and 0219 are included in the phylogenetic analysis. Bars representing 0.1 changes per nucleotide sequence are shown. Insets show J chain assignments for all sequences within the neutralizing subtree identified by the exogenous donor sequences. **(B)** Phylogenetically inferred maturation intermediates. Backbone ribbon representations are shown for HIV-1 gp120 (red) and the heavy chain variable domains (green). Critical intermediates defined from the phylogenetic tree in (A) are labeled I₄₅, II₄₅, III₄₅, I₇₄ and II₇₄. The number of VH-gene mutations is provided (e.g. I₄₅: 23), and the location of these is highlighted in the surface representation and colored according to their chemistry.

Figure 6. Analysis of the heavy chain antibodyome of donor 74 and identification of heavy chains with HIV-1 neutralizing activity. Identity/diversity-grid analysis, cross-donor phylogenetic analysis, and CDR H3 analysis when coupled to functional characterization of selected heavy chain sequences, provides a means for identification of novel heavy chains with HIV-1 neutralizing activity. **(A)** Identity/diversity-grid analysis. The location of the 70 synthesized heavy chains from donor 74 is shown, including neutralizing (red stars) and non-neutralizing (black stars) sequences. **(B)** Cross-donor phylogenetic analysis and CDR H3 lineage analysis. A maximum-likelihood phylogenetic tree of the 70 synthesized heavy chain sequences is rooted in the putative reverted unmutated ancestor of VRC-PG04. The probe-identified VRC-PG and VRC-CH antibodies are shown in red text. Grid location and CDR H3 class is specified for neutralizing and non-neutralizing sequences. Within each CDR H3 class, all sequences with

identical CDR H3s are highlighted in orange in the far right grids (with the number of total sequences corresponding to each CDR H3 class shown). (C) Expression levels of selected heavy chains reconstituted with the light chain of VRC-PG04 versus breadth of neutralization. (D) Neutralization potency of reconstituted phylogenetically-predicted antibodies on seven HIV-1 isolates. (E) CDR H3 analysis of donor 74 heavy chain sequences. For each of the 110,386 sequences with derived from the IGHV1-2*02 allele, the CDR H3 was determined and its percent identity to that of the VRC-PG04 heavy chain was graphed. The sequences with high CDR H3 identity to VRC-PG04 reside in regions of high overall heavy chain sequence identity, even for sequences with a low divergence from IGHV1-2*02.

Figure 7. Maturation lineages of four unique VRC01-like heavy chains in donor 74. The CDR H3 sequence, a product of V(D)J gene recombination and N nucleotide addition and removal, provides a signature to trace the lineage of a particular B cell. (A) Lineage analysis of CDR H3 class 3. Grid positions are displayed for the 390 heavy chain sequences with a CDR H3 sequence identical to the identified CDR H3 class 3. These sequences cluster into an elongated family of sequences with moderate identity to VRC-PG04. Representative sequences ranging from low to high IGHV1-2*02 sequence divergence (representing low to high levels of affinity maturation) are shown as structural models of the heavy chain variable domain, with maturation changes highlighted in surface mode colored by chemistry as in Fig. 5B. Sequences of displayed structures are shown in Fig. S14. Overall neutralization breadth and potency for sequence ID 13826_2 was assessed on a 20-isolate HIV-1 panel, with individual neutralization results tabulated in Table S15. (B) Lineage analysis of CDR H3 class 6 was performed as described above. The sequence ID 10731_1 that was selected in the grid analysis and found to be

neutralizing is shown as a member of this family. (C) CDR H3 classes 7 and 8. Analysis of the CDR H3 of classes 7 and 8 suggest that these might be clonally related (Fig. S13b). Sequences from these related classes segregate in similar ways, suggestive of related maturational pathways.

SUPPORTING MATERIAL

Supplementary Materials and Methods

Human specimens. The sera and peripheral blood mononuclear cells (PBMCs) of donor 45 (*S1-2*) and donors from the international AIDS-vaccine initiative (IAVI) protocol G (*S3-4*), and donor 0219 from the center for HIV/AIDS vaccine immunology (CHAVI) 001 cohort (*S5-6*) have been described previously. Donor 45, from whom monoclonal antibodies (mAbs) VRC01, VRC02 and VRC03 were isolated (*S1*), was infected with an HIV-1 clade B virus. The IAVI protocol G donor 74, from whom mAbs VRC-PG04 and VRC-PG04b were isolated, was infected with a A/D recombinant virus. Donor 0219, from whom mAbs VRC-CH30, VRC-CH31 and VRC-CH32 were isolated, was infected with a clade A virus. These three donors were chronically infected and had not initiated antiretroviral treatment at the time of PBMC sampling. All human samples were collected with informed consent under clinical protocols approved by the appropriate institutional review board (IRB).

Protein expression and purification. Monomeric gp120s, gp120 with the CD4-binding site knockout mutation D368R (*S2, 7*), gp120 cores, RSC3 and Δ RSC3 (*S1*) were expressed by transient transfection of 293F cells as previously described (*S1*). Briefly, genes encoding the proteins of interest were each synthesized with a C-terminal His tag (GeneArt, Regensburg, Germany), and cloned into a mammalian CMV/R expression vector (*S8*). Proteins were produced by transient transfection using 293fectin (Invitrogen, Carlsbad, CA) in 293F cells (Invitrogen) maintained in serum-free free-style medium (Invitrogen). Culture supernatants were harvested 5 - 6 days after transfection, filtered through a 0.45 μ m filter, and concentrated with buffer-exchange into 500 mM NaCl, 50 mM Tris (pH 8.0). Proteins were purified by Co-NTA (cobalt-nitrilotriacetic acid) chromatography method using a HiTrap IMAC HP column (GE Healthcare, Piscataway, NJ). The peak fractions were collected, and further purified by gel-filtration using a HiLoad 16/60 Superdex 200 pg column (GE Healthcare). The fractions containing monomers of each protein were combined, concentrated and flash frozen at -80°C.

Antibodies, plasmids, antibody and protein expression and purification. Anti-gp120 mAb 2G12 was purchased from Polymun Scientific Inc. (Vienna, Austria). Anti-CD4bs mAbs b12, VRC01 and VRC03 were described (*S1, 9*). The mAb 17b, directed to the co-receptor region of

gp120, was provided by James Robinson (Tulane University). Other antibody sequences were synthesized and cloned into the CMV/R expression vector containing the constant regions of IgG1. Full-length IgGs were expressed from transient transfection of 293F cells, and purified by affinity chromatography using HiTrap Protein A HP Columns (GE Healthcare). The CD4-Ig plasmid construct was provided by Joseph Sodroski (Dana Farber Cancer Institute) and the fusion protein was expressed by transient transfection as described above.

Isolation of antigen-specific memory B cells by fluorescence activated cell sorting (FACS).

As described previously (*SI*), the Avi-tagged RSC3 and Δ RSC3 were expressed, purified, and biotinylated using the biotin ligase Bir A (Avidity, Denver, CO). Biotinylation of the RSC proteins was confirmed by ELISA. The proteins were then conjugated with the streptavidin-fluorochrome reagents, streptavidin-allophycocyanin (SA-APC) (Invitrogen) for RSC3 and streptavidin-phycoerythrin (SA-PE) (Sigma) for Δ RSC3. About 20 million donor PBMC were stained with RSC3-APC, Δ RSC3-PE, and an antibody cocktail consisting of anti-CD3-APC-Cy7 (BD Pharmingen), CD8-Qdot705 (VRC), CD19-Qdot585 (VRC), CD20-Pacific Blue (VRC), CD27-APC-AlexaFluor700 (Beckman Coulter), CD14-Qdot800 (VRC), IgG-FITC (BD Pharmingen), and IgM-PE-Cy5 (BD Pharmingen). In addition, aqua blue (Invitrogen) was used to exclude dead cells. The stained PBMC were washed with PBS, then analyzed and sorted using a modified 3-laser FACS Aria cell sorter (configuration in **fig. S1**) using the FACSDiva software (BD Biosciences). Single cells with the phenotype of CD3⁻, CD8⁻, aqua blue⁻, CD14⁻, CD19⁺, CD20⁺, IgG⁺, IgM⁻, RSC3⁺ and Δ RSC3⁻ were sorted into 96-well PCR plates containing 20 μ l of lysis buffer per well. The lysis buffer contained 0.5 μ l of RNase Out (Invitrogen), 5 μ l of 5x first strand buffer (Invitrogen), 1.25 μ l of 0.1M DTT (Invitrogen) and 0.0625 μ l of Igepal (Sigma). The PCR plates with sorted cells were stored at -80°C. The total content of the donor PBMC sample passing through the sorter was saved in FCS files for further analysis with FlowJo software (TreeStar, Cupertino, CA).

Single B-cell immunoglobulin gene amplification and cloning. As described previously (*SI*), the frozen plates with single B-cell RNA were thawed at room temperature, and the reverse-transcription was carried out by adding 3 μ l of random hexamers (Gene Link, Hawthorne, NY) at 150 ng/ μ l, 2 μ l of dNTP mix, each at 10 mM, and 1 μ l of SuperScript III (Invitrogen) into each

well. The thermocycle for reverse-transcription was 42°C for 10 min, 25°C for 10 min, 50°C for 60 min and 94°C for 5 min. The cDNA plates were stored at -20°C, and the IgH, Igκ and Igλ variable region genes were amplified independently by nested PCR starting from 5 μl of cDNA as template. All PCRs were performed in 96-well PCR plates in a total volume of 50 μl containing water, 5 μl of 10x buffer, 1 μl of dNTP mix, each at 10 mM, 1 μl of MgCl₂ at 25 mM (Qiagen, Valencia, CA) for 1st round PCR or 10 μl 5x Q-Solution (Qiagen) for 2nd round PCR, 1 μl of primer or primer mix (*S10*) for each direction at 25 μM, and 0.4 μl of HotStar Taq DNA polymerase (Qiagen). Each round of PCR was initiated at 94°C for 5 min, followed by 50 cycles of 94°C for 30 sec, 58°C for IgH and Igκ or 60°C for Igλ for 30 sec, and 72°C for 1 min, followed by 72°C for 10 min. The positive 2nd round PCR products were cherry-picked for direct sequencing with both forward and reverse PCR primers. PCR products that gave a productive IgH, Igκ or Igλ rearranged sequence were re-amplified from the 1st round PCR using custom primers containing unique restriction digest sites and subsequently cloned into the corresponding Igγ1, Igκ and Igλ expression vectors as previously described (*S10*). The full-length IgG1 was expressed by co-transfection of 293F cells with equal amount of the paired heavy and light chain plasmids, and purified using a recombinant protein-A column (GE Healthcare).

IgG gene family analysis. The IgG heavy and light chain nucleotide sequences of the variable region were analyzed with JoinSolver® (<http://Joinsolver.niaid.nih.gov>) (*S11*) and IMGT/V-Quest (http://www.imgt.org/IMGT_vquest/share/textes/) (*S12*). The VRC mAb V_K gene use was determined by homology to germline genes in the major 2p11.2 IGK locus (*S13*). The VRC mAb D gene use was determined by homology to genes in the major 14q32.33 IGH locus. A combination of consecutive matching length with a +1/-2.02 scoring algorithm in the context of the V to J distance was applied for determining IGHD alignments and VD and DJ junctions in mutated sequences. Immunoglobulin rearrangements were grouped into classes based upon the VDJ gene use, similarity of replacement and silent mutations and the CDR3 identity.

ELISA analyses. As previously described (*S1*), each antigen in PBS at 2 μg/ml was used to coat plates overnight at 4°C. Coated plates were blocked with B3T buffer (150 mM NaCl, 50 mM Tris-HCl, 1 mM EDTA, 3.3% fetal bovine serum, 2% bovine albumin, 0.07% Tween 20) for 1

hour at 37°C, followed by incubation with antibody serially diluted in B3T buffer for 1 hour at 37°C. Horseradish peroxidase (HRP)-conjugated goat anti-human IgG Fc antibody (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) at 1:10,000 was added for 1 hour at 37°C. All volumes were 100 µl/well except that 200 µl/well was used for blocking. Plates were washed between each step with 0.1% Tween 20 in PBS. Plates were developed using either 3,3',5,5'-tetramethylbenzidine (TMB) (Kirkegaard & Perry Laboratories) and read at 450 nm. For competitive ELISA analyses, plates were coated with 1 µg/ml of a sheep anti-gp120 C5 antibody, D7324 (Cliniqa Corp., Fallbrook, CA) or 10 µg/ml of *Galanthus nivalis* lectin (Sigma) to capture 2 µg/ml of purified YU2 gp120 or RSC3 respectively. After blocking, serial dilutions of the competitor antibodies or CD4-Ig were added to the captured gp120 or RSC3 in 50 µl of B3T buffer, followed by adding 50 µl of biotin-labeled antibody or CD4-Ig at fixed concentrations: 200 ng/ml of VRC-PG04 and 500 ng/ml of VRC-CH31 to bind to YU2 gp120 or RSC3, 150 ng/ml of CD4-Ig and 80 ng/ml of 17b to bind to YU2 gp120. The plates were incubated at 37°C for 1 hour, followed by incubation with 250 ng/ml of streptavidin-HRP (Sigma) at room temperature for 30 min, and developed with TMB as described above.

HIV-1 neutralization and protein competition assays. Neutralization was measured using single-round-of-infection HIV-1 Env-pseudoviruses and TZM-bl target cells, as described previously (S14-16). Neutralization curves were fit by nonlinear regression using a 5-parameter hill slope equation as previously described (S15). The 50% and 80% inhibitory concentrations (IC₅₀ and IC₈₀) were reported as the antibody concentrations required to inhibit infection by 50% and 80% respectively. Competition of serum or mAb neutralization (S1) was assessed by adding a fixed concentration (25 µg/ml) of the RSC3 or ΔRSC3 glycoprotein to serial dilutions of antibody for 15 min prior to the addition of virus. The resulting IC₅₀ values were compared to the control with mock PBS added. The neutralization blocking effect of the proteins was calculated as the percent reduction in the ID₅₀ (50% inhibitory dilution) value of the serum in the presence of protein compared to PBS.

Construction of the HIV-1 envelope sequence phylogenetic trees. HIV-1 gp160 protein sequences of the 180 isolates used in the neutralization assays were aligned using MUSCLE, for multiple sequence comparison by log-expectation (S17-18). The protein distance matrix was

calculated by “protdist” and the dendrogram was constructed using the neighbor-joining method (S19) by “Neighbor”. All analysis and the programs used were performed at the NIAID Biocluster (<https://niaid-biocluster.niaid.nih.gov/>). The tree was displayed with Dendroscope (S20).

Crystallization of the gp120:VRC-PG04 and gp120:VRC03 complexes. The same HIV-1 clade A/E 93TH057 Δ V123 gp120 that crystallized with VRC01 (S21) was used to form complexes with antibodies VRC03 and VRC-PG04 for crystallization trials. The gp120 was expressed, purified and deglycosylated as previously described (S21). The antigen-binding fragments (Fabs) of VRC-PG04 and VRC03 were generated by LyS-C (Roche) digestion of IgG1 (S21). The gp120: VRC-PG04 or gp120:VRC03 complexes were formed by mixing deglycosylated 93TH057 gp120 and antibody Fabs (1:1.2 molar ratio) at room temperature and purified by size exclusion chromatography (Hiload 26/60 Superdex S200 prep grade, GE Healthcare) with buffer containing 0.35 M NaCl, 2.5 mM Tris pH 7.0, 0.02% NaN₃. Fractions with gp120:antibody complexes were concentrated to ~10 mg/ml, flash frozen with liquid nitrogen before storing at -80°C and used for crystallization screening experiments.

Three commercially available screens, Hampton Crystal Screen (Hampton Research), Precipitant Synergy Screen (Emerald BioSystems), and Wizard Screen (Emerald BioSystems), were used for initial crystallization trials of the gp120:antibody complexes. Vapor-diffusion sitting drops were set up robotically by mixing 0.1 μ l of protein with an equal volume of precipitant solutions (Honeybee, DigiLab). Droplets were allowed to equilibrate at 20° C and imaged at scheduled times with RockImager (Formulatrix.). Robotic crystal hits were optimized manually using the hanging drop vapor-diffusion method. Crystals of diffraction-quality for the gp120:VRC03 complex were obtained at 9 % PEG 4000, 200 mM Li₂SO₄, 100 mM Tris/Cl⁻, pH 8.5. For the gp120:VRC-PG04 complex, best crystals were grown in 9.9% PEG 4000, 9.0 % isopropanol, 100 mM Li₂SO₄, 100 mM HEPES, pH 7.5.

X-ray data collection, structure determination and refinement for the gp120:VRC-PG04 and gp120:VRC03 complexes. Diffraction data of the gp120:VRC03 and gp120:VRC-PG04 crystals were collected under cryogenic conditions. Best cryo-protectant conditions were obtained

by screening several commonly used cryo-protectants as described previously (S21). X-ray diffraction data were collected at beam-line ID-22 (SER-CAT) at the Advanced Photon Source, Argonne National Laboratory, with 1.0000 Å radiation, processed and reduced with HKL2000 (S22). For the gp120:VRC-PG04 crystals, a 2.0 Å data set was collected using a cryoprotectant solution containing 18.0 % PEG 4000, 10.0 % isopropanol, 100 mM Li₂SO₄, 100 mM HEPES, pH 7.5, 12.5 % glycerol and 7.5 % 2R,3R-butanediol. For the gp120:VRC03 crystals, a 1.9 Å data set was collected using a cryoprotectant solution containing 15% PEG4000, 200 mM Li₂SO₄, 100 mM Tris/Cl⁻, pH 8.5 and 30% ethylene glycol.

The crystal structures of gp120:VRC-PG04 and gp120:VRC03 complexes were solved by molecular replacement using Phaser (S23) in the CCP4 Program Suite (S24). The gp120:VRC-PG04 crystal was in a P212121 space group with dimensions a=61.8, b=66.5, c=237.3, $\alpha=\beta=\gamma=90.0$. The gp120:VRC03 crystal also belonged to a space group P212121 with cell dimensions a=61.0, b=70.3, c=217.9, $\alpha=\beta=\gamma=90.0$. Both crystals contained only one molecule per asymmetric unit (**table S4**). The structure of 93TH057 gp120 in the previously solved VRC01 complex (PDB ID 3NGB) was used as an initial model to place gp120 in the complexes. With gp120 fixed in the search model, a variable domain of antibody Fab was then used to locate antibody VRC03 or VRC-PG04 in the complexes.

Further refinements were carried out with PHENIX (S25). Starting with torsion-angle simulated annealing with slow cooling, iterative manual model building was carried out on Xtalview (S26) and COOT (S27) with maps generated from combinations of standard positional, individual *B*-factor, TLS refinement algorithms and non-crystallographic symmetry (NCS) restraints. Ordered solvents were added during each macro cycle. Throughout the refinement processes, a cross validation (R_{free}) test set consisting of 5% of the data was used and hydrogens were included as riding model. Structure validations were performed periodically during the model building/refinement process with MolProbity (S28) and pdb-care (S29). X-ray crystallographic data and refinement statistics are summarized in **table S4**.

Numbering of amino acid residues in antibody. We follow the Kabat (S30) nomenclature for amino acid sequences in antibodies.

Protein structure analysis and graphical representations. GRASP (S31) and APBS (S32) were used in calculations of molecular surfaces, volumes, and electrostatic potentials. PISA (S33) was used to perform protein-protein interfaces analysis. CCP4 (S27) was used for structural alignments. All graphical representation with protein crystal structures were made with Pymol (S34).

Analysis of structural convergence vs. binding interactions. To evaluate antibody structural convergence, the gp120 molecules from the three complex structures (with VRC01, VRC03, and VRC-PG04) were aligned. Residue correspondence in the three antibodies was determined based on the resulting structural alignment (rather than a sequence alignment). Residues in a given antibody that were not structurally aligned to residues in the other two antibodies were discarded from further analysis. For each of the three pairs of structures, C α RMSD was computed for the six CDR regions, while C α deviation was computed for each residue. Structural convergence for each CDR was then evaluated based on the average of the three pairwise C α RMSDs for the given CDR. Structural convergence for the per-residue comparisons was evaluated based on the average of the three pairwise C α deviation values for each residue. Residue numbering was based on the VRC-PG04 structure.

Interface surface areas and hydrophobic interactions were computed using the PISA server (<http://www.sciencedirect.com/science/article/B6WK7-4NR18NH-1/2/4675d51c7fc094d424ffbcf323c0aa05>). CDR interface surface areas for each antibody were computed as the sum of the interface surface areas of the corresponding residues. The average of the interface surface areas for each paratope residue was computed over the three structures. The average of the solvation energy values Δ^iG for each paratope residue i (as obtained from the PISA Interface Residues Table) was also computed over the three structures. Residues with positive average PISA Δ^iG were deemed to participate in hydrophobic interactions and were included in the correlation analysis against the respective per-residue C α deviations.

Analysis of neutralization breadth vs. targeting precision. The CD4-defined initial site of vulnerability included the following gp120 residues (*S21*): 257, 279, 280, 281, 282, 283, 365, 366, 367, 368, 370, 371, 455, 456, 457, 458, 459, 460, 469, 472, 473, 474, 475, 476, 477. For each antibody, the interface surface areas on gp120 were determined using the PISA server. In each case, the interface surface area corresponding to the residues from the initial site of vulnerability was termed 'Inside', while the remaining interface surface area was termed 'Outside'. Targeting precision was defined as the function 'Inside - Outside'. The neutralization breadth of CD4-Ig and the different antibodies was determined using IC₈₀ values for Tier 2 viruses, as obtained from: (*S1*) (VRC01, VRC03, b12, and CD4-Ig), (*S35*) (b13 and F105), and the present study (VRC-PG04).

Sample preparation for 454 pyrosequencing. Briefly, mRNA was extracted from 20 million PBMC into 200 µl of elution buffer (Oligotex kit, Qiagen), then concentrated to 10-30 µl by centrifuging the buffer through a 30 kD micron filter (Millipore). The reverse-transcription was performed in one or multiple 35 µl-reactions, each composed of 13 µl of mRNA, 3 µl of oligo(dT)12-18 at 0.5 µg/µl (Invitrogen), 7 µl of 5x first strand buffer (Invitrogen), 3 µl of RNase Out (Invitrogen), 3 µl of 0.1M DTT (Invitrogen), 3 µl of dNTP mix, each at 10 mM, and 3 µl of SuperScript II (Invitrogen). The reactions were incubated at 42°C for 2 hours. The cDNAs from each sample were combined, cleaned up and eluted in 20 µl of elution buffer (NucleoSpin Extract II kit, Clontech). Therefore, 1 µl of the cDNA was equivalent of transcripts from 1 million PBMC. The immunoglobulin gene-specific PCRs were set up using 5 µl of the cDNA as template (equivalent of transcripts from 5 million PBMC), using the Platinum Taq DNA Polymerase High Fidelity system (Invitrogen) in a total volume of 50 µl. The reaction mix was composed of water, 5 µl of 10x buffer, 2 µl of dNTP mix, each at 10 mM, 2 µl of MgSO₄, 1 µl of each primer at 25 µM, and 1 µl of platinum Taq DNA polymerase high fidelity. The forward primers for VH1 gene amplification were 5'-L-VH1, 5'-ACAGGTGCCCCACTCCCAGGTGCAG 3'; 5'-L-VH1#2, 5'-GCAGCCACAGGTGCCCCACTCC3'; 5'-L-VH1-24, 5'-CAGCAGCTACAGG CACCCACGC3'; 5'-L-VH1-69, 5'-GGCAGCAGCTACAGGTGTCCAGTCC3'; the reverse primers were 3'-C_γ-CH1, 5'-GGGGGAAGACCGATGGGCCCTTGGTGG3', and 3'-C_μ-CH1, 5'-GGGAATTCTCACAGGAGACGA3'. The forward primer for VK3 amplification was 5'-L-

VK3, 5'CTCTTCCTCCTGCTACTCTGGCTCCCAG3'; the reverse primer was 3'CK494, 5'GTGCTGTCCTTGCTGTCCTGCT3'. The PCRs were initiated at 95°C for 2 min, followed by 25 cycles of 95°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min, followed by 72°C for 10 min. The PCR products at the expected size (450-500bp) were gel purified (Qiagen), followed by phenol/chloroform extraction.

454 library preparation. PCR products were quantified using Qubit (Life Technologies, Carlsbad, CA). Following end repair 454 adapters were added by ligation. Library concentrations were determined using the KAPA Biosystems qPCR system (Woburn, MA) with 454 standards provided in the KAPA system.

454 pyrosequencing. 454 pyrosequencing of the PCR products was performed on a GS FLX sequencing instrument (Roche-454 Life Sciences, Bradford, CT) using the manufacturer's suggested methods and reagents. Initial image collection was performed on the GS FLX instrument and subsequent signal processing, quality filtering, and generation of nucleotide sequence and quality scores were performed on an off-instrument linux cluster using 454 application software (version 2.5.3). The amplicon quality filtering parameters were adjusted based on the manufacturer's recommendations (Roche-454 Life Sciences Application Brief No. 001-2010). Quality scores were assigned to each nucleotide using methodologies incorporated into the 454 application software to convert flowgram intensity values to Phred-based quality scores and as described (S36). The quality of each run was assessed by analysis of internal control sequences included in the 454 sequencing reagents. Reports were generated for each region of the PicoTiterPlate (PTP) for both the internal controls and the samples.

Bioinformatics analysis of 454-pyrosequencing-determined antibodyomes. A general bioinformatics pipeline has been developed to process and analyze 454 pyrosequencing-determined antibodyomes. The information generated in each step of the process was used to characterize the basic features of antibodyomes as well as to identify potential neutralizing antibody sequences for functional validation. Specifically, each sequence read was (1) reformatted and labeled with a unique index number; (2) assigned to variable (V) gene family and allele using an in-house implementation of IgBLAST (<http://www.ncbi.nlm.nih.gov/igblast/>);

(3) compared with the germline V-gene and known VRC01-like antibodies using nucleotide sequences and a global alignment module implemented in CLUSTALW2 (S37); (4) subjected to a template-based error correction scheme where 454 homopolymer errors in V gene were detected and corrected based on the alignment to germline sequence; (5) translated to amino acid sequence, which was further compared with known VRC01-like antibodies; (6) filtered using characteristic sequence motifs in variable domain sequence such as QVQ (or other possible triplets) at the N-terminus, CAR (or other possible triplets) at the end of V region, WGXX at the end of CDR H3, and VSS (or other possible triplets) at the C-terminus of variable domain. As an optional step, the structural compatibility of a 454-pyrosequencing-derived heavy- or light-chain sequence with known VRC01-like antibody/gp120 complex structures can be evaluated by threading (S38-39).

Phylogenetic analysis of donor antibodyomes. Three phylogenetic analyses were performed for donor 45 and donor 74 2008 heavy-chain antibodyomes. The first analysis was performed on a small set of “representative” sequences selected from the IGHV1-2*02 family. The sequence selection was done by first dividing the full-length sequences into 50 bins with an increment of 0.7% germline divergence and then randomly selecting a sequence from each bin, resulting in 38 sequences for donor 45 and 50 for donor 74. After incorporating the inferred germline sequence of VRC03 or VRC-PG04 and matured VRC01-like mAb sequences into the data set, phylogenetic analysis was performed using maximum-likelihood (ML) method assuming a constant rate of mutation, as implemented in the *dnamlk* program of PHYLIP package (<http://evolution.genetics.washington.edu/phylip.html>). 1,000 bootstrapped sets were then generated using the *seqboot* program and the majority-rule consensus tree was calculated using the *consense* program. Bootstrap values of the key intermediate states shown in **Fig. 5** were extracted from the *consense* output. In the second analysis, “VRC01-like” antibody heavy-chain sequences in an antibodyome were obtained using an iterative screening procedure. Briefly, in each round the full-length sequences of IGHV1-2*02 origin were divided into subsets with each having no more than 5,000 sequences; a neighbor-joining (NJ) tree was constructed for each subset using the “*Phylogenetic trees*” option in CLUSTALW2 (S37); after rooted at the inferred germline of VRC01 (for donor 45) or VRC-PG04 (for donor 74) the sequences residing on the smallest branch that contains VRC01, VRC02, VRC03 and VRC-PG04 were extracted from the

NJ tree and deposited into a new data set for the next round of analysis. Using this approach, we obtained 109 VRC03-like sequences and 5,047 VRC-PG04-like sequences from donor 45 and 74 antibodyomes, respectively. From these two data sets, 45 and 1,889 non-redundant sequences were identified using the *blastclust* module in NCBI BLAST package (S40). Third, after error correction using VRC03 or VRC-PG04 as a template, the ancestral sequences of V region were inferred for the key intermediate states shown in **Fig. 5** from the maximum-likelihood (ML) trees of non-redundant VRC03-like or VRC-PG04 like sequences. The calculation was done using the *dnamlk* program of PHYLIP package.

Analysis of CDR H3 lineage. Due to the sequence variation, we adopted a template-based approach to CDR H3 identification for 454-pyrosequencing-determined heavy chain sequences. Specifically, a 454-derived heavy chain sequence was aligned to the VRC01 heavy chain sequence using CLUSTALW2 (S37); then the nucleotide sequences of two motifs that define the CDR H3 in VRC01 – CTR and WGXXG – were used as “anchors” to locate the CDR H3 region in the 454-derived heavy chain sequence. For sequences with long CDR H3s, gap insertion may occur in the two motif regions and cause ambiguities in the CDR H3 identification, which were dealt with by allowing a maximum of 10 gaps between two adjacent nucleotides in the motif recognition. Using this template-based approach, the CDR H3 sequence and length were calculated for all full-length sequences in the IGHV1-2*02 family. In the CDR H3 lineage analysis, the 35 expressed and experimentally tested heavy-chain sequences shown in **Fig. 6** were divided into 9 CDR H3 groups, allowing no more than 5-nucleotide difference between members within the group. For each lineage, the characteristic CDR H3 sequences were used to search for other sequences with the same CDR H3s from the IGHV1-2*02 family. The number of sequences in each CDR H3 lineage was listed in **Fig. 6**.

Analysis of J chain. 109 VRC03-like and 5,047 VRC-PG04-like heavy-chain sequences identified using iterative phylogenetic analysis were submitted to the SoDA2 (S41) server (<https://hippocrates.duhs.duke.edu/soda/GetInput.aspx>) for assignment of variable (V), diverse (D), and joining (J) germline genes and junction analysis. For 14 VRC03-like sequences with non-IGHJ1*01 assignment and 66 VRC-PG04-like sequences with non-IGHJ2*01 assignment, the J segment was manually alignment to IGHJ1*01 or IGHJ2*01 for comparison.

Statistical analysis. Statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software Inc.).

Table S1. ELISA binding profiles of VRC-PG04, VRC-PG04b, VRC-CH30, VRC-CH31 and VRC-CH32 compared to a panel of known CD4bs mAbs.

	YU2 gp120 based proteins*						HXB2 gp120 based proteins		Antigenically resurfaced proteins		
	gp120 WT	gp120 D368R	gp120 I420R	gp120 K121D	gp120 D368R/I420R	gp120 M475S/R476A	gp120 WT	stabilized core	RSC3	Δ RSC3 (Δ 371I)	Δ RSC3** (P363N Δ 371I)
VRC-PG04	++++ [#]	+++	++++	++++	++	++++	++++	++++	++++	++	++
VRC-PG04b	++++	+	++++	++++	+	+++	++++	++++	++++	++	-
VRC-CH30	+++	+	+++	+++	+	++	++	++++	+++	-	-
VRC-CH31	++++	++	+++	+++	++	+++	+++	++++	++++	+	-
VRC-CH32	+++	-	+++	+++	+	+++	+++	++++	+++	+	-
CD4-Ig	++++	-	++++	++++	-	-	+	++++	-	-	-
VRC01	++++	+++	++++	++++	+++	+++	++++	++++	++++	++	+
VRC02	++++	+++	++++	++++	+++	+++	++++	++++	++++	++	+
VRC03	+++	-	++	+++	-	-	+++	++++	++++	-	-
b12	++++	-	++++	++++	-	++	++++	++++	++++	-	-

*Mutant residue numbers are based on the HXB2 sequence.

** This is a double mutant of the Δ 371I deletion together with the P363N mutation, which adds an N-linked glycan on the β 15 strand near the CD4 binding loop.

[#]Binding was categorized based on the OD₄₅₀ values at the highest concentration of antibody tested (5 μ g/ml for mAbs and CD4-Ig) and the 50% effective concentration (EC₅₀) values as shown below:

++++ OD₄₅₀ $\geq$ 3.0 and EC₅₀ $\leq$ 0.1
 +++ OD₄₅₀ $\geq$ 3.0 and EC₅₀ $>$ 0.1
 ++ 1.0 $\leq$ OD₄₅₀ $<$ 3.0
 + 0.2 $\leq$ OD₄₅₀ $<$ 1.0
 - OD₄₅₀ $<$ 0.2

Table S2. Gene family analysis of mAbs VRC-PG04, VRC-PG04b, VRC-CH30, VRC-CH31 and VRC-CH32.

Heavy chain					
	IGHV	IGHD	IGHJ	CDR3 length	VH mutation frequency
VRC01	1-2*02	3-16*01	1*01	14	91/288 (32%)
VRC03	1-2*02	HD3 family	1*01	16	86/288 (30%)
VRC-PG04	1-2*02	5-12*01 [#]	2*01	16	86/288 (30%)
VRC-PG04b	1-2*02	5-12*01 [#]	2*01	16	85/288 (30%)
VRC-CH30	1-2*02	3-16*01	4*02	15	66/288 (23%)
VRC-CH31	1-2*02	3-16*01	4*02	15	68/288 (24%)
VRC-CH32	1-2*02	3-16*01	4*02	15	65/288 (23%)
Light chain					
	IGKV		IGKJ	CDR3 length	VK mutation frequency
VRC01	3-11*01		2*01	5	45/264 (17%)
VRC03	3-20*01		2*01	5	53/267 (20%)
VRC-PG04	3-20*01		5*01	5	51/267 (19%)
VRC-PG04b	3-20*01		5*01	5	50/267 (19%)
VRC-CH30	1-33*01		2*01	5	41/264 (16%)
VRC-CH31	1-33*01		2*01	5	40/264 (15%)
VRC-CH32	1-33*01		2*01	5	44/264 (17%)

[#] See detailed CDRH3 analysis in **fig. S13b**.

Table S3a: Summary of the breadth and potency of antibody neutralization against 180 HIV-1 Env-pseudoviruses

Clade	Measured by IC ₅₀ (µg/ml)							Measured by IC ₈₀ (µg/ml)						
	VRC-PG04	VRC-PG04b	VRC-CH30 [#]	VRC-CH31 [#]	VRC01 [#]	VRC03 [#]	CD4-Ig [#]	VRC-PG04	VRC-PG04b	VRC-CH30 [#]	VRC-CH31 [#]	VRC01 [#]	VRC03 [#]	CD4-Ig [#]
Total	Titer < 50	77%	69%	87%	85%	89%	93%	72%	62%	85%	79%	85%	43%	66%
(N = 179)	Titer < 1	65%	56%	79%	75%	75%	34%	41%	31%	62%	63%	47%	23%	11%
	Geometric mean*	0.211	0.225	0.084	0.068	0.270	1.8	0.771	0.850	0.446	0.259	0.838	0.929	5.2
A	Titer < 50	96%	92%	100%	94%	100%	89%	96%	92%	100%	88%	96%	58%	53%
(N = 27)	Titer < 1	93%	92%	94%	89%	96%	32%	78%	73%	83%	83%	74%	26%	11%
	Geometric mean*	0.092	0.062	0.049	0.040	0.136	2.6	0.399	0.294	0.229	0.091	0.430	0.781	5.6
B	Titer < 50	82%	77%	83%	83%	92%	93%	82%	72%	83%	83%	92%	74%	59%
(N = 39)	Titer < 1	72%	64%	67%	83%	82%	41%	46%	39%	33%	50%	49%	48%	22%
	Geometric mean*	0.215	0.193	0.140	0.094	0.254	1.2	0.879	0.979	1.3	0.484	0.902	0.746	2.3
C	Titer < 50	74%	65%	71%	71%	85%	94%	67%	56%	57%	71%	81%	39%	64%
(N = 54)	Titer < 1	65%	50%	71%	57%	67%	42%	33%	24%	57%	57%	42%	17%	8%
	Geometric mean*	0.251	0.360	0.105	0.062	0.330	1.3	0.848	1.1	0.316	0.497	0.988	1.5	5.8
D	Titer < 50	56%	50%	75%	75%	88%	100%	56%	38%	75%	75%	78%	13%	88%
(N = 9)	Titer < 1	33%	25%	75%	75%	56%	38%	11%	0%	50%	50%	33%	13%	25%
	Geometric mean*	0.549	0.992	0.344	0.247	0.643	1.4	2.8	4.7	1.3	0.755	1.7	0.190	2.8
CRF01_AE	Titer < 50	81%	80%	100%	100%	93%	87%	75%	67%	100%	100%	88%	27%	60%
(N = 16)	Titer < 1	69%	67%	78%	89%	75%	27%	38%	33%	78%	78%	25%	7%	0%
	Geometric mean*	0.215	0.234	0.067	0.058	0.459	4.7	1.1	0.824	0.511	0.483	1.4	5.7	13.4
CRF02_AG	Titer < 50	69%	56%	100%	100%	81%	100%	56%	50%	100%	100%	81%	20%	73%
(N = 16)	Titer < 1	50%	50%	100%	100%	62%	27%	31%	13%	100%	100%	44%	13%	13%
	Geometric mean*	0.263	0.382	0.050	0.023	0.228	1.8	0.580	1.7	0.205	0.107	0.815	0.462	5.5
Other	Titer < 50	67%	50%	50%	50%	78%	93%	55%	39%	50%	33%	72%	21%	79%
(N = 18)	Titer < 1	50%	28%	50%	33%	72%	21%	28%	11%	0%	17%	39%	21%	0%
	Geometric mean*	0.367	0.557	0.393	0.695	0.264	3.2	0.917	1.7	2.4	0.849	0.677	0.216	8.6

[#]VRC-CH30 and VRC-CH31 were tested on a subset of 52 viruses, including 18 clade A, 6 clade B, 7 clade C, 4 clade D, 9 CRF01_AE, 1 CRF02_AG, 5 other recombinant viruses, and 1 clade G. The VRC01, VRC03 and CD4-Ig data (*SI*) were included for comparison.

*Geometric means were calculated for viruses neutralized with an IC₅₀ (or IC₈₀) value < 50 µg/ml.

Table S3b: Antibody neutralization data against 28 HIV-1 clade A Env-pseudoviruses

Virus ID	Clade	Origin	Neutralization IC ₅₀ (µg/ml)							Neutralization IC ₈₀ (µg/ml)						
			VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig	VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig
0260.v5.c36	A	Tanzania	0.615	0.402			0.760	0.020	>50	2.2	1.2			2.0	0.070	>50
0330.v4.c3	A	Tanzania	0.155	0.090			0.070	>50	0.970	0.593	0.478			0.210	>50	4.7
0439.v5.c1	A	Tanzania	0.104	0.097			0.052			0.493	0.328			0.236		
3365.v2.c20	A	Tanzania	0.096	0.079	0.021	0.010	0.060	0.980	0.050	0.288	0.297	0.096	0.046	0.170	9.7	0.230
3415.v1.c1	A	Tanzania	0.204	0.086			0.060	0.020	12.6	0.801	0.439			0.150	0.080	>50
3718.v3.c11	A	Tanzania	0.057	0.041			1.2	>50	14.6	0.285	0.215			>50	>50	>50
398-F1_F6_20	A	Tanzania	0.048	0.061			0.270	0.490	11.7	0.259	0.247			0.740	2.0	>50
BB201.B42	A	Kenya	0.157	0.037	0.075	0.011	0.343			0.468	0.254	0.291	0.077	1.1		
BB539.2B13	A	Kenya	1.5	>50	0.295	>50	0.094			16.9	>50	3.4	>50	0.330		
BI369.9A	A	Kenya	0.052	0.016	0.020	0.009	0.149			0.254	0.117	0.119	0.044	0.657		
BS208.B1	A	Kenya	0.009	0.011	0.003	0.007	0.019	0.297	0.246	0.052	0.051	0.020	0.009	0.078	2.6	20.8
KER2008.12	A	Kenya	0.224	0.225	0.201	0.107	0.379	0.403	0.649	1.3	1.4	0.844	0.406	1.7	1.7	4.0
KER2018.11	A	Kenya	0.711	0.401	1.4	0.385	0.852	0.389	3.3	2.7	2.2	6.2	2.3	2.3	1.3	15.9
KNH1209.18	A	Kenya	0.062	0.052	0.130	0.022	0.087	45.0	6.0	0.220	0.215	0.508	0.099	0.296	>50	>50
MB201.A1	A	Kenya	0.034	0.036	0.052	0.015	0.237			0.214	0.190	0.182	0.067	0.478		
MB539.2B7	A	Kenya	0.304	0.606	0.231	0.156	0.544			0.956	3.0	0.821	0.448	1.5		
MI369.A5	A	Kenya	0.072	0.029	0.029	0.025	0.162			0.331	0.239	0.126	0.081	0.769		
MS208.A1	A	Kenya	0.480	0.355			0.101	>50	7.9	0.599	0.789			0.462	>50	40.9
Q168.a2	A	Kenya	0.050	0.048	0.064	0.025	0.115	3.4	11.6	0.190	0.161	0.248	0.102	0.362	27.8	>50
Q23.17	A	Kenya	0.048	0.044			0.085	0.065	12.7	0.133	0.119			0.261	0.202	>50
Q259.w6	A	Kenya	>50	>50	0.023	7.2	0.170	0.055	0.708	>50	>50	0.091	>50	0.543	0.178	2.8
Q461.e2	A	Kenya	0.271	0.268	0.366	0.146	0.492	>50	25.4	1.0	1.3	1.4	0.430	1.6	>50	>50
Q769.h5	A	Kenya	0.017	0.008	0.023	0.017	0.084	0.034	1.3	0.130	0.065	0.123	0.093	0.289	0.140	5.7
Q842.d12	A	Kenya	0.012	0.011	0.007	0.003	0.030	>50	>50	0.045	0.045	0.031	0.012	0.096	>50	>50
QH209.14M.A2	A	Kenya	0.037				0.024			0.247				0.078		
RW020.2	A	Rwanda	0.052	0.033	0.011	0.004	0.224	>50	11.7	0.266	0.205	0.059	0.027	0.883	>50	46.1
UG037.8	A	Uganda	0.023	0.013	0.020	0.004	0.079	12.1	0.134	0.119	0.083	0.093	0.054	0.313	>50	0.721
Breadth	N=27	Titer < 50	96%	92%	100%	94%	100%	68%	89%	96%	92%	100%	88%	96%	58%	53%
		Titer < 1	93%	92%	94%	89%	96%	53%	32%	78%	73%	83%	83%	74%	26%	11%
Geometric mean [#]			0.092	0.062	0.052	0.029	0.141	0.364	2.6	0.398	0.294	0.241	0.091	0.439	0.781	5.6

* Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.

Geometric means were calculated for neutralization sensitive viruses with an IC₅₀ (or IC₈₀) value < 50 µg/ml.

Table S3c: Antibody neutralization data against 39 HIV-1 clade B Env-pseudoviruses

Virus ID	Clade	Origin	Neutralization IC ₅₀ (µg/ml)					Neutralization IC ₈₀ (µg/ml)							
			VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig	VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03
3988.25	B	USA	0.362	0.102		0.220	2.5	49.4	1.7	0.932			1.2	12.0	>50
5768.04	B	USA	0.050	0.028		0.166	0.382	0.756	0.595	0.326			0.829	0.995	>50
6101.10	B	USA	0.151	0.115		0.111	0.094	2.7	0.828	0.720			0.315	0.184	5.3
6535.3	B	USA	1.4	3.6		0.539	0.438	2.5	4.4	25.6			2.7	2.4	16.3
7165.18	B	USA	>50	>50		>50	>50	2.9	>50	>50			>50	>50	33.0
89.6	B	USA	0.079	0.030	0.022	0.014	0.187	0.242	0.501	0.242	0.237	0.151	2.3	0.589	0.752
AC10.29	B	USA	>50	>50		2.2	>50	10.7	>50	>50			6.5	>50	>50
ADA	B	USA	0.412	0.210		0.424			1.4	1.1			1.4		
BaL.01	B	USA	0.116	0.102		0.102			0.601	>50			0.318		
BG1168.01	B	USA	0.792	1.1	1.6	0.653	0.276		2.4	9.3	6.0	2.5	1.5	>50	>50
BL01	B	USA	>50	>50	>50	>50	>50	13.4	>50	>50	>50	>50	>50	>50	>50
BR07	B	USA	0.543	0.475	0.442	0.298	1.2	3.4	5.3	8.2	8.4	5.8	5.2	12.8	0.625
BX08_16	B	USA	0.363	1.2				0.046	1.3	9.2			0.680		0.211
CAAN.A2	B	USA	2.7	1.7		8.3	>50	>50	5.9	3.4			2.8	47.6	>50
HO86.8	B	Peru	>50	>50		>50	>50	9.8	>50	>50			>50	>50	>50
HT593.1	B	Haiti	0.253	0.197	0.219	0.170	0.334	0.323	1.1	1.1	1.6	0.760	1.8	0.741	4.5
HXB2	B	USA	0.064	0.203		0.040			0.104	0.676			0.077		
JR-CSF	B	USA	0.138	0.489		0.093	0.093	0.186	0.621	>50			0.544	0.517	1.7
JR-FL	B	USA	0.104	0.413		0.031	0.009	0.247	0.740	13.7			0.093	0.025	0.967
MN.3	B	USA	>50	>50		0.033			>50	>50			0.070		
PVO.04	B	Italy	0.234	0.162		0.216	0.328	20.1	0.724	0.467			1.2	1.7	>50
QH0515.01	B	Trinidad	0.520			0.386	0.187	1.8	4.2				2.9	0.668	>50
QH0692.42	B	Trinidad	1.3	1.3		1.5	0.954	0.603	7.1	7.8			4.8	2.1	2.6
R2	B	USA	0.226			0.198	0.035	0.016	1.2				0.931	0.126	0.063
REJO.67	B	USA	0.020	0.027		0.062	0.059	1.2	0.059	0.052			0.251	0.196	11.5
RHPA.7	B	USA	0.048	<0.023		0.060	1.1	1.1	0.062	0.088			0.185	6.6	13.9
SC422.8	B	Trinidad	0.122	0.129		0.076	0.036	5.2	0.534	0.604			0.265	0.105	>50
SF162	B	USA	0.088	0.081		0.237			0.249	0.227			0.627		
SS1196.01	B	USA	0.138	0.110		0.276			0.356	0.301			0.650		
THRO.18	B	USA	>50	>50		2.3	>50	0.509	>50	>50			23.0	>50	2.5
TRJO.58	B	USA	0.072	0.094		0.083	0.043	22.1	0.180	0.278			0.207	0.098	>50
TRO.11	B	Italy	0.291	0.659		0.207	0.055	>50	0.781	3.4			0.832	0.342	>50
WITO.33	B	USA	1.2	>50		0.148	>50	2.2	29.9	>50			0.412	>50	13.2
YU2	B	USA	0.019	0.007	<0.016	0.126	0.037	0.102	0.103	0.058	0.184	<0.016	0.372	0.115	0.314
CNE10	B'	China	0.619	0.355		0.776			1.9	1.1			1.9		
CNE12	B'	China	0.268	>50		0.785			0.938	>50			2.2		
CNE14	B'	China	>50	>50		0.389			>50	>50			0.978		
CNE4	B'	China	0.618	0.386		0.871			1.9	1.4			2.4		
CNE57	B'	China	0.126	0.162		0.535			0.310	0.357			1.3		
Breadth	N=39	Titer < 50	82%	77%	83%	83%	92%	93%	82%	72%	83%	83%	92%	74%	59%
		Titer < 1	72%	64%	67%	83%	82%	41%	46%	39%	50%	50%	49%	48%	22%
Geometric mean [#]			0.215	0.193	0.140	0.094	0.254	1.2	0.879	0.979	1.3	0.484	0.902	0.746	2.3

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.

#Geometric means were calculated for neutralization sensitive viruses with an IC₅₀ (or IC₈₀) value < 50 µg/ml.

Table S3d: Antibody neutralization data against 54 HIV-1 clade C Env-pseudoviruses

Virus ID	Clade	Origin	Neutralization IC ₅₀ (µg/ml)							Neutralization IC ₈₀ (µg/ml)						
			VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig	VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig
286.36	C	Ethiopia	0.419	0.399			0.188	1.8	7.3				0.839	18.4	39.1	
288.38	C	Ethiopia	0.455	0.255			0.992	0.342	0.459				4.0	1.2	2.1	
0013095-2.11	C	India	0.196	0.146			0.110	0.800	0.300				0.330	2.9	1.3	
001428-2.42	C	India	0.011	0.009			0.020	0.020	0.490				0.060	0.020	2.1	
0077_V1_C16	C	India	>50	>50			1.0						3.7			
00836-2.5	C	India	>50	>50			0.128						0.520			
0921.v2.c14	C	India	0.227	0.609												
16055-2.3	C	India	>50	>50			0.080	0.110	1.3				0.260	1.9	11.6	
16845-2.22	C	India	>50	>50			2.8	48.5	0.210				12.7	>50	0.930	
16936-2.21	C	India	0.216	0.161			0.190	0.080	0.810				0.630	0.390	5.3	
25710-2.43	C	India	0.745	1.8			0.430	0.170	0.320				1.4	0.610	2.1	
25711-2.4	C	India	0.454	0.367			1.0	0.990	13.8				2.4	3.7	>50	
25925-2.22	C	India	0.359	0.909			0.530	0.210	2.6				1.6	1.0	20.2	
26191-2.48	C	India	0.256	0.267			0.190	>50	1.3				0.670	>50	10.3	
3168_V4_C10	C	India	0.190	0.158			0.131						0.279			
3637_V5_C3	C	India	>50	>50			4.1						11.0			
3873_V1_C24	C	India	0.203	0.642			0.954						0.326			
6322_V4_C1	C	India	>50	>50			>50						>50			
6471_V1_C16	C	India	>50	>50			>50						>50			
6631_V3_C10	C	India	>50	>50			>50						>50			
6644_V2_C33	C	India	0.398	>50			0.164						0.528			
6785_V5_C14	C	India	0.219	0.478			0.332						0.869			
6838_V1_C35	C	India	0.545	2.9												
96ZM651_02	C	Zambia	3.0	17.1			0.525						1.9			
BR025.9	C	Brazil	7.2	>50	0.463	1.1	0.115	>50	0.064				49.0	>50	3.6	
CAP210.E8	C	South Africa	>50	>50			>50	>50	1.5				>50	>50	8.3	
CAP244.D3	C	South Africa	0.541	0.286			0.428	47.1	2.6				2.7	>50	17.5	
CAP45.G3	C	South Africa	>50	>50			2.3	>50	2.1				>50	>50	>50	
CNE30	C	China	0.626	2.9			0.927						2.6			
CNE31	C	China	0.336	1.2			0.962						2.2			

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Table S3d: Antibody neutralization data against 54 HIV-1 clade C Env-pseudoviruses (continued)

Virus ID	Clade	Origin	Neutralization IC ₅₀ (µg/ml)						Neutralization IC ₈₀ (µg/ml)							
			VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig	VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig
CNE53	C	China	0.108	0.064			0.108			0.274	0.222			0.280		
CNE58	C	China	0.738	>50			0.124			2.6	>50			0.313		
DU123.06	C	South Africa	>50	>50			18.2	>50	0.142	>50	>50			>50	>50	0.938
DU151.02	C	South Africa	0.081	29.0			3.2	34.6	1.4	0.281	>50			46.5	>50	6.0
DU156.12	C	South Africa	<0.023	<0.023			0.089	>50	14.5	<0.023	<0.023			0.193	>50	>50
DU172.17	C	South Africa	0.194	0.554			>50	>50	0.260	1.2	6.3			>50	>50	1.8
DU422.01	C	South Africa	>50	>50			>50	>50	11.5	>50	>50			>50	>50	>50
MW965.26	C	Malawi	0.034	0.312			0.038			0.110	4.2			0.116		
SO18.18	C	Malawi	0.042	0.039	0.253	0.066	0.069	0.083	9.9	0.171	0.138	0.992	0.318	0.178	0.324	>50
TV1.29	C	South Africa	>50	>50	>50	>50	>50	>50	0.405	>50	>50	>50	>50	>50	>50	1.0
TZA125.17	C	Tanzania	>50	>50	>50	>50	>50	>50	0.125	>50	>50	>50	>50	>50	>50	39.5
TZBD.02	C	Tanzania	0.054	0.032	0.184	0.091	0.109	1.3	0.895	0.271	0.243	0.538	0.342	0.328	22.1	5.7
ZA012.29	C	South Africa	0.106	0.103	0.055	0.018	0.305	9.2	5.4	0.669	1.4	0.296	0.103	1.0	>50	45.7
ZM106.9	C	Zambia	0.137	0.340			0.489	0.150	>50	0.388	1.3			1.3	0.428	>50
ZM109.4	C	Zambia	0.040	0.090			0.128	>50	0.028	0.143	0.309			0.754	>50	0.281
ZM135.10a	C	Zambia	>50	>50			0.346	>50	0.296	>50	>50			2.7	>50	9.8
ZM176.66	C	Zambia	0.070	0.025	0.011	0.008	0.055	0.033	0.212	10.2	1.5	0.063	0.055	0.258	0.150	35.4
ZM197.7	C	Zambia	2.3	>50			0.360	2.1	28.3	7.5	>50			1.6	9.2	>50
ZM214.15	C	Zambia	1.6	1.2			0.440	18.8	26.6	4.8	5.6			2.6	>50	>50
ZM215.8	C	Zambia	0.172	0.149			0.095	>50	1.2	0.527	0.387			0.527	>50	>50
ZM233.6	C	Zambia	>50	>50			2.0	>50	3.4	>50	>50			9.3	>50	12.4
ZM249.1	C	Zambia	<0.023	0.070			0.048	8.6	11.1	0.109	0.260			0.232	>50	>50
ZM53.12	C	Zambia	6.6	22.1			1.3	10.3	8.6	15.6	>50			4.0	45.6	32.2
ZM55.28a	C	Zambia	0.639	0.795			0.340	>50	>50	2.3	3.6			1.2	>50	>50
Breadth	N=54	Titer < 50	74%	65%	71%	71%	85%	58%	94%	67%	56%	57%	71%	81%	39%	64%
		Titer < 1	65%	50%	71%	57%	67%	31%	42%	33%	24%	57%	57%	42%	17%	8%
Geometric mean [#]			0.251	0.360	0.105	0.062	0.330	1.1	1.3	0.848	1.1	0.315	0.497	0.988	1.5	5.8

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.

[#]Geometric means were calculated for neutralization sensitive viruses with an IC₅₀ (or IC₈₀) value < 50 µg/ml.

Table S3e: Antibody neutralization data against 9 HIV-1 clade D Env-pseudoviruses

Virus ID	Clade	Origin	Neutralization IC ₅₀ (µg/ml)						Neutralization IC ₈₀ (µg/ml)							
			VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig	VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig
231965.c1	D	Uganda	>50	>50			0.340	32.6	2.5					1.2	>50	7.1
247-23	D	Cameroon	>50	>50	>50	>50	2.8	>50	1.1				>50	>50	>50	6.7
3016.v5.c45	D	Tanzania	0.130	0.215			0.160	>50	0.720					0.420	>50	3.2
57128.vrc15	D	Uganda	>50	>50			>50	>50	0.112					>50	>50	0.855
6405.v4.c34	D	Tanzania	2.3	9.8			2.2	>50	41.8					6.1	>50	>50
A03349M1.vrc4a	D	Uganda	3.5				3.7	>50	4.0					11.0	>50	17.3
NKU3006.ec1	D	Kenya	0.379	0.417	0.194	0.108	0.570	0.060	6.5				0.693	1.7	0.190	21.4
UG021.16	D	Uganda	>50	>50	0.321	0.172	0.266						0.759	0.970		
UG024.2	D	Uganda	0.126	1.1	0.655	0.807	0.156	>50	0.009				4.1	0.674	>50	0.025
Breadth	N=9	Titer < 50	56%	50%	75%	75%	88%	25%	100%	56%	38%	75%	75%	78%	13%	88%
		Titer < 1	33%	25%	75%	75%	56%	13%	38%	11%	0%	50%	50%	33%	13%	25%
Geometric mean [#]			0.549	0.992	0.344	0.247	0.843	1.4	1.1	2.8	4.7	1.3	0.755	1.7	0.190	2.8

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.
#Geometric means were calculated for neutralization sensitive viruses with an IC₅₀ (or IC₈₀) value < 50 µg/ml.

Table S3f: Antibody neutralization data against 16 HIV-1 CRF01_AE Env-pseudoviruses

Virus ID	Clade	Origin	Neutralization IC ₅₀ (µg/ml)							Neutralization IC ₈₀ (µg/ml)						
			VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig	VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig
620345.c1	AE	Thailand	>50	>50		>50	>50	>50		>50				>50		>50
C1080.c3	AE	Thailand	2.3	17.2	2.0	2.9	3.4									
C2101.c1	AE	Thailand	0.044	0.631	0.033	0.047	0.360									
C3347.c11	AE	Thailand	>50	>50	0.011	0.010	0.170									
C4118.09	AE	Thailand	0.092	0.029	0.043	0.039	0.110									
CNE3	AE	China	>50	>50			17.7									
CNE5	AE	China	0.494	3.3			0.370									
CNE55	AE	China	0.272	0.150			0.390									
CNE56	AE	China	0.478	0.681			0.430									
CNE59	AE	China	0.345	0.269			0.540									
M02138	AE	Thailand	0.277	0.211	1.1	0.503	0.348									
R1166.c1	AE	Thailand	0.788	0.461	0.146	0.114	1.7									
R2184.c4	AE	Thailand	0.011	0.009	0.008	0.007	0.080									
R3265.c6	AE	Thailand	1.7				0.450									
TH966.8	AE	Thailand	0.154	0.083	0.049	0.037	0.334									
TH976.17	AE	Thailand	0.016	0.013	0.014	0.010	0.087									
Breadth	N=16	Titer < 50	81%	80%	100%	100%	93%	27%	87%	75%	67%	100%	100%	88%	27%	60%
		Titer < 1	69%	67%	78%	89%	75%	13%	27%	38%	33%	78%	78%	25%	7%	0%
Geometric mean [#]			0.215	0.234	0.067	0.058	0.459	0.919	4.7	1.1	0.824	0.511	0.483	1.4	5.7	13.4

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.
#Geometric means were calculated for neutralization sensitive viruses with an IC₅₀ (or IC₈₀) value < 50 µg/ml.

Table S3g: Antibody neutralization against 16 CRF02_AG Env-pseudoviruses

Virus ID	Clade	Origin	Neutralization IC ₅₀ (µg/ml)										Neutralization IC ₈₀ (µg/ml)						
			VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig	VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig			
235-47	AG	Cameroon	0.010	0.015			0.040	0.110	29.0	0.045	0.044			0.170	1.5	>50			
242-14	AG	Cameroon	>50	>50			>50	>50	1.9	>50	>50			>50	>50	15.5			
250-4	AG	Cameroon	>50	>50			>50	>50	6.0	>50	>50			>50	>50	43.1			
251-18	AG	Cameroon	0.516	0.718			2.5	>50	3.1	1.5	2.1			11.2	>50	27.3			
255-34	AG	Cameroon	1.4	>50			0.700	>50	0.190	13.9	>50			2.7	>50	1.1			
257-31	AG	Cameroon	0.433	0.411			2.8	>50	20.1	1.3	1.4			8.7	>50	>50			
263-8	AG	Cameroon	>50	>50			0.200	0.110	1.0	>50	>50			0.550	0.410	8.3			
266-60	AG	Cameroon	1.3	0.887			1.2	>50	2.5	4.5	4.8			4.3	>50	>50			
269-12	AG	Cameroon	>50	>50			0.230	>50	4.5	>50	>50			0.660	>50	22.9			
271-11	AG	Cameroon	0.057	0.432	0.050	0.023	0.060	>50	0.020	0.358	13.0	0.205	0.107	0.220	>50	0.070			
278-50	AG	Cameroon	>50	>50			>50	>50	1.6	>50	>50			>50	>50	8.0			
280-5	AG	Cameroon	0.090	0.886			0.020	0.020	4.2	0.564	22.1			0.150	0.160	37.5			
928-28	AG	Cote d'Ivoire	12.2	>50			0.410	>50	0.400	>50	>50			1.7	>50	3.3			
DJ263.8	AG	Kenya	0.802	11.0			0.080	>50	0.088	>50	>50			0.553	>50	0.557			
T253-11	AG	Cameroon	0.088	0.234			0.470	>50	43.1	0.290	1.1			1.4	>50	>50			
T33-7	AG	Cameroon	<0.023	0.045			<0.023	>50		<0.023	0.346			<0.023	>50	>50			
Breadth	N=16	Titer < 50	69%	56%	100%	100%	81%	20%	100%	56%	50%	100%	100%	81%	20%	73%			
		Titer < 1	50%	50%	100%	100%	62%	20%	27%	31%	13%	100%	100%	44%	13%	13%			
Geometric mean*			0.253	0.382	0.050	0.023	0.228	0.062	1.8	0.580	1.7	0.205	0.107	0.815	0.462	5.5			

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.
#Geometric means were calculated for neutralization sensitive viruses with an IC₅₀ (or IC₈₀) value < 50 µg/ml.

Table S3h: Antibody neutralization against 17 HIV-1 recombinant and 1 clade G Env-pseudoviruses

Virus ID	Clade	Origin	Neutralization IC ₅₀ (µg/ml)							Neutralization IC ₈₀ (µg/ml)						
			VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig	VRC-PG04	VRC-PG04b	VRC-CH30	VRC-CH31	VRC01	VRC03	CD4-Ig
3301_V1_C24	AC	Tanzania	>50	>50			0.140	0.390	2.9	>50	>50			0.320	0.230	10.1
3589_V1_C4	AC	Tanzania	16.7	>50			0.120	>50	13.0	>50	>50			0.470	>50	47.1
6540.v4.c1	AC	Tanzania	>50	>50			>50	>50	3.7	>50	>50			>50	>50	11.7
6545.v3.c13	AC	Tanzania	>50	>50			>50	>50	2.99	>50	>50			>50	>50	11.43
0815_V3_C3	ACD	Tanzania	0.054	2.6			0.060	0.320	3.0	0.162	>50			0.130	0.070	9.4
6095_V1_C10	ACD	Tanzania	0.781	1.2			0.464			2.5	4.6			1.5		
3468_V1_C12	AD	Tanzania	0.038	1.1			0.050	>50	7.8	0.228	6.9			0.170	>50	>50
CH038.12	BC	China	0.182	0.157	>50		0.520	>50	3.8	0.545	0.582	>50	>50	1.6	>50	17.1
CH070.1	BC	China	0.226	0.178	0.333	8.1	37.7	>50	0.570	1.6	2.1	4.5	>50	>50	>50	2.9
CH117.4	BC	China	0.026	>50	>50	>50	0.140	0.140	3.9	0.193	>50	>50	>50	0.340	0.630	16.9
CH181.12	BC	China	0.160	0.158	0.319	0.137	0.490	7.8	0.990	1.4	1.1	1.2	0.601	1.7	>50	3.5
CNE15	BC	China	0.054	0.157			0.080			0.244	0.445			0.280		
CNE7	BC	China	16.0	>50			0.540			>50	>50			1.4		
CNE40	BC	China	0.502	0.463			0.425			1.6	1.8			4.4		
3326_V4_C3	CD	Tanzania	>50	>50			0.100	>50	50.0	>50	>50			2.3	>50	>50
3337_V2_C6	CD	Tanzania	>50	>50			0.140	>50	0.320	>50	>50			0.340	>50	1.3
3817.v2.c59	CD	Tanzania	3.0	4.7	0.570	0.303	>50	>50	>50	49.4	>50	2.7	1.2	>50	>50	>50
X2088_c9	G	Ghana	>50	>50	>50	>50	>50	>50	2.4	>50	>50	>50	>50	>50	>50	8.8
Breadth	N=18	Titer < 50	67%	50%	50%	50%	78%	29%	93%	55%	39%	50%	33%	72%	21%	79%
		Titer < 1	50%	28%	50%	33%	72%	21%	21%	28%	11%	0%	17%	39%	21%	0%
Geometric mean [#]			0.367	0.557	0.393	0.695	0.264	0.211	3.2	0.917	1.7	2.4	0.849	0.677	0.216	8.6

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.

[#]Geometric means were calculated for neutralization sensitive viruses with an IC₅₀ (or IC₈₀) value < 50 µg/ml.

Table S4: X-ray crystallographic data and refinement statistics for VRC-PG04:gp120 and VRC03:gp120 complexes.

Crystal	VRC-PG04:gp120	VRC03:gp120
Data collection		
Space group	P212121	P212121
Wavelength, Å	1.0000	1.0000
Unit cell dimensions		
a (Å)	61.8	62.0
b (Å)	66.5	70.2
c (Å)	237.3	216.5
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Complexes per ASU	1	1
Resolution, Å	2.0	1.9
Completeness, %*	88.3 (64.1)	90.3 (54.8)
Redundancy	8.6 (6.2)	3.2 (2.4)
No. of total reflections	510537	213952
No. of unique reflections	59364	69237
I/σ [†]	24.4 (1.9)	6.3 (1.7)
R _{sym} ^{*,†}	0.097	0.145
Refinement statistics (F >0 σ)		
Resolution, Å	2.0	1.9
R _{work} /R _{free} , % ^{‡,§}	19.85/23.84	18.79/22.84
RMSD bond length, Å	0.007	0.008
RMSD bond angles, °	1.04	1.11
Average B-factor, Å ²	47.3	51.0
Ramachandran analysis		
Favored, %	96.2	96.9
Allowed, %	100.0	99.5
PDB ID	To be deposited	To be deposited

* Values in parentheses are for the highest resolution shell.

[†] $R_{\text{sym}} = \sum |I - \langle I \rangle| / \sum \langle I \rangle$, where I is the observed intensity, and $\langle I \rangle$ is the average intensity of multiple observations of symmetry related reflections.

[‡] $R = \sum_{\text{hkl}} ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum_{\text{hkl}} |F_{\text{obs}}|$

[§] R_{free} calculated from 5% of the reflections excluded from refinement.

Table S5a. List of VRC-PG04 heavy chain residues that interact with HIV-1 gp120**VRC-PG04 Heavy chain interaction with HIV-1 gp120**

Chain:Residue	Bond*	ASA*	BSA*	ΔiG^*
H:GLU 33		53.36	3.67	-0.06
H:LEU 34		21.93	12.73	0.2
H:TRP 47		70.89	15.95	0.26
H:TRP 50		30.54	28.68	0.29
H:LYS 52	H	72.66	34.11	-0.9
H:VAL 52B		67.97	16.54	0.21
H:THR 53	H	96	68.09	0.45
H:GLY 54		21.99	17.94	-0.07
H:ALA 55		43.91	25.01	0.4
H:VAL 56		56.95	39.54	0.46
H:ASN 57	H	55.11	54.19	-0.57
H:PHE 58		21.93	15.66	-0.11
H:GLY 59		39.34	20.78	-0.08
H:SER 60		36.11	7.23	0.1
H:PRO 61		113.23	13.72	0.22
H:ARG 64	HS	133	87.93	0.14
H:ARG 71	HS	68.07	25.63	-0.66
H:ARG 73	H	127.33	35.80	-0.81
H:ASP 74		121.27	35.10	-0.25
H:TYR 98		197.37	22.99	0.05
H:THR 99		25.49	12.75	-0.15
H:GLY 100		70.66	65.09	0.39
H:GLY 100A		88.7	34.21	0.15
H:GLY 100C		37.99	8.05	0.08
H:TRP 100D		145.06	53.21	0.13

VRC-PG04 Heavy chain interaction with glycan on HIV-1 gp120

Chain:Residue	Bond*	ASA*	BSA*	ΔiG^*
H:GLY 100A	H	88.7	41.21	-0.06
H:GLN 100B		126.62	1.74	0.03
H:GLY 100C		37.99	14.38	0.22

* Bond type: H: Hydrogen, D Disulphide bond, S : Salt bridge C: Covalent link

ASA: Accessible Surface Area, Å²BSA: Buried Surface Area, Å² ΔiG : Solvation energy effect, kcal/mol

|||: Buried area percentage, one bar per 10%

Detailed gp120:VRC-PG04 interface data was calculated on the EBI PISA server (http://www.ebi.ac.uk/msdsrv/prot_int/cgi-bin/piserver)

Table 5b. List of VRC-PG04 light chain residues that interact with HIV-1 gp120**VRC-PG04 light chain interaction with HIV-1 gp120**

Chain:Residue	Bond*	ASA*	BSA*	ΔiG^*
L:GLU 1	H	203.92	70.35	-0.4
L:VAL 3		53.76	17.01	0.27
L:SER 27		67.84	4.02	0.06
L:TYR 30		137.09	0.12	0
L:LEU 91		143.97	63.81	1.02
L:GLU 96	H	121.88	37.92	-0.18
L:PHE 97		96.7	30.09	0.48

VRC-PG04 light chain interaction with glycan on HIV-1 gp120

Chain:Residue	Bond*	ASA*	BSA*	ΔiG^*
L:TYR 30		137.09	6.92	-0.03
L:GLY 31		25.42	22.81	0.34
L:HIS 32		132.41	46.34	-0.02
L:GLN 90		16.81	0.31	0.01
L:LEU 91		143.97	27.15	0.43

* **Bond type:** H: Hydrogen, D Disulphide bond, S : Salt bridge C: Covalent link

ASA: Accessible Surface Area, Å²

BSA: Buried Surface Area, Å²

ΔiG : Solvation energy effect, kcal/mol

||||: Buried area percentage, one bar per 10%

Detailed gp120:VRC-PG04 interface data was calculated on the EBI PISA server (http://www.ebi.ac.uk/msdsrv/prot_int/cgi-bin/piserver)

Table S5c. List of HIV-1 gp120 residues that interact with VRC-PG04 heavy chain

Chain:Residue	Bond*	ASA*	BSA*	ΔG^*
G:LYS 97		152.45	23.66	0.03
G:GLU 102		58.18	1.11	-0.02
G:LEU 122		65.89	4.36	0.07
G:GLY 124		85.12	29.55	-0.18
G:GLU 275		53.04	20.76	-0.05
G:ASN 276		81.38	17.75	-0.27
G:ASN 279		61.51	31.36	-0.32
G:ASN 280	H	72.13	43.77	-0.48
G:ALA 281	H	90.09	76.09	0.73
G:LYS 282		72.43	31.85	-0.53
G:THR 283		30.9	9.48	-0.11
G:SER 365	H	96.21	45.24	0.48
G:GLY 366		48.7	23.28	0.13
G:GLY 367		56.22	23.64	0.27
G:ASP 368	HS	79.6	50.25	-0.48
G:ILE 371		55.28	45.39	0.73
G:MET 426		15.29	0.12	0
G:TRP 427		31.25	1.23	-0.01
G:GLY 429	H	68.44	47.50	0.15
G:GLY 431		27.77	6.35	0.07
G:THR 455		58.29	31.36	0.39
G:ARG 456	H	31.19	1.84	-0.02
G:ASP 457	S	40.84	25.79	0.06
G:GLY 458	H	47.21	36.29	0.01
G:GLY 459		88.91	47.28	0.22
G:ARG 469		49.96	18.57	-0.24
G:GLY 472		22.43	4.52	-0.05
G:GLY 473		29.7	17.90	0.2
G:ASP 474		73.97	4.42	-0.07
G:LYS 476		59.25	21.78	-0.81
G:NAG 776	H	360.8	64.91	-1.95

* **Bond type:** H: Hydrogen, D Disulphide bond, S : Salt bridge C: Covalent link

ASA: Accessible Surface Area, Å²

BSA: Buried Surface Area, Å²

ΔG : Solvation energy effect, kcal/mol

||||: Buried area percentage, one bar per 10%

Detailed gp120:VRC-PG04 interface data was calculated on the EBI PISA server (http://www.ebi.ac.uk/msdsrv/prot_int/cgi-bin/piserver)

Table S5d. List of HIV-1 gp120 residues that interact with VRC-PG04 light chain

Chain:Residue	Bond*	ASA*	BSA*	ΔiG^*
G:ASN 276		81.38	0.35	0.01
G:THR 278		121.08	34.02	-0.11
G:ASN 279		61.51	21.74	0.07
G:ASN 280	H	72.13	21.42	-0.24
G:GLY 458		47.21	4.53	0.07
G:GLY 459		88.91	9.98	-0.06
G:ASN 461	H	138.25	82.82	-0.45
G:ASN 462	H	152.6	42.36	-0.4
G:NAG 776		360.8	125.10	-2.59

* **Bond type:** H: Hydrogen, D Disulphide bond, S : Salt bridge C: Covalent link

ASA: Accessible Surface Area, Å²

BSA: Buried Surface Area, Å²

ΔiG : Solvation energy effect, kcal/mol

|||: Buried area percentage, one bar per 10%

Detailed gp120:VRC-PG04 interface data was calculated on the EBI PISA server (http://www.ebi.ac.uk/msdsrv/prot_int/cgi-bin/piserver)

Table S6a. List of VRC03 heavy chain residues that interact with HIV-1 gp120

Chain:Residue	Bond*	ASA*	BSA*	ΔiG^*
H:ARG 30		101.57	57.04	-0.36
H:HIS 35		4.62	1.18	-0.03
H:GLU 46		38.15	0.31	0.01
H:TRP 47		68.77	28.74	0.46
H:TRP 50		55.82	51.92	0.36
H:LYS 52	H	96.54	42.97	-0.86
H:LEU 53		95.76	53.25	0.76
H:TRP 54	H	205.01	199.06	2.01
H:GLY 55		16.03	13.27	-0.1
H:ALA 56		46.42	34.02	0.54
H:VAL 57		55.92	42.38	0.35
H:SER 58		38.45	32.58	-0.13
H:TYR 59		50.99	30.31	0.21
H:ALA 60		5.02	5.02	0.08
H:ARG 61	H	213.64	156.18	-1.08
H:GLN 62	H	114.87	59.94	0.09
H:GLN 64	H	107.9	46.05	-0.52
H:ARG 71	HS	72.06	24.95	-0.67
H:LEU 73		30.12	0.67	0.01
H:SER 74		10.73	7.86	-0.09
H:GLN 75		144.81	33.96	-0.25
H:ASP 76		62.88	1.68	0.01
H:PRO 76A		125.61	45.28	0.72
H:PRO 76D		65.16	19.85	0.27
H:ASP 99		143.04	17.15	-0.16
H:TYR 100		212.68	16.56	0.26
H:GLY 100B		33.36	2.27	0.04
H:ASP 100C	HS	87.28	62.64	-0.31
H:PHE 100D		111.1	33.72	0.54

* **Bond type:** H: Hydrogen, D Disulphide bond, S : Salt bridge C: Covalent link

ASA: Accessible Surface Area, Å²

BSA: Buried Surface Area, Å²

ΔiG : Solvation energy effect, kcal/mol

||||: Buried area percentage, one bar per 10%

Detailed gp120:VRC-03 interface data was calculated on the EBI PISA server (http://www.ebi.ac.uk/msdsrv/prot_int/cgi-bin/piserver)

Table S6b. List of VRC03 light chain residues that interact with HIV-1 gp120**VRC03 light chain interaction with HIV-1 gp120**

Chain:Residue	Bond*	ASA*	BSA*	ΔiG^*
L:GLU 1		153.88	74.77	-0.2
L:ILE 2		15.71	8.21	0.13
L:GLN 27		83.2	6.51	-0.11
L:ASN 31		40.33	12.91	-0.2
L:PHE 91		131.73	54.69	0.88
L:GLU 96	H	122.84	62.03	-0.31
L:PHE 97		46.56	25.17	0.4

VRC03 light chain interaction with glycan on HIV-1 gp120

Chain:Residue	Bond*	ASA*	BSA*	ΔiG^*
L:ASN 31		40.33	9.18	-0.02
L:ALA 32		47.78	32.39	0.21
L:ASP 50		69.43	12.21	-0.16
L:GLN 90		3.66	0.84	0.01
L:PHE 91		131.73	23.55	0.37

* **Bond type:** H: Hydrogen, D Disulphide bond, S : Salt bridge C: Covalent link

ASA: Accessible Surface Area, Å²

BSA: Buried Surface Area, Å²

ΔiG : Solvation energy effect, kcal/mol

|||: Buried area percentage, one bar per 10%

Detailed gp120:VRC-03 interface data was calculated on the EBI PISA server

(http://www.ebi.ac.uk/msdsrv/prot_int/cgi-bin/piserver)

Table S6c. List of HIV-1 gp120 residues that interact with VRC03 heavy chain

Chain:Residue	Bond*	ASA*	BSA*	ΔiG^*
G:LYS 97		158.83	40.59	-0.92
G:THR 123		48.84	5.77	0.09
G:GLY 124		87.08	80.77	0.31
G:GLY 198		91.23	50.65	0.33
G:SER 199		54.24	15.88	0.25
G:THR 257		8.68	7.17	0.11
G:ASN 279	H	60.35	20.99	-0.17
G:ASN 280		77.1	54.49	-0.56
G:ALA 281	H	84.01	70.75	0.64
G:LYS 282	S	75.7	17.37	0.03
G:THR 283		22.48	7.61	-0.09
G:SER 365		95.12	58.00	0.45
G:GLY 366		46.67	22.26	0.09
G:GLY 367		62.05	26.17	0.34
G:ASP 368	HS	81.49	54.28	-0.38
G:GLU 370		19.22	17.10	0.27
G:ILE 371		38.73	35.22	0.56
G:HIS 375		14.16	2.73	0.01
G:ASN 425		49.86	11.65	0.11
G:MET 426		23.72	7.83	-0.08
G:TRP 427		50.55	34.07	0.21
G:GLY 429		69.61	49.16	0.19
G:THR 430		54.07	1.87	0.03
G:GLY 431		30.64	14.86	0.18
G:THR 455		43.78	23.07	0.32
G:ARG 456		32.9	3.51	-0.03
G:ASP 457	H	53.35	46.31	0.2
G:GLY 458	H	44.89	37.44	-0.26
G:GLY 459		77.9	37.58	0.45
G:ALA 460	H	111.5	29.52	-0.11
G:ASN 461		119.23	35.73	0.17
G:THR 463		77.05	26.95	0.04
G:ASN 465	H	42.83	11.56	-0.17
G:GLU 466		28.03	3.34	-0.01
G:THR 467		24.58	15.75	-0.09
G:ARG 469	H	50.3	22.19	-0.59
G:GLY 472		31.48	22.96	-0.21
G:GLY 473		32.75	28.94	-0.08
G:ASP 474		71.26	2.68	0.04
G:ILE 475		8.14	0.67	0.01

* **Bond type:** H: Hydrogen, D Disulphide bond, S : Salt bridge C: Covalent link

ASA: Accessible Surface Area, Å²

BSA: Buried Surface Area, Å²

ΔiG : Solvation energy effect, kcal/mol

||||: Buried area percentage, one bar per 10%

Detailed gp120:VRC-03 interface data was calculated on the EBI PISA server

(http://www.ebi.ac.uk/msdsrv/prot_int/cgi-bin/piserver)

Table S6d. List of HIV-1 gp120 residues that interact with VRC03 light chain

Chain:Residue	Bond*	ASA*	BSA*	ΔiG^*
G:ASN 276		75.77	12.98	-0.15
G:THR 278		127.43	74.78	0.69
G:ASN 279		60.35	14.54	0.08
G:ASN 280	H	77.1	22.61	-0.26
G:ARG 456		32.9	2.42	-0.06
G:GLY 458		44.89	3.36	0.05
G:GLY 459	H	77.9	35.49	-0.11
G:ALA 460		111.5	32.16	0.51
G:ASN 461		119.23	2.58	-0.01
G:ASN 462		149.19	39.53	0.08
G:NAG 776		351.09	107.06	-1.06

* **Bond type:** H: Hydrogen, D: Disulphide bond, S : Salt bridge C: Covalent link

ASA: Accessible Surface Area, Å²

BSA: Buried Surface Area, Å²

ΔiG : Solvation energy effect, kcal/mol

||||: Buried area percentage, one bar per 10%

Detailed gp120:VRC-03 interface data was calculated on the EBI PISA server

(http://www.ebi.ac.uk/msdsrv/prot_int/cgi-bin/piserver)

Table S7. Comparison of gp120 recognition by CD4-induced antibodies derived from a common IGVH1-69 allele.

Heavy chain			
RMSD(Å) /Angle(°)	17b	412d	X5
17b		8.9/37.7	21.4/85.5
412d			24.6/109.2
X5			
Light chain			
RMSD(Å) /Angle(°)	17b	412d	X5
17b			
412d	24.2/40.9		
X5	38.1/87.1	48.0/87.6	

Pair-wise RMSDs and angles for both heavy and light chains of all antibodies were calculated after gp120s in the complexes were superposed. Corresponding fragments in the frameworks1, 2, 3 and 4 of the heavy and light chains were used in the computation. Though sharing a common VH1-69 gene in their heavy chains, CD4-induced antibodies 17b, 412d and X5 had substantial variation in gp120 recognition.

Table S8. Orientations of RSC3-reactive CD4-binding site antibodies in gp120:antibody complexes.

Heavy chain					
RMSD(Å) /Angle(°)	VRC01	VRC03	VRC-PG04	b12	b13
VRC01		1.9/62	2.1/88	24.0/125.6	25.5/120.5
VRC03			2.4/12.0	24.0/120.9	25.7/118.7
VRC-PG04				24.0/123.9	25.6/116.8
b12					7.0/12.9
b13					
Light chain					
RMSD(Å) /Angle(°)	VRC01	VRC03	VRC-PG04	b12	b13
VRC01					
VRC03	4.9/11.1				
VRC-PG04	5.2/33.7	1.6/7.3			
b12	48.6/68.2	50.1/73.9	49.5/70.2		
b13	53.4/60.5	54.7/67.5	54.1/63.0	8.3/11.4	

To compare how different CD4-binding site antibodies approach HIV-1 gp120, pairwise RMSDs and angles for both heavy and light chains of all antibodies were calculated after gp120s in the complexes were superposed. Only corresponding fragments in the frameworks 1, 2, 3 and 4 of the heavy and light chains were used in the computation. Pairs with RMSD<10 Å were colored red and those with RMSD>10 Å were colored green. The results clearly showed that VRC01, VRC03 and VRC-PG04 had very similar modes of approach towards HIV-1 gp120, while other RSC-reactive CD4-binding site antibodies such as b12, b13 had different orientations in recognition.

Table S9. Heavy/Light-chain complementation of VRC01-like antibody

Heavy chain	Light chain	Antibody yield (mg/L culture supernatant)
VRC01	VRC03	77.08
VRC01	VRC04	73.60
VRC03	VRC01	70.52
VRC03	VRC-PG04	73.80
VRC-PG04	VRC01	67.24
VRC-PG04	VRC03	60.68
VRC-CH31	VRC01	25.32
VRC-CH31	VRC03	28.44
VRC-CH31	VRC04	15.00
VRC01	VRC-CH31	10.32
VRC03	VRC-CH31	5.76
VRC04	VRC-CH31	22.32

Table S10. Neutralization IC₅₀ titers* (µg/ml) of chimeric antibodies derived from known VRC01-like antibodies against 20 HIV-1 clade A, B and C Env-pseudoviruses

mAb designation	Clade A (n=6)						Clade B (n=8)						Clade C (n=6)						IC ₅₀ < 50 µg/ml	IC ₅₀ < 1 µg/ml	geometric mean [#] (ug/ml)		
	Q23.17	Q842.d12	UG037.8	Q168.12	KER2008.12	KER2018.11	YU2	JR-FL	JR-CSF	7165.18	BG1168.1	CAAN.A2	PVO.4	TRO.11	Du156.12	ZM109.4	ZM106.9	TV1.29				ZM176.66	SO18.18
VRC01	0.085	0.030	0.079	0.115	0.379	0.852	0.128	0.031	0.093	>50	0.276	0.824	0.216	0.207	0.089	0.128	0.489	>50	0.055	0.069	90%	90%	0.140
VRC01H/VRC-PG04L	0.307	0.031	0.045	0.116	25.3	0.402	0.117	0.299	0.146	>50	28.1	0.968	0.344	2.9	0.123	0.326	3.0	>50	4.0	0.060	65%	90%	0.482
VRC01H/VRC-CH31L	>50	1.2	2.0	5.9	>50	>50	3.2	1.5	>50	>50	11.4	>50	>50	>50	>50	>50	>50	>50	>50	11.5	0%	35%	3.7
VRC03	0.065	>50	12.1	3.4	0.403	0.389	0.037	0.009	0.093	>50	>50	8.3	0.328	0.055	>50	>50	0.150	>50	0.053	0.063	55%	70%	0.224
VRC03H/VRC01L	0.018	0.013	0.019	0.038	0.489	0.260	0.024	0.010	0.016	>50	12.4	2.5	0.213	0.059	1.6	3.8	0.060	>50	>50	0.062	65%	85%	0.132
VRC03H/VRC-PG04L	0.017	0.011	0.021	0.049	0.724	0.349	0.028	0.008	0.032	>50	0.847	1.8	0.107	0.044	1.8	>50	0.393	>50	5.4	0.035	70%	85%	0.119
VRC03H/VRC-CH31K	0.031	0.017	0.034	0.224	1.3	3.0	0.035	0.010	0.117	>50	0.818	8.1	0.552	0.168	>50	>50	1.2	>50	0.063	0.182	60%	80%	0.196
VRC-PG04	0.048	0.012	0.023	0.050	0.224	0.711	0.019	0.104	0.138	>50	0.792	2.7	0.234	0.291	<0.023	0.040	0.137	>50	0.070	0.042	85%	90%	0.102
VRC-PG04H/VRC01L	0.051	0.043	0.148	0.051	6.2	1.1	0.096	>50	0.043	>50	>50	6.9	0.431	>50	0.166	0.179	1.3	>50	>50	0.176	50%	70%	0.264
VRC-PG04H/VRC03L	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	0%	0%	>50
VRC-PG04H/VRC-CH31L	0.139	0.088	0.416	0.370	>50	>50	0.743	0.230	>50	>50	5.8	>50	2.4	>50	>50	>50	>50	>50	>50	0.425	35%	45%	0.499
VRC-CH31	0.014	0.003	0.004	0.025	0.107	0.385	<0.016	0.014	0.132	>50	0.653	>50	0.194	0.180	3.5	0.069	7.6	>50	0.008	0.065	75%	85%	0.085
VRC-CH31H/VRC01L	0.085	0.029	0.177	0.423	0.701	2.9	0.848	>50	7.7	>50	>50	>50	1.7	2.5	0.493	>50	>50	>50	>50	2.5	30%	60%	0.680
VRC-CH31H/VRC03L	1.4	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	5%	5%	1.4
VRC-CH31H/VRC-PG04L	0.031	0.013	0.067	0.295	0.621	1.4	1.4	>50	>50	>50	>50	>50	0.904	3.2	0.163	>50	>50	>50	>50	2.1	35%	55%	0.350

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green.

#Geometric means were calculated for neutralization sensitive viruses with an IC₅₀ value < 50 µg/ml.

Table S11. Sequences selected from the IGHV1-2*02 family of donor 45 heavy-chain 2008 antibodyome with high predicted structural compatibility with known VRC01-like antibody-gp120 complexes.^a

Seq. Index	IGHV1-2*02 divergence (%)	VRC01 threading score	VRC03 threading score	VRC-PG04 threading score	VRC01 SeqID (%)	VRC03 SeqID (%)	VRC-PG04 SeqID (%)
VRC01	32.09	0.093	0.122	0.156	100.00	64.62	60.80
VRC03	30.74	0.310	0.031	0.206	68.87	100.00	62.13
VRC-PG04	28.72	0.185	0.219	0.092	62.81	60.26	100.00
65030	1.01	0.110	0.087	0.127	67.22	64.10	66.40
61272	4.39	0.128	0.090	0.136	69.97	62.82	64.80
103787	8.45	0.171	0.084	0.125	65.84	64.62	66.13
70542	9.46	0.160	0.089	0.122	65.01	63.59	65.33
87722	13.85	0.092	0.132	0.104	65.01	63.08	63.73
80585	15.88	0.093	0.133	0.108	63.36	62.31	62.13
22425	17.69	0.147	0.094	0.137	64.19	58.97	64.00
18761	23.31	0.155	0.107	0.145	60.06	53.59	60.80
49433	30.95	0.309	0.041	0.199	68.32	99.23	61.87

^a The germline divergence of sequences in the IGHV1-2*02 family was divided into 12 bins ranging from 0 to 36%. In each divergence bin, the sequence that has the lowest threading score to any of the VRC01-, VRC03- and VRC-PG04-gp120 complex structures was selected as candidate for synthesis and listed in this table. Note that only 9 sequences remained because the divergence bin 24-27% was empty, the sequence selected from the 27-30% bin was identical to VRC03, and the sequence from the 33-36% bin was discarded due to severe sequencing errors. For each sequence, the listed columns include index number, germline divergence, normalized DFIRE threading score(S39, 43) to VRC01, VRC03 and VRC-PG04 complex structures, and nucleotide sequence identities to VRC01, VRC03 and VRC-PG04 heavy chains.

Table S12. Sequences selected from the non-IGHV1-2*02 families of donor 45 heavy-chain 2008 antibodyome with high germline divergence and large family size. ^a

Seq. Index	V-gene family	Germline divergence (%)	VRC01 SeqID (%)	VRC03 SeqID (%)	VRC-PG04 SeqID (%)	Family size
96362	IGHV1-18*01	20.61	60.06	59.49	64.00	4
61822	IGHV1-24*01	8.45	61.71	59.49	60.53	5
7863	IGHV1-3*02	11.82	61.43	62.05	61.07	1
28241	IGHV1-45*02	20.07	59.78	57.69	59.20	3
19891	IGHV1-46*02	17.23	61.98	58.72	63.47	4
70085	IGHV1-58*02	12.16	62.53	60.77	60.53	6
153849	IGHV1-69*01	20.27	60.06	57.95	62.67	2
146940	IGHV1-8*01	27.70	60.06	54.36	60.80	10
5827	IGHV1-c*01	13.95	63.09	58.97	61.07	3

^a For each of the 9 non-IGHV1-2*02 germline families of donor 45 heavy-chain 2008 antibodyome, the most divergent 10 sequences were subjected to a clustering procedure using a sequence identity cutoff of 75%. The center of the cluster that has at least two members was selected as candidate for synthesis and listed in this table. For each sequence, the listed columns include index number, V-gene family name, germline divergence, and nucleotide sequence identities to VRC01, VRC03 and VRC-PG04 heavy chains.

Table S13. Expression of antibodies with selected heavy chains derived from donor 45, 2008

Sequence ID	Paired with	Yield* (mg/L culture sup)	Neutralization* (Y/N)	Amino acid sequence of heavy chain V domain
65030	VRC01L	12.24	N	QVQLVQSGAEVKKPGASVKVSCKASGYTFGTYYHHVVRQAPGGGLEWMGWINPNSGGTNYAQKFGQGRVTMTROTSTISAYMELSLRSDDTAVYYCARLRRDSSGFYYFDYWGQGLTVTVSS
61272	VRC01L	15.00	N	QVQLVQSGAEVKKPGASVKVSCKASGYTFDTYYIHVVRQAPGGGLEWMGWINPNSGGTNYAQKFGQGTVTVTROTSTISGTAYMELSLRSDDTAVYYCARGDYDTNSGRFDPWGQGLWSPPQ
103787	VRC01L	16.80	N	QVQLVQSGPEVKKPGASVKVSCKASGYTYNTYYHHVVRQAPGGGLEWMGWINPNSGGTNYAQRQTRVDMTTRDTSINTVYMELSGLTSDDTAVYYCVTFDFNGGSSPFTLTITWGQGLTVTVSS
70542	VRC01L	28.32	N	QVQLVQSGAEVKKPGASVKVSCKASGYTFDTYYIHVVRQVPQGQGLEWGWHPNSGRATHQKFGQGRVTMTSGTSTVYMELTGLTPDDTAVYYCARDNRYNNVWVLYTFDYWGQGLTVTVSS
87722	VRC01L	no expression	n/a	QVQLVPSGAEVKKPGASMRVSCQTSGYTFNSYYIHWMRQVPQGQGLEWMGWINPNSGYTNTYQKQDRVTMTTRDTSINTAYLELSALRSDETAIVYYCARDFSDGWPSYFDFWGQGLTVTVSS
80585	VRC01L	11.52	N	QVQLVQSGAEVKKPGASMRVSCQTSGYTFNSYYIHWMRQVPQGQGLEWMGWINPNSGYTNTYQKFGQGRVTMTTRDTSINTAYLELSALRSDETAIVYYCARDFSDGWPSYFDFWGQGLTVTVSS
22425	VRC01L	15.84	N	QVQLVQSGAEVKKPGASVKVSCKASGYRFTDQHINWIRQAPGGQFEWLGREFNPANGGTDLAQKFGQGRVSMTRNMSITTAYLELSRLTSDDTATYYCLNSGWTNEYHYDHWGQGTPTVTVSS
18761	VRC01L	no expression	n/a	QVQLVESGREVKKPGSVKVSCKASGYRFTDQHINWIRQAPGGQFEWLGREFNPANGGTDLAQKFGQGRVSMTRNMSITTAYLELSRLTSDDTATYYCLNSGWTNEYHYDHWGQGTPTVTVSS
19891	VRC01L	0.93	N	QVQLVQSGAEVKKPGASLKLSCKTSKTFNTYYWQVVRQAPGQGRLEWLVFSPRDAVTKYARAFQGRLTVTRDTISGTITTYMELSSLRVDDTAMYYCATSLVAAPDRILLTIWGQGTMTMIVVSSA
146940	VRC01L	no expression	n/a	QVQLVQSGAELKRPASVRVSCQTSQNTFNNDVNWIRQAPGGGLEWMGWINPVKFGGSGHHKFDHRHTFNRRDTTINAAYLDLKNLKVDDTAFYYCVVATGSAIDYDWGHGTLVSVSSA
96362	VRC01L	4.05	N	QVQLVQSGPEAGRPASVKVSCKASGYIFTSPYIGVVRQAPGQRLEWMGWINPENGYYKSAQEFQDRLLTLSTNNSAHTAYMELSLTSDDTAIFYCARVQFDSKYITFFDLWGQGLTVTVSSA
7863	VRC01L	no expression	n/a	QVQLVQSGAEMKKPGASVKVSCKSSGYTFTYSIHHVVRQAPQGQGLEWMGRSNTASDNTKYSQEFQVRVAITRDTSANITTYVELSSLRSDAVDVYYCGRVSWHRCRGIGYWGQGLTVTVSS
153849	VRC01L	5.28	N	QVQLVQSGAEVKKVGSVTLCKISGGTFTYDFAVNWRQAPQGQGLEWVAVIPMGFTPYPPKFRHRVTYSSYSGSMITTYVMELNLTFTDITAIYYCARDGREGVNPACQWLDPWGQGLTVTVSSIT
28241	VRC01L	no expression	n/a	QVQLVQPGAEVKKPASVSVKVSCKASRYTFTKHFTQWVRRGPGQGHEWLACFKPYNINHTHYAQNFNWGRLLTSTDRSVHTAYMDLSSVRSEDMRVFLCAIPEVERLKTLLVWIRWGQGLTVTVASG
70085	VRC01L	3.78	N	QVQLVQSGPVKKPGTSVQVSCKASGFTFSSYVICWVRQTRGQRLEWIGWVWGSGITNCAQNFQERVTLIRDMSTDITAYMELNSLGSDELTALYYCAAEPILRFPVGVWSIPWVGQGLTVTVSSA
61822	VRC01L	3.63	N	QVQLVQSGVEVKKPGASVKVSCKVSGYFLSKISIHVVRQAPKEKLEWMGGFDEAEQGETVYAQKFGQGRVTVTEDTSTDTAYMDVSSLRSEDTAVYYCATGIRGRPLISWGQGTVTVTVSSA
5827	VRC01L	no expression	n/a	QVQLVAGLGRVKKVWGSVKVSCSFGTITSYGIHWVQQSPGGQGLEWMGWINPGNSPSYAKKFGQGRFTMTDRDMSTTTTAYTDLSSLTSEDMAVYYYAYPGFPSYYDSSGYYYEPLLWYWGQGLTVTVSSG

*no expression, yield was less than 0.60mg/L; Y, yes; N, no; n/a, not available.

Table S14. Expression of antibodies with selected heavy chains from donor V74, 2008, paired with VRC-PG04 light chain

Sequence ID	Yield* (mg/L culture sup)	Neutralization* (Y/N)	Amino acid sequence of heavy chain V domain
10731_1	1.56	Y	QSGSGVKKPGASVRSVCRASEDLFGDEIHYDDEVIHWLRQVPGORPEWMGMWRPKTGARNQARQFQPRISLTRDRALSTAYLDNLSLTSADSGTYFCARQTEKPDFFADQQGWSFNLWGRGAHFIVSSAST
124918_2	12.24	N	QSGPEVRKPGASVTVSCKTSGYTFTDNYFHWLRQAPGMGLEWGMWINPHNGGSAKKFQGRITMTROTTHIDTAYMELSGLTSDDTAFYFCAREGGHSSGFDYWGGQALVTVTSSAST
132797_4	no expression	n/a	SRGAEVKKPGASVKVSKASGYTFTQYIHWVRQAPQGQLEWGWKIVSGTVNFASLDFRNRIISRDRDLSTAYMDIRGLTQDDTATYFCARQKFASRYSGDQGSYFDLWGRGTLIVVSSAST
143251_3	12.96	N	QAGGGVKKPGASVTLSCKTADQEDVFAAYMHVWRQAPGQTFEFLGWMPKPTGAVSYARKFQGRVSFYMTRELGMAYMDLRNLRFDLTAVYVCARVGGGAADDGSGYTEPPSPDYWGQGTIVTVSSAST
164202_3	18.12	Y	QSASGVRRPGASVRSVCWTSEDIFERSELIYVWRQAP>GRGLEWIGWIKLVSGAVNFGSVDFRDRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFASRYSGDQGSYFDLWGRGTLIVVSSAST
166726_3	8.28	N	QSGAEVKKPGASVKVSKASGYTFTGYMHVWRQAPGQTFEFLGWMPKPTGAVNARQFQGRVSFYRTRELGIAYMDLRDLKFDDTAVYFCARKTKADVSGDDRGFFFDLWGRGTRVIVSSAST
168509_2	no expression	n/a	QSGSGVKKPGTSVRVSCWTSEDIFERTELIHWVRQAPGQGLEWGWVKTVTGAVNFGSPNFRHRVSLTRDRDLFTAHMDIRGLTQGDATYVCARDWGATVWVYLLDSWGQGTPTVTVSSAST
179400_4	11.40	Y	QSGAEVKKPGASVTLSCKTADQEDVFAAYMHVWRQAPGQTFEFLGWMPKPTGAVSYARKFQGRVSFYMTRELGMAYMDLRNLRFDLTAVYFCARKTAGDVSGDNRGYFFDLWGRGSRVIVSSAST
179500_4	6.84	N	QSGSAMKKPGASVRSVCWTSEDIFDTTELIYVWRQAPGQGLEWGWKIVSGTVNFARQFQGRVSFYRTRELGIAYMDLRDLKFDDTAVYFCARKTKGDVSGDDRGFFFDLWGRGTRVIVSSAST
179888_3	21.12	N	ESGPEVRKPGASVKISCKTSGYIFTDNYFHWLRQAPAVGLEWGMWINPHNGYTKSAKKFQGRITMTRODTAVDTAYMELIDLTSDDTAIYFCAREGGHSSGFDYWGGQALVTVTSSAST
184939_4	no expression	n/a	QSGSAMKKPGRSVKVSCWTSEDIFTHRVDLVWRQAPGQGLEWGWKIVSGTVNFASLDFRNRIISRDRDLSTAYMDIRGLTQDDTATYFCARQKFASRYSGDQGSYFDLWGRGTLIVVSSAST
185961_4	14.28	N	QSGSGVKKPGASVRSVCWTSEDIFEKSELIHWVRQAPGQGLEWGMWINPRTGVANNAQKFQDRVTMTRTSITTAAYMELTNLRSDDSATYYCALGDUICDTRTCSYNSFEPWGGQTLITVSSAST
186275_2	1.80	Y	QSGAEVKKPGASVKVSKASGYTFTGYMHVWRQAPGQGLEWGWVKTGAVNFGSLNFRHRVSLTRDRDLFTAHMDIRGLTQGDATYFCARQKFARGDQGWFFDLWGRGTLIVVSSAST
193526_4	no expression	n/a	QAGGMKKPGASMTVSCETADEDIFDAAYMHVWRQVPGQTFEFLGWMPKPTGAVNARKFQGRISFYRTRELAIAVMMDLRDLRFDDTAVYFCARKTVGDVSGDGRGHFFDLWGRGTRVISSAST
193896_4	no expression	n/a	QSGSAMKKPGASVRSVCWTSEDIFDTTELIYVWRQAPGQGLEWGWKIVSGTVNFASSDFRNRIISRDRDLSTAHMDIRGLTQDDTATYFCARQKFERYRGRDQGSYFDLWGRGTVIISAST
196147_4	11.04	Y	QSGAEVKKPGASVKVSKASGYTFTSYMHVWRQAPGQGLEWGWKPVTVGAVNFGSPNFRHRVSLTRDRDLFTAHMDIRGLTQGDATYFCARQKYAGQGQWYFDLWGRGTLIVVSSAST
28936_1	no expression	n/a	QSGAEVKKPGSSIKVSKASGDTISRVAINWVRQAPGQGLEWGWKTVTGAVNFGSLDFRHRISLTRDRDLFTAHMDIRGLTQGDATYFCARQKFESRYTGQGQWYFDLWGRGTHIWSSAST
30263_2	21.12	N	QSGAEVKKPGASAKVSKASGYTFSGYHIHWVRQAPGQGLEWGWINPNSGATQCARKFQERVAMTRDTTNNTVYVELNRLTSDDTATYYCARDWGATVWVYLLDSWGQGTPTVTVSSAST
43243_3	no expression	n/a	QSGAEVKKPGASVKVSKASGYTTYTGVSWLQVPGORPEWMGMWRPKTGARNQARQFQPRISLTRDRALSTAYLDNLSLTSADSGTYFCARQTEKPDFFADQQGWSFNLWGRGAHFIVSSAST
43359_2	6.12	N	QSGAEVKKRPGASVRSVCRASGYTFNFMYYVWRQAPGQGLEVYGVWINPNTGTTKYAQKFQGWVTLTDTSTITTAYLEMGRLTDPDDTALFYCATYAGPAADEAFDVWGQGTMTVTVSSAST
46260_1	no expression	n/a	QSGSGVKKPGASVRSVCWTSEDIFERTELIHWVRQAPGQGLEWGWVKTVTGTVNYARKFQGRVSFYRTRELGIAYMDLRNLRFDLTAVYFCARKTAGDVSGDKRGFFFDLWGRGSRVIVSSAST
47890_1	7.32	N	QSGSAMKKPGASVRSVCWTSEDIFDTTELIYVWRQAPGQGLEWGWKIVSGTVNFASLDFRNRIISRDRDLSTAHMDIRGLTDDTGYIYCARGPMGGSHVYWGGSLVTVSSAST
69713_1	11.52	Y	QSGSGVKKPGASVRSVCWTSEGEFEKSELIHWVRQAPGQGLEWGWVKTVTGAVNFGHQISDRVSLTRDRDLFTAHMDIRGLTQGDATYFCARQKFERRGGQWIFDLWGRGTLIAVSSAST
70679_1	14.88	N	QSGAEVKKRPGASLNVSCHASGYLFNYYIHWVRQAPGQGLEWGMWINPTTKITNLPKFERGVTLTREPSKILYLGLNGLTPDDTAIYFCARSEQEQLAHLDFWGGQSLTVSSAST
71632_2	22.96	Y	QSGAEVKKRPGASVKVSCWTSEDIFERTELIHWVRQAPGQGLEWGWVKTGAVNFGSPNFRHRVSLTRDRDLFTAHMDIRGLTQGDATYFCARQKFERRGGQWYFDLWGRGTLIVVSSAST
74400_3	12.12	N	QSGAEVRRPGASVKVSKASGYNFIAHYHVWRQAPGQGLTWMGWINPDSGDTVYAQNFVDRVMMTRNTSIQTWVLEINVLTEDTAIFYCATPDKKSLGWFDTWGCGSQVTVSSAST

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Table S14. Expression of antibodies with selected heavy chains derived from donor V74, 2008, paired with VRC-PG04 light chain (continued)

Sequence ID	Yield* (mg/L culture sup)	Neutralization* (Y/N)	Amino acid sequence of heavy chain V domain
86984_2	1.92	Y	QSGAEVKKPGASVQVSCKASGYPTFKYMHVVRQAPGQGLEWIGWKA VSGAVNYGSLDFRIHRVSLTRDRDLSTAHMDIRGLTQDDTATYFCARQKFARGDQGWFFDLWGRGTLIVSSAST
94565_1	no expression	n/a	QAGGGMKKPGASMTVSCKTADEDIFDAAYMHVVRQAPGQTFEWLGWMKPVTVGAVNYARQFQGRVSYFRTRELGIAYMDLRDLKFDDTAVYYCARRLPSTYYVSSVMSCDYWGQGTIVTVSSAST
96298_1	13.32	Y	QSGLEVKKPGASVRVSCWTSIEDIFDTTELIYVVRQAPGQGLEWIGWVKIVSGTVNFASLDFRNRIISLRDRDLSTA-HMDIRGLTQDDTATYFCARQKFASRYSGDQGSYFDLWGRGTLIVSSAST
9815_2	22.32	Y	QSGSAMKKPGASVRVSCWTSIEDIFDTTELIYVVRQAPGQGLEWIGWVKIVSGTVNFASLDFRNRIISLRDRDLSTA-HMDIRGLTQGGDTATYFCARQKFYAGGQGWYFDLWGRGTLIVSSAST
104625_2	23.52	N	QSGAEVRKPGASVKVSCKASGYNFIAVYVHVRQAPGQGLTWGMWINPDSGDTVYAQNFLDRVYMTNRTSITTVYLELKDILTADTAIYYCATPKKDDLGWFDTWGQGSQVTVSSAST
43555_1	no expression	n/a	QSGSAMKKPGASVRVSCWTSIEDIFDTTELIYVVRQAPGQGLEWIGWVKIVSGTVNFASLDFRNRIISLRDRDLSTA-HMDIRGLTQDDTAVYYCTSDRRSGNSYVPDHHYHYGMDVWVGQGTIVTVSSAST
76927_2	no expression	n/a	QSGSGVKILGASVRVSCWTSIEDIFERTELIHVVVRQAP ³ GQGLEWWMGWINPNSGGTYAQFQGRVTMTTDTSTNTAYMELRSRDDTAIYYCARDVLRYFDWFLGVEYFEYWGQGTIVTVSSAST
99473_3	no expression	n/a	QSGSGVKILGASVRVSCWTSIEDIFERTELIHVVVRQAP ³ GQGLEWIGWKA VSGAVNYAQDFKGRVAMTRDTSINTAYMQLAGLRSDDTAVYFCARDGGTGPPRYFLWVGQGTLLTVSSAST
121325_4	2.88	N	AGRGVKKPGASVTLSCKTADEDVFDAAYMHVVRQAPGQGTFEWLGWMKPVTVGAVNYARKFQGRVSYFRTRELGIAYMDLRDLRFTDDTAIYYCARAGVWFGELPHWVSGVGGMDVWVGQGTIVTVSSAST
13826_2	10.68	Y	QSEAEVKKPGASMTVSCETADEDIFDAAYMHVVRQAPGQTFEWLGWMKPVTVGAVNYARKFQGRISFYRTRELAIAIYMDLRDLRFDDTAVYFCARKTAGDVSGDNRGYFFDLWGRGSRVIVSSAST
151901_4	10.68	Y	PSGSGVKKPGASVRVSCWTSIEDIFERTELIHVVVRQAP ³ GQGLEWIGWVKTVTGAVNFGSLDFRHRISLTRDRDLFTA-HMDIRGLTQGGDTATYFCARQKFESRYTGCGGWYFDLWGRGTHIVVSSAST
165478_2	24.72	Y	QSGSGVKKPGASVRVSCWTSIEDIFEGSELIHVVVRQAPGQGLEWIGWVKTVTGAVNFGSAYFRHRVSLTRDRDLFTA-HMDIRGLTQGGDTATYFCARQKFMISGGQGWYFDLWGRGTIVVSSAST
17720_4	20.28	Y	QSGAEVKKPGASVRVSCWTSIEDIFDTTELIYVVRQAP ³ GQGLEWIGWVKIVSGTVNFASLDFRNRIISLRDRDLSTA-HMDIRGLTQDDTATYFCARQKFESRYTGCGGWYFDLWGRGTHIVVSSAST
164922_3	21.48	N	QSGAAVKKPGSSVKVSCKASGYIFTGYIHHVIRQAP ³ QGLEWWMGWINPSTGDTKFAHQFQGRVSYFRTRELGIAYMDLRDLKFDDTAVYFCARKTKGDSVGGDRGFFFDLWGRGTRVIVSSAST
186640_2	14.64	Y	QSGAEVKKPGSSVRVSCWTSIEDIFERTELIHVVVRQAP ³ GQGLEWIGWVKTVTGAVNFGSPNFRHRVSLTRDRDLFTA-HMDIRGLTQGGDTATYFCARQKFESRYSGDQGSYFDLWGRGTLIVSSAST
61048_1	22.44	Y	QSGAEVKKPGASVRVSCWTSIEDIFKSELIHVVVRQAPGQGLEWIGWVKTVTGAVNFGSSDFRQRVSLTRDRDLFTA-HMDIRGLTQGGDTATYFCARQKFERGGQGWYFDLWGRGTLIVVSSAST
105239_4	no expression	n/a	QSGAEVKKPGASVKVSCKASGYTFTVYMHVVRQAPRGLEWIGWKA VSGAVNYGSLDFRHRVSLTRDRDLFTA-HMDIRGLTQDDTATYFCARQKFARGDQGWFFDLWGRGTLIVVSSAST
120119_4	9.12	N	QSGAEVKKPGSSVKVSCVSGGVFTSYAVTWVRQAPGQGLEWIGWKA VSGAVNYGSLDFRHRVSLTRDRDLFTA-HMDIRGLTQDDTAIYFCARQKFARGDQGWFFDLWGRGTLIVSSAST
127586_4	19.84	N	QSGAEVKKPGASVKVSCKASGYTFTTYGISVVRQAPGQGLEWIGWVKTVTGAVNFGHQISDRVSLTRDRDLFTA-HMDIRGLTQGGDTATYFCARQKFYAGGQGWYFDLWGRGTLIVVSSAST
156858_3	no expression	n/a	QSGSAMKKPGASVRVSCWTSIEDIFDTTELIYVVRQAPGQGLEWIGWVKIVSGTVNFASLDFRNRIISLRDRDLSTAYMDIRGLTQDDTALYYCAREWRYCTGGSPCSEYLOHWGQGTIVKVSAST
178037_3	no expression	n/a	QSGSATEKPGASVRVSCWTSIEDIFDTTELIYVVRQAPGQGLEWIGWVKIVSGTVNFGSSDFRNRIISLRDRDLSTA-HMDIRGLTQDDTATYFCARQKFESRYGQGSYFDLWGRGTLIVSSAST
186066_4	no expression	n/a	QAGGGMKKPGASMTVSCKTADEDIFDAAYMHVVRQAPGQTFEWLGWMKPVTVGAVNYARRFQGRVSYFRTRELGIAYMDLRDLKFDDTAVYFCARKTKGDSVGGDRGFVSDLWGRGTRVIVSSAST
89680_4	no expression	n/a	QSGAEVKKPGASVKVSCRASGYTFGNHAIISVVRQAPGQGLEWWMGWIRPKTGARNQARQFQPRISLTRDRALSTAYLDNLSADSGLTYFCARQTKPDPFYADQGWSPNLWGRGAHFIVSSAST
149768_4	6.24	N	QSGAEMKKPGSSVKVSCKASGGTFRHSPISVVRQAP ³ GQGLEWIGWKA VSGAVNYGSLDFRHRVSLTRDRDLSTA-HMDIRGLTQDDTATYFCARQKFARGDQGWFFDLWGRGTLIVVSSAST
169094_4	no expression	n/a	QSGSTQMKKPGASVRVPCWTSIEDIFDTTELIYVVRQAPGQGLEWIGVRKIVSGTVNFASLDFRNRIISLRDRDLSTA-HMDIRGLTQDDTATYFCARQKFESRYRGDQGSYFDLWGRGTLIVSSAST

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Table S14. Expression of antibodies with selected heavy chains derived from donor V74, 2008, paired with VRC-PG04 light chain (continued)

Sequence ID	Yield* (mg/L culture sup)	Neutralization* (Y/N)	Amino acid sequence of heavy chain V domain
190244_4	no expression	n/a	NTGSAMKKPGASVRVSCWTSIEDIFDTTELIYWVRQAPGGGLEWIGWVKIVSGTVNFASLDFRNRLSLRDRDLSTAYMDIRGLTQDDTATYFCARQKFAFRYSGDQGSYFDLWGRGTLIVSSAST
196283_4	no expression	n/a	QSGSGVKKLGASVRVSCWTSIEDIFERTELIHWVRQA²QGGLWIGWVKSCQCAVNYGSLDFRQSLSLTRDRDLSTAHMDIRGLTQDDTATYFCARQKFCGEGHQGWFFDLWGRGTLIVSSAST
24972_4	19.84	Y	QSGGGVKKPGTSASFSCRTSDDIYDNEFFDSAFMHVWVRQIPQRPFWMGWMNPRSGAVNVARQLQPRVSMYRDRDLSTAYMEFKSLTSDTGTYFCARKKRGDGFNLVFDLWGRGSQVIVSSAST
74511_1	no expression	n/a	QSGSGVKKPGASVRVSCWTSIEDIFERTELIHWVRQAPGGGLEWIGWVKPVTGAVNYARKFOGRVSFYRTRELGIAYMDLRDLKDDTAVYFCARKTGKDVSGDGRGFFDLWGRGTRVIVSSAST
95589_2	24.12	Y	QSGGGVKKPGASASFSCRTSEDPDNEFFDFSEFMHVVRLTPGQRPWVGWWMNPRSGGVNYAGQFRPRMSMWRDLSTAYMELRDLTADTGLYFCARRKDDYDWWYDLWGRGAHIIVSSAST
43567_2	12.12	N	QSGAEVKKPGASVKVSCKASGYTFTSYDINWVRQATGGGLEWIGWVKTVTGAVNFGSSDFRQVSLTRDRDLFTAHMDIRGLTQGDATYFCARQKFYAGGGQWYFDLWGRGTLIVSSAS
57729_2	18.24	N	QSGAELKKPGSSVRVSCKTSGGSFNINWVRQAPGGGLEWIGWVKIVSGTVNFASLDFRNRLSLRDRDPSTAYMDIRGLTQDDTATYFCARQKFAFRYSGDQGSYFDLWGRGTLIVSSAST
8460_4	no expression	n/a	QFGAEVKKPGSSVKVSCKASGGTFSNNAFSWVRQAPGGGLEWIGWVKTVTGAVNFGSLDFRHRISLTRDRDLFTAHMDIRGLTQGDATYFCARQKFEFRYTGGQGWYFDLWGRGTHIVSSAST
31458_3	13.40	N	QSGAEVKKPGASVKVSCKASGYTFTTYSIGISWVRQAPGGGLEWIGWVKTVTGAVNFGSSDFRQVSLTRDRDLFTAHMDIRGLTQGDATYFCARQKFYAGGGQWYFDLWGRGTLIVSSAST
12467_3	no expression	n/a	QSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGRGLEWIGWVKTVSGAVNFGSVDFRDRVSLTRDRDLFTAHMDIRGLTQGDATYFCARQKFEKLYSDDQGLYFDLWGRGTLIVSSAST
195462_4	2.16	N	QVRDEVKKPGSSMKVSCVTASRGTFESSVAISWVRQAFGGGLEWIGWVKTVTGAVNFGSAVFRHRVSLTRDRDLFTAHMDIRGLTQGDATYFCARQKFKYGGQGWYFDLWGRGTLIVSSAST
86343_1	no expression	n/a	QSGAEVKRPGASVMVSCKASGYTFLNYAFSWVRQAPGGGLEWIGWVKTVSGAVNFGSTDFRERVSLTRDRDLFTAHMDIRGLTQGDATYFCARQKFEKLYTGDQGLYFDLWGRGTLIVSSAST
149590_4	21.48	Y	QSGSGVKKVGASVRVSCWTSIEDIFERTELIHWVRQAPGGGLEWIGWVKAVSGAVNYGSLDFRHRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFEYAGGGQWYFDLWGRGTLIVSSAST
86277_2	no expression	n/a	QSGSGVKKLGASVRVSCWTSIEDIFERTELIHWVRQAPGGGLEWIGWVKAVSGAVNYGSLDFRHRVSLTRDRDLSTAHMDIRGLTQDDTATYFCARQKFAFGDQGWFFDLWGRGTLIVSSAST
18278_1	7.68	Y	QSGSAMKKPGASVRVSCWTSIEDIFERTELIWVRQAPGRGLEWIGWVKTVSGTVNFGSSDFERNRLSLTRDRDLSTAHMDIRGLTQDDTATYFCARQKFESLYSDQGSYFDLWGRGTLIVSSAST
167612_4	20.52	Y	QAGGGVKKPGASVTLCKTADVEDVDAAYMHVVRQAPGQTFEWLGLWMKPVTGAVNYARKFQGRVSFYRTRELGIAYMDLRNLRFDOTAVYFCARKTAGDVSGDKRGFFDLWGRGSRVIVSSAST
99989_1	no expression	n/a	QAGGGMKKPGASMTVCKTADVEDVDAAYMHVVRQAPGQTFEWLGLWMKPVTGAVNYARKFQGRVSFYRTRELGIAYMDLRDPKDDTAVYFCARKTKGDVSGDGRGFFDLWGRGTRVIVSSAST
135083_3	no expression	n/a	QSGSGVKKLGASVRVSCWTSIEDIFERTELIHWVRQAPGGGLEWIGWVKAVTGTGVNFGSLNFRQVSLTRDRDLFTAHMDIRGLTQGDATYFCARQKFEKLYTGGQGWYFDLWGRGTLIVSSAST
53821_1	24.60	Y	QSGSAMKKPGASVRVSCWTSIEDIFDTTELIHWVRQAPGGGLEWIGWVKAVSGAVNYGSLDFRHRVSLTRDRDLSTAHMDIRGLTQDDTATYFCARQKFAFGDQGWFFDLWGRGTLIVSSAST

*no expression, yield was less than 0.60mg/L; Y, yes; N, no; n/a, not available.

Table S15. Neutralization IC₅₀ titers* (μg/ml) of antibodies derived from 454 pyrosequencing against 20 HIV-1 clade A, B and C Env-pseudoviruses

		Clade A (n=6)						Clade B (n=8)						Clade C (n=6)						IC ₅₀ < 50 μg/ml	IC ₅₀ < 1 μg/ml	geometric mean [#] (μg/ml)			
mAb designation	CDRH3 class	Q23.17	Q842.d12	UG037.8	Q168.12	KER2008.12	KER2018.11	YU2	JR-FL	JR-CSF	7165.18	BG1168.1	CAAN.A2	PVO.4	TRO.11	Du156.12	ZM109.4	ZM106.9	TV1.29	ZM176.66	SO18.18				
45-08-104575H/03L		0.840	>50	>50	>50	0.684	0.633	0.085	0.015	2.4	>50	>50	45.9	3.6	1.6	>50	>50	>50	3.9	>50	0.196	2.6	60%	30%	0.956
45-08-42644H/03L		0.126	>50	>50	5.3	0.580	0.494	0.076	0.023	0.366	>50	>50	14.3	1.2	0.268	>50	>50	0.341	>50	0.055	0.200	65%	50%	0.371	
45-01-181371L/01H		0.035	0.014	0.034	0.067	0.225	0.486	0.054	0.013	0.287	>50	0.335	0.436	0.311	0.107	0.070	0.066	0.181	>50	0.008	0.038	90%	90%	0.062	
45-01-181371L/03H		0.010	0.004	0.008	0.045	0.239	0.282	0.011	0.002	0.044	>50	27.2	1.0	0.123	0.014	28.3	3.5	0.066	>50	>50	0.026	85%	65%	0.098	
45-01-181371L/04H		0.085	0.016	1.0	0.083	>50	1.5	0.097	>50	>50	>50	>50	15.4	3.1	>50	1.2	0.127	13.7	>50	>50	0.204	60%	30%	0.513	
45-01-223454L/01H		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	0%	0%	>50	
45-01-223454L/03H		0.804	>50	>50	>50	1.7	0.367	0.039	0.012	18.4	>50	>50	>50	15.7	1.1	>50	>50	11.6	>50	1.2	0.686	55%	25%	0.970	
45-01-223454L/04H		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	0%	0%	>50	
V74-08-179400H/04L	CDRH3-3	>50	>50	12.7	3.3	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	15%	0%	7.5	
V74-08-13826H/04L	CDRH3-3	0.474	0.082	0.319	0.533	>50	>50	0.768	>50	>50	>50	>50	>50	>50	>50	8.1	0.144	4.0	>50	>50	>50	9.9	40%	25%	0.663
V74-08-167612H/04L	CDRH3-3	0.149	0.034	0.123	>50	>50	>50	0.089	>50	>50	>50	>50	>50	1.1	>50	>50	4.2	>50	>50	>50	>50	>50	55%	40%	0.380
V74-08-95589H/04L	CDRH3-4	0.065	0.026	0.162	0.104	>50	0.801	0.119	1.1	0.108	>50	3.4	8.7	0.155	0.159	2.3	0.116	0.790	>50	0.849	0.525	85%	65%	0.354	
V74-08-24972H/04L	CDRH3-5	0.033	0.016	0.065	0.034	0.191	0.272	0.037	0.023	0.040	>50	29.0	1.4	0.060	0.076	1.9	0.080	0.857	>50	1.1	0.470	90%	70%	0.180	
V74-08-10731H/04L	CDRH3-6	0.228	0.067	0.279	0.470	3.3	6.4	0.867	10.9	>50	>50	3.1	>50	3.7	8.7	0.621	0.567	>50	>50	>50	>50	70%	35%	1.2	
V74-08-186275H/04L	CDRH3-7	3.9	0.804	10.9	5.0	>50	>50	2.7	>50	>50	>50	>50	24.9	>50	>50	7.5	>50	>50	>50	>50	>50	40%	5%	4.8	
V74-08-86984H/04L	CDRH3-7	1.3	0.766	2.6	7.9	>50	>50	4.4	>50	>50	>50	>50	39.7	>50	>50	14.2	>50	>50	>50	>50	>50	40%	5%	4.5	
V74-08-196147H/04L	CDRH3-7	>50	4.1	8.2	>50	>50	49.6	18.5	>50	>50	>50	>50	9.8	>50	>50	0.673	>50	>50	>50	>50	>50	35%	5%	6.8	
V74-08-149590H/04L	CDRH3-7	0.047	0.029	0.089	0.130	0.420	0.709	0.143	0.151	0.155	>50	1.0	3.7	0.368	0.282	0.089	0.093	0.820	>50	0.209	0.225	90%	80%	0.225	
V74-08-53821H/04L	CDRH3-7	0.071	0.020	0.104	0.102	0.221	0.703	0.125	0.065	0.162	>50	1.2	8.3	0.282	0.161	0.206	0.109	2.7	>50	>50	0.324	85%	70%	0.235	
V74-08-165478H/04L	CDRH3-7	0.053	0.018	0.106	0.071	0.263	0.341	0.067	0.046	0.593	>50	1.2	4.0	0.463	0.298	0.074	0.077	1.1	>50	46.3	0.226	90%	70%	0.268	
V74-08-71632H/04L	CDRH3-7	0.100	0.050	0.077	0.231	0.445	0.987	0.186	0.119	1.6	>50	2.0	3.3	0.514	0.891	0.122	0.102	5.7	>50	3.0	0.236	90%	65%	0.425	
V74-08-61048H/04L	CDRH3-7	0.060	0.031	0.120	0.228	0.861	0.823	0.223	0.160	0.692	>50	4.0	6.9	0.299	1.2	0.094	0.105	1.8	>50	0.422	0.227	90%	70%	0.375	
V74-08-69713H/04L	CDRH3-7	>50	0.425	0.430	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	10%	10%	0.427	
V74-08-9815H/04L	CDRH3-7	1.4	0.047	21.8	0.158	>50	1.8	22.0	>50	>50	>50	>50	26.5	>50	>50	>50	0.196	>50	>50	>50	40.6	45%	15%	2.3	
V74-08-186640H/04L	CDRH3-8	0.146	0.025	0.118	0.152	>50	>50	0.593	>50	>50	>50	>50	>50	0.472	>50	>50	>50	>50	>50	>50	0.408	35%	35%	0.185	
V74-08-151901H/04L	CDRH3-8	0.114	0.076	0.201	0.849	1.9	4.0	0.481	0.124	0.510	>50	2.8	8.9	1.5	0.945	0.240	0.195	1.8	>50	13.0	0.767	90%	35%	0.778	
V74-08-164202H/04L	CDRH3-8	3.6	0.030	0.091	0.247	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	25%	15%	0.457	
V74-08-96298H/04L	CDRH3-8	>50	0.022	46.3	0.548	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	15%	10%	0.824	
V74-08-18278H/04L	CDRH3-8	0.584	0.027	0.117	0.114	4.5	>50	0.222	>50	>50	>50	>50	>50	0.308	4.2	>50	>50	>50	>50	>50	>50	45%	30%	0.464	
V74-08-17720H/04L	CDRH3-8	13.5	0.090	3.2	0.472	>50	>50	23.4	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	30%	10%	3.1	

*Values < 1 μg/ml are highlighted in red, and values 1 – 50 μg/ml are in green.

#Geometric means were calculated for neutralization sensitive viruses with an IC₅₀ value < 50 μg/ml.

Table S16. Expression of antibodies with phylogenetic-segregation selected light chains

Donor	Year	Sequence ID	Heavy(H) /Light(L) chain	Paired with	Antibody yield (mg/L culture supernatant)
45	2001	223454	L	VRC01H	14.16
45	2001	223454	L	VRC03H	17.52
45	2001	223454	L	VRC-PG04H	19.84
45	2001	181371	L	VRC01H	29.16
45	2001	181371	L	VRC03H	18.96
45	2001	181371	L	VRC-PG04H	25.08

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Appendix

The bioinformatics analysis of four antibodyomes obtained from 454 pyrosequencing of PBMCs of two HIV-1 infected individuals, donor 45 and donor 74, is summarized in this Appendix. As described in the Methods section, a computational pipeline has been developed to process and analyze the 454-pyrosequencing-determined antibodyomes. The results obtained from each step of the pipeline can be used to characterize the basic features of antibodyome and to identify potentially neutralizing antibodies for experimental validation. For each antibodyome, the following analyses are shown in this appendix: read length distribution, germline family distribution, query/germline alignment coverage, germline divergence distribution, sequence identity distribution, gap opening distribution, error-correction/improvement correlation, sequence-quality improvement distribution, and sequence identity/protein length distribution.

1. Analysis of donor 45 heavy-chain 2008 antibodyome (BC) (Figures A-1 to A-13)
2. Analysis of donor 74 heavy-chain 2008 antibodyome (NISC) (Figures A-14 to A-24)
3. Analysis of donor 45 light-chain 2001 antibodyome (BC) (Figures A-25 to A-37)
4. Analysis of donor 74 heavy-chain 2008 antibodyome (BC) (Figures A-38 to A-48)

EXAMPLE 2

Development and Ontogeny of CD4 Binding Site Broad Neutralizing Antibodies

Described below is the natural clone of CH30-34 clonal lineage with the reverted unmutated common ancestors (RUAs) and the clonal lineage intermediates (IAs). These RUAs and IAs are needed for B cell lineage design for design of immunogens that bind well to these RUAs and IAs. (See, e.g., U.S. Prov. 61/542,469 filed October 3, 2011.) The RUAs do not bind well gp120 Envs that the IAs and mature antibodies do. Thus, the RUAs can be used as templates for vaccine design to start of a B cell clone, like the CH30-34 clonal lineage.

Fig. 1-Ex.2 shows the clonal lineage of the broadly neutralizing antibodies CH30-34 with unmutated common ancestors and intermediate antibodies (I1, I2, I3, I4), as well as mature antibodies (CH30, CH31, CH32, CH33, CH34). The RUAs and IAs are inferred models of the B cell receptors of precursors of mature CH30-CH34 antibodies. The figure shows the Kds of binding of the antibodies in the clonal lineage to the E.A244 gp120 Delta 11 recombinant Env as measured in surface plasmon resonance. The sequences shown are the sequences of the clonal lineage heavy chains.

Fig. 2-Ex.2 shows the same binding data as in Fig. 1-Ex.2 but with sequences of the clonal lineage light chains.

Fig. 3-Ex.2 shows the progressive increase in potency of neutralizing antibodies against HIV-1 isolate MN with progressive decrease in inhibitory concentration 50s as affinity maturation progresses. Sequence data provided are a repeat of the VH sequences. Also shown are indications of what antibodies mediate ADCC as + or – (see Fig. 4-Ex.2).

Fig. 4-Ex.2 shows antibody dependent cellular cytotoxicity assay curves of RUAs, IAs and CH31 antibody against CM235 HIV infected CD4 T cells.

Figs. 5-Ex.2, 6-Ex.2 and 7-Ex.2 show binding curves of the members of the clonal lineage to the E.A244 gp120 recombinant Env protein (Fig. Ex.2), to the resurfaced core protein (RSC) (Fig. 6-Ex.2) and to the group M consensus Env CONS gp120 protein (Fig. 7-Ex.2). All three figures show that the RUAs do not react with these envs while the IAs and CH31 do react. These data imply that what is needed to induce these broad neutralizing antibodies are immunogens designed using the RUAs as templates.

Fig. 8-Ex.2 shows the steps of a B cell lineage-based approach (see also U.S. Prov. 61/542,469).

What is claimed is:

1. An antibody against the CD4 binding site of HIV-1 comprising a heavy or light chain amino acid sequence of CH30, CH31 or CH32 as shown in Figure 1C, or fragment of said antibody.
2. The antibody according to claim 1 wherein said fragment is an antigen-binding fragment.
3. The antibody according to claim 1 wherein said fragment comprises a complementarity determining region (CDR) of said heavy or light chain amino acid sequence of CH30, CH31 or CH 32.
4. A composition comprising the antibody according to claim 1, or said fragment thereof, and a carrier.
5. An isolated nucleic acid encoding the antibody according to claim 1, or said fragment thereof.
6. A vector comprising the nucleic acid according to claim 5, wherein said nucleic acid is present in said vector in operable linkage with a promoter.
7. An isolated host cell comprising the vector according to clam 6.
8. A composition comprising the vector according to claim 6 and a carrier.
9. A method of inhibiting HIV-1 infection in a patient comprising administering to said patient said antibody according to claim 1, or said fragment thereof, in an amount sufficient to inhibit said infection.

10. The method according to claim 9 wherein said antibody is administered parenterally.

11. The method according to claim 9 wherein a nucleic acid encoding said antibody, or said fragment thereof, is administered to said patient under conditions such that said nucleic acid is expressed and said antibody, or said fragment thereof, is produced.

12. A method of treating HIV-1 infection in a patient comprising administering to said patient said antibody according to claim 1, or said fragment thereof, in an amount sufficient to effect said treatment.

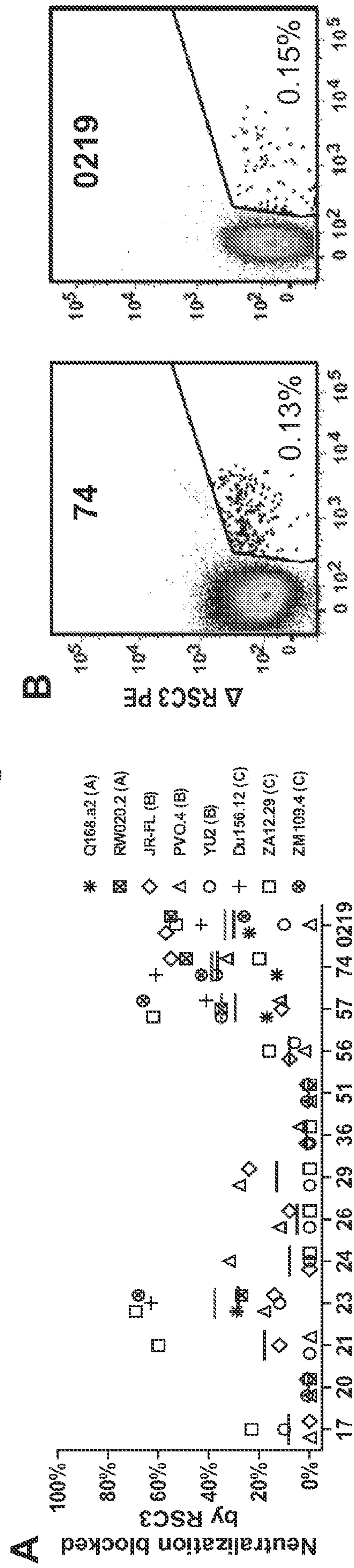
13. The method according to claim 12 wherein said antibody is administered parenterally.

14. The method according to claim 12 wherein a nucleic acid encoding said antibody, or said fragment thereof, is administered to said patient under conditions such that said nucleic acid is expressed and said antibody, or said fragment thereof, is produced.

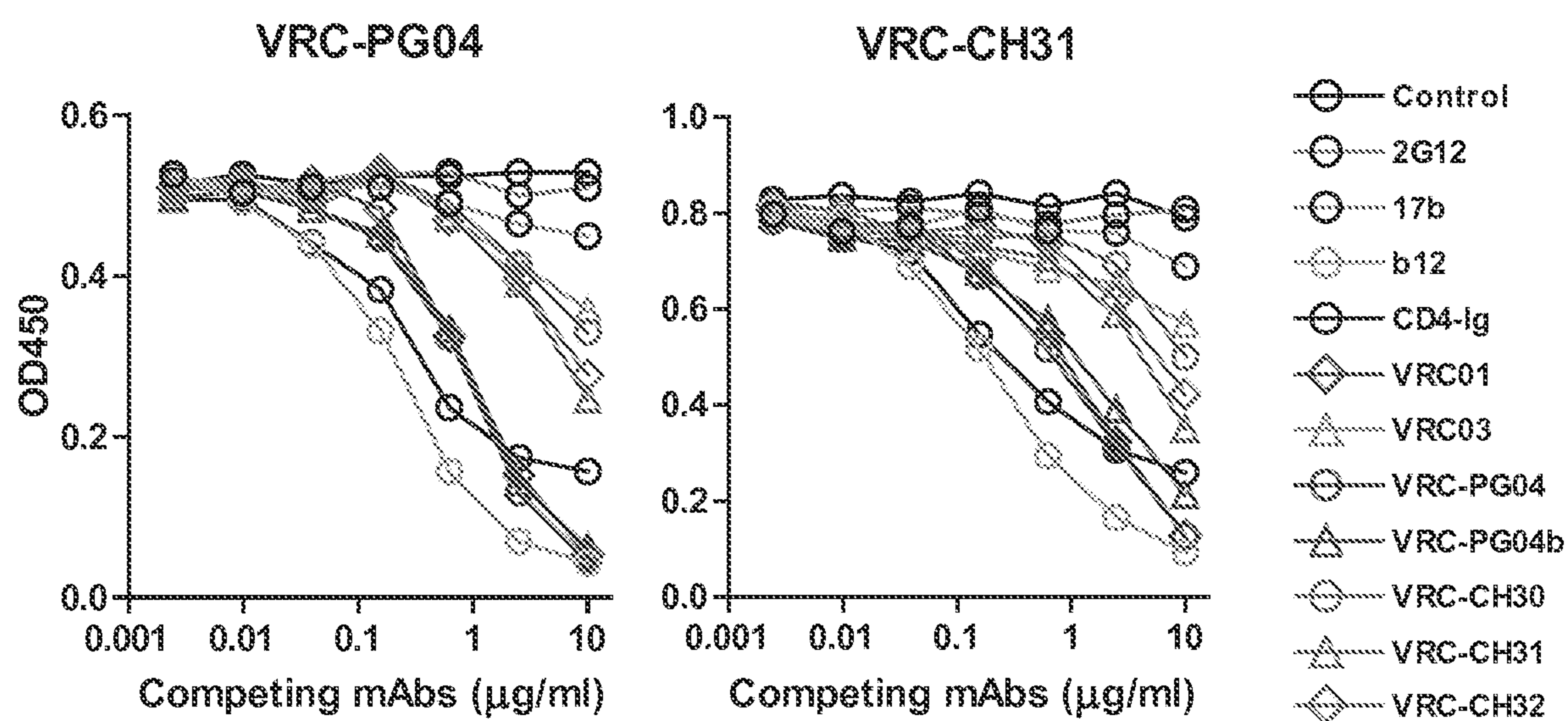
15. A method for selecting a candidate immunogen suitable for use in producing broadly neutralizing antibodies against HIV-1 in a patient comprising screening test immunogens for their ability to bind to reverted unmutated common ancestor antibodies (UAs) or clonal lineage intermediate antibodies (IAs) of the clonal lineage of CH30, CH31 or CH32 and identifying test immunogens that bind to said UAs or IAs, wherein test immunogens that bind to said UAs or IAs are said candidate immunogens.

16. The method according to claim 15 wherein said UAs or IAs comprise a heavy or light chain amino acid sequence as shown in Figure 1-Ex.2, Figure 2-Ex.2 or Figure 3-Ex.2.

Figure 1

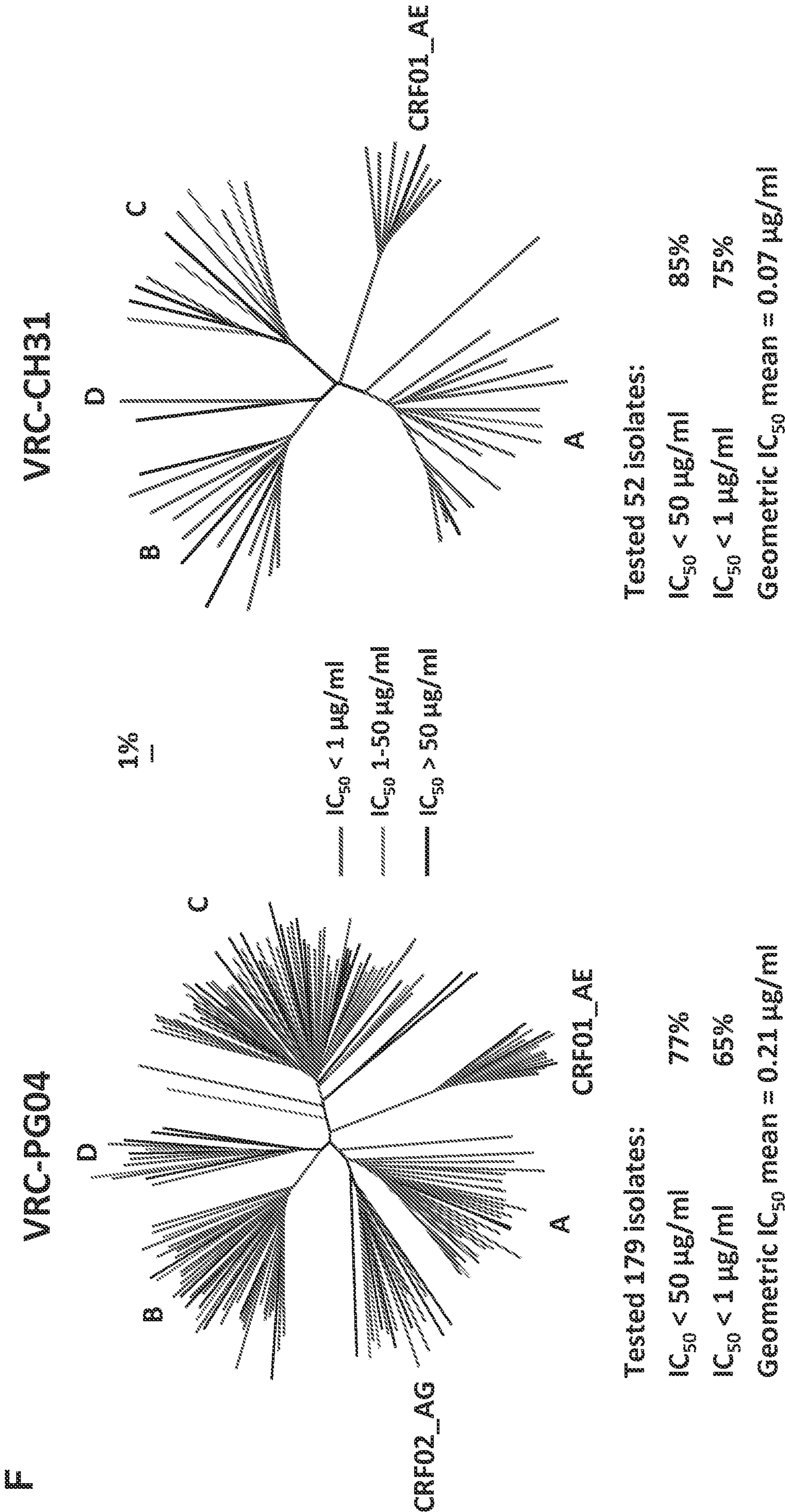


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Figure 1**D****E**

mAb ID	Specificity	Sequence identity to VRC-PG04		Sequence identity to VRC-CH31	
		heavy	light	heavy	light
VRC01	CD4bs	50%	64%	48%	51%
VRC03	CD4bs	51%	66%	48%	47%
VRC-PG04	CD4bs	100%	100%	48%	47%
VRC-PG04b	CD4bs	98%	98%	49%	47%
VRC-CH30	CD4bs	49%	50%	95%	97%
VRC-CH31	CD4bs	51%	48%	100%	100%
VRC-CH32	CD4bs	50%	48%	95%	96%
b12	CD4bs	54%	66%	53%	48%
b13	CD4bs	46%	50%	45%	77%
F105	CD4bs	37%	69%	38%	55%
17b	CD4i	51%	61%	49%	62%
48d	CD4i	43%	51%	50%	72%
X5	CD4i	50%	67%	49%	56%

Figure 1



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Figure 1G

>Vf4 Light Chain (VRC-CH30)

GACATCCAGATGACCCAGTCTCCATCGTCCTTGTCTGCATCACTCGGAGACAGAGTCACCATCACTTGTGAGGCGA
GTCGGGGCATTGGTAAAGATTTAAATTGGTACCAGCAGAAACCGGGAAAGGCCCTAAGTTACTGGTCTCTGATG
CATCCATTTTGGAAAGGGGGGGTCCCATCAAGGTTCAAGTGGGAGTGGATTTACCAAAATTTTAGTCTGACCATCAG
CAGCCTGCAGCCTGAGGATGTTGCGACATACTTCTGTGAGCAGTACGAGACTTTTGGCCAGGGGACCAAAGTGGA
CATCAAA

>Vf4 Heavy Chain (VRC-CH30)

CAGGTGCAGCTGGTGCAGTCAGGGGCTGCCGTGAGGAAGCCTGGGGCCTCAGTGACTGTCTCCTGCAAATTCGCT
GAAGACGACGACTACTCTCCACACTGGGTGAATCCGGCCCCTGAACACTATATTCACTTTCTACGACAGGGCCCCTG
GACAGCAACTGGAGTGGTTGGCATGGATGAACCCTACGAATGGCGCCGTCAATTATGCATGGCAGCTTCATGGCA
GGCTCACGGCGACAGAGACGGGTCCATGACTACAGCCTTTTTGGAAGTGAGGAGTCTAAGATCTGACGACACG
GCCGTCTATTATTGTGCGAGGGGCCAGAAAAGGGGGCGGAGTGAATGGGCCTACGCCCACTGGGGCCAGGGGAAC
CCCGGTCGCGTCTCCTCA

>VRC-CH31 (vf5) Light Chain

GACATCCAGATGACCCAGTCTCCATCGTCCCTGTCTGCATCACTCGGGGACAGAGTCACCATCACTTGCCAGGCGA
GTCGGGGCATTGGCAAAGATTTAAATTGGTACCAGCAGAAAGCGGGAAAAGGCCCTAAGTTACTGGTCTCTGATG
CATCCACTTTTGGAAAGGGGGGGTCCCATCAAGGTTCAAGTGGGAGTGGATTTACCAAAATTTTAGTCTGACTATCAG
CAGCCTGCAGGCTGAGGATGTTGCAACATACTTCTGTCAACAATACGAGACTTTTGGCCAGGGGACCAAGGTGGA
CATCAAA

>VRC-CH31 (vf5) Heavy Chain

CAGGTGCAGCTGGTGCAGTCAGGGGCTGCCGTGAGGAAGCCTGGGGCCTCAGTGACTGTCTCCTGTAAATTCGCT
GAAGACGACGACTACTCTCCATACTGGGTGAATCCGGCCCCTGAACATTTTATTCACTTTTTTCGACAGGGCCCCTG
GACAACAAC TAGAGTGGCTGGCATGGATGAACCCAACGAATGGCGCCGTTAATTATGCATGGTACCTTAATGGCA
GGGTACAGGGCGACAGGGACAGGTCCATGACTACAGCCTTTTTGGAAGTGAAGAGTCTAAGATCTGACGACACG
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TCCGGTCGTCGTCTCGTCA

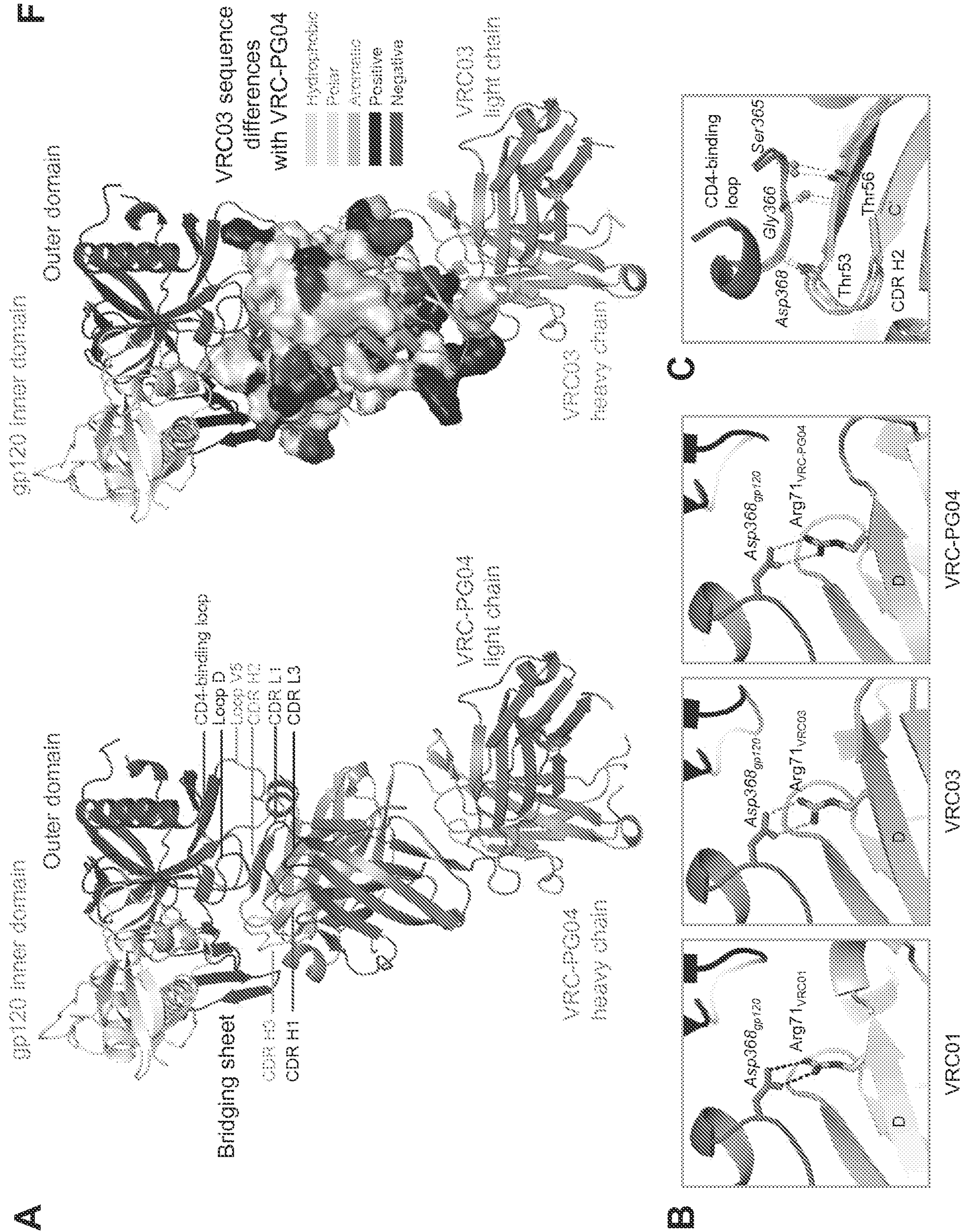
>Vf6 Light Chain (VRC-CH32)

GACATCCAGATGACCCAGTCTCCATCGTCCCTGTCTGCATCACTCGGAGACAGAGTCACCATCACTTGCCAGGCGA
GTCGGGGCATTGGCAAAGATTTAAATTGGTACCAACAGAAACCGGGAAAGAGCCCCTAAGTTACTAGTCTCTGATG
CATCCATTTTGGAAAGGGGGGGTCCCAACGAGATTCAGTGGGAGTGGATTTACCAAAACTTTAGCCTGACCATCA
GCAGCCTGCAGGCTGAGGATGTTGCAACATATTTCTGTGAGCAATACGAAACTTTTGGCCAGGGGACCAAGGTGG
ACATCAAA

>Vf6 Heavy Chain (VRC-CH32)

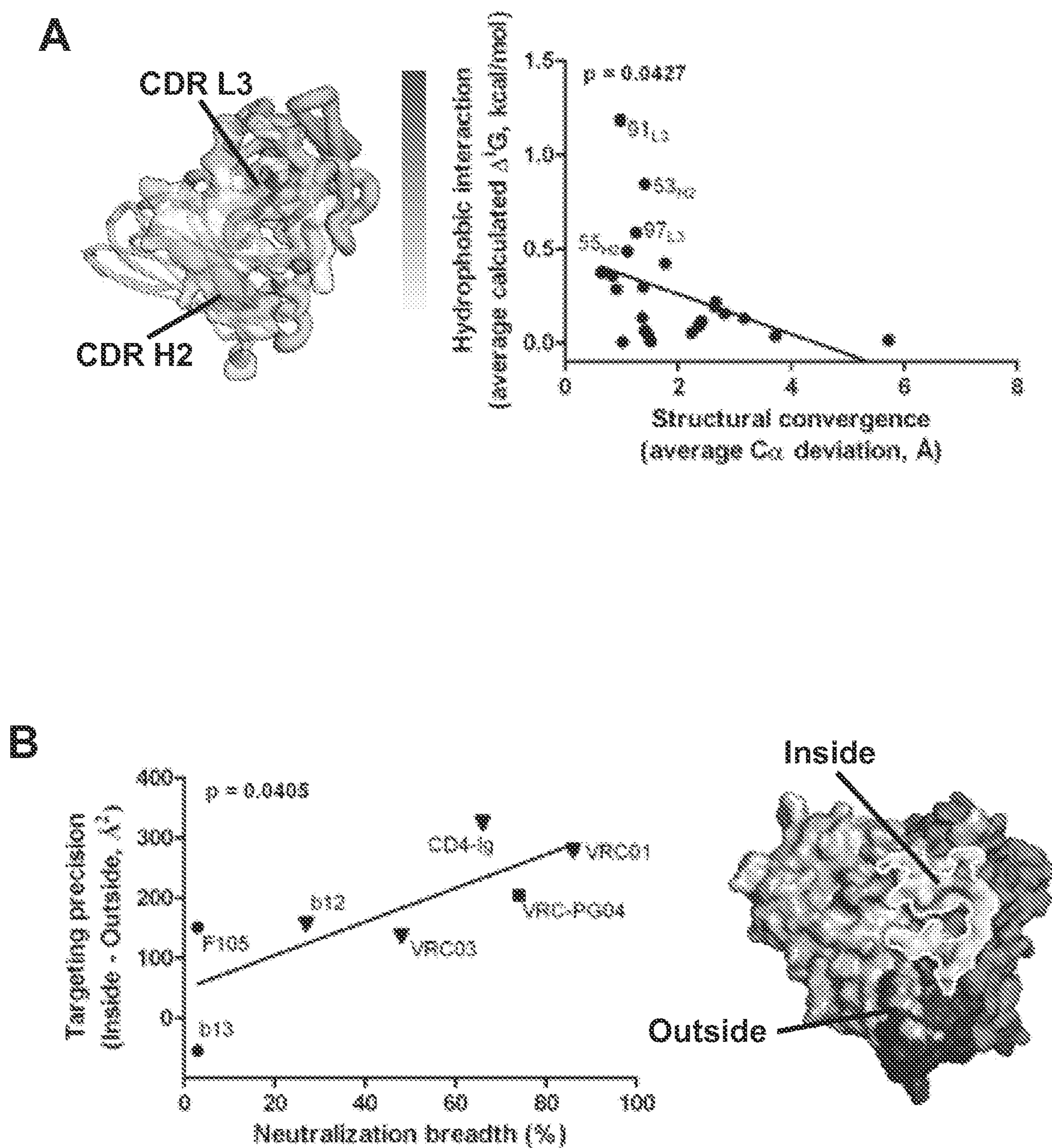
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GAAGACGACGACTTCTCTCCACACTGGGTGAATCCGGCCCCTGAACACTATATTCATTTTCTGCGACAGGCACCTG
GACAACAAC TAGAGTGGTTGGCATGGATGAAGCCTACGAATGGTGCCGTCAATTATGCATGGCAACTTCAGGGCA
GGGTACAGGTGACAGGGACAGGTCCAGACTACAGCCTTTTTGGAAGTTAAGAATCTGAGATCTGACGACACGG
CCGTCTATTATTGTGCGAGGGGCCAGAAAAGGGGGCGCAGCGAGTGGGCCTATGCCCACTGGGGCCAGGGGAACC
CCGGTCGTCATCTCCGCA

Figure 2



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Figure 3



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Figure 3

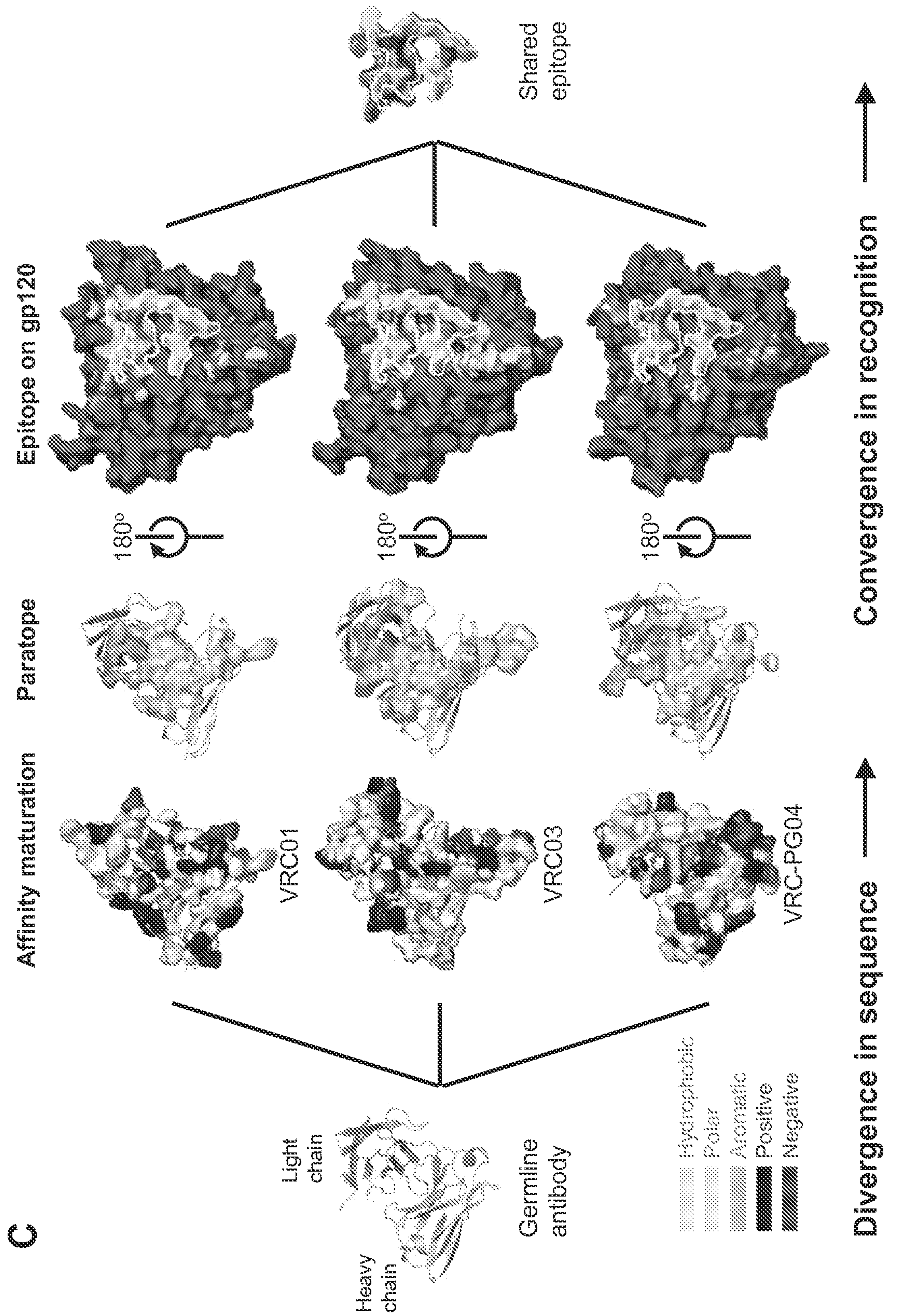
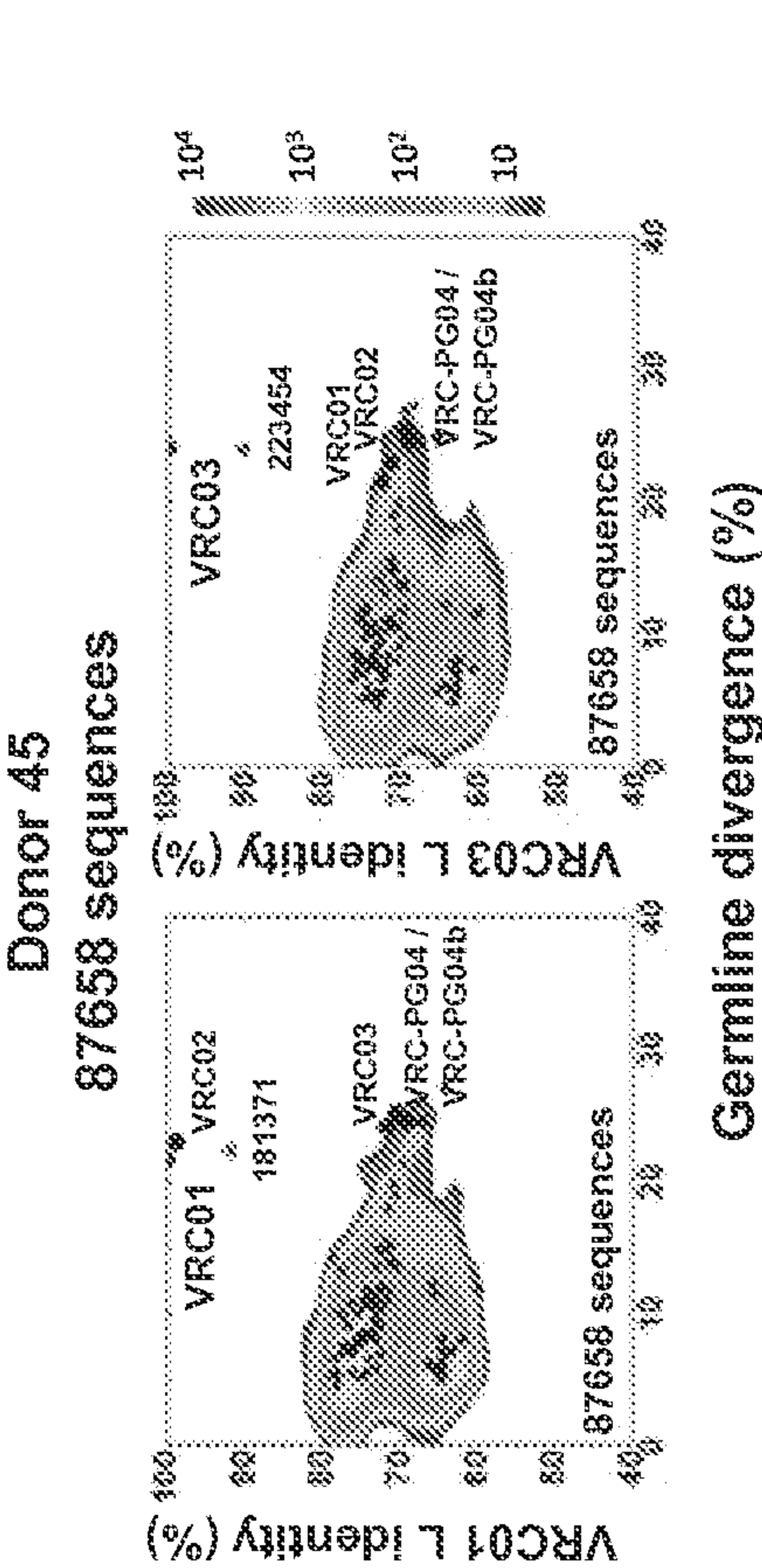
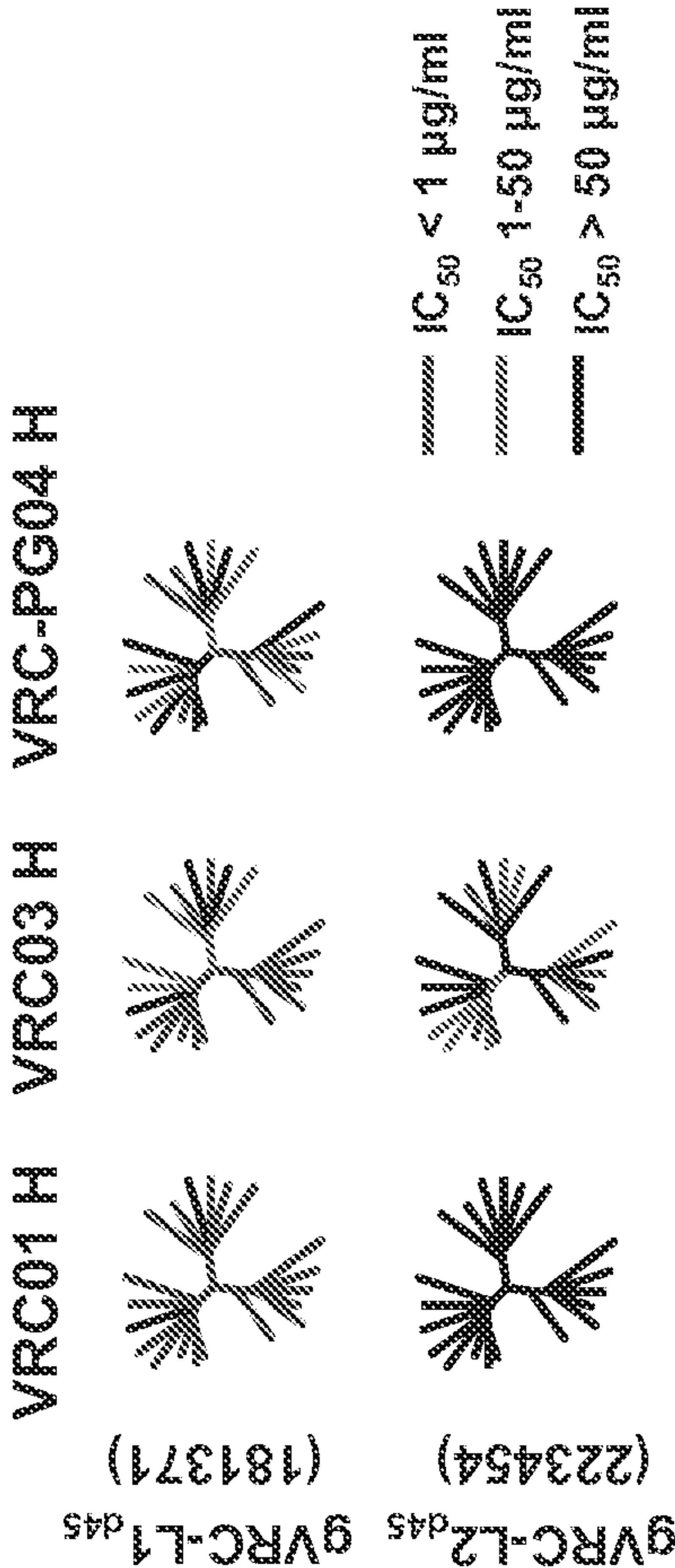


Figure 4

C Light-chain antibodyome



D Light-chain neutralization



A Heavy-/Light-chain complementation

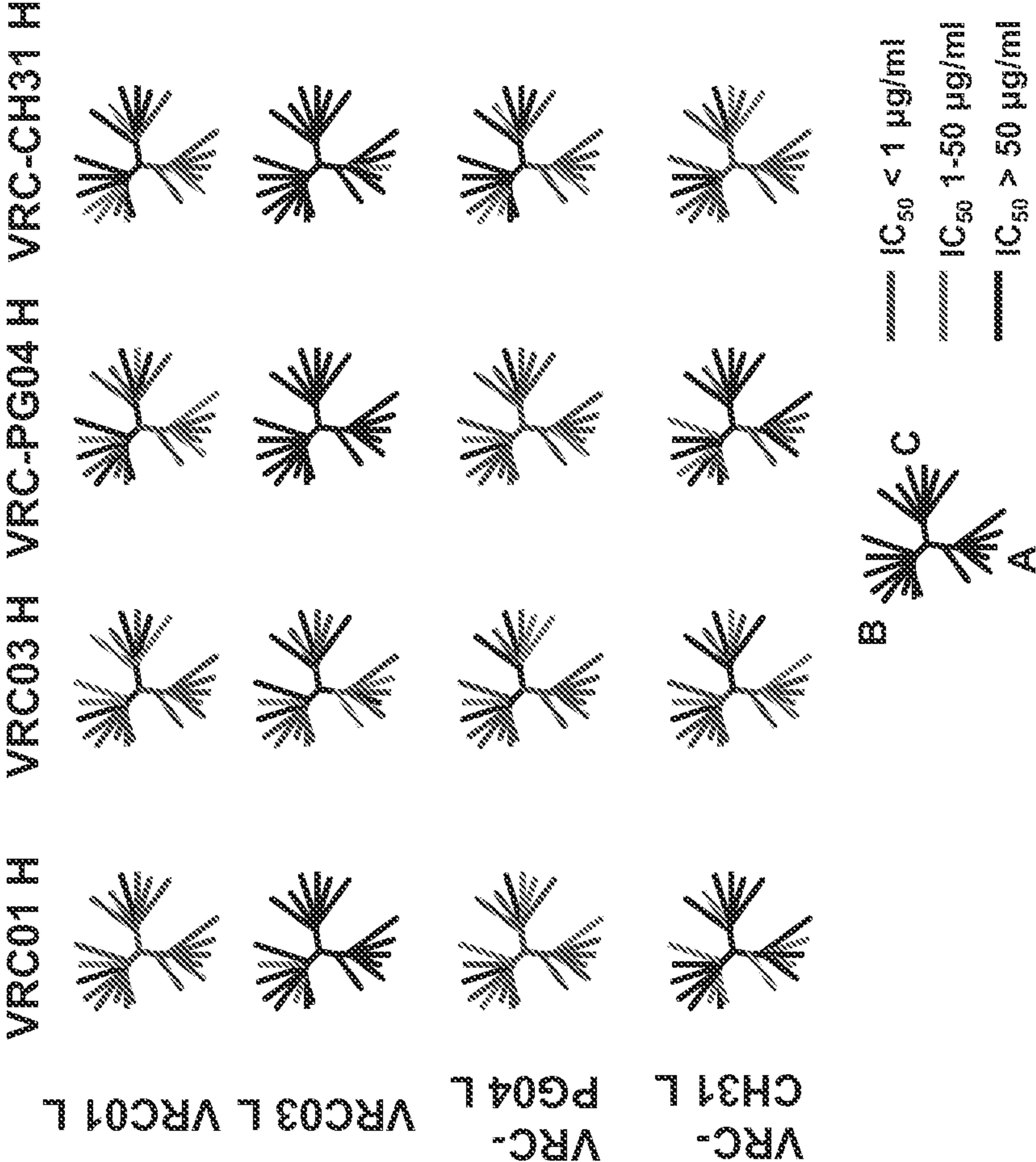


Figure 4

B Heavy-chain antibodyome

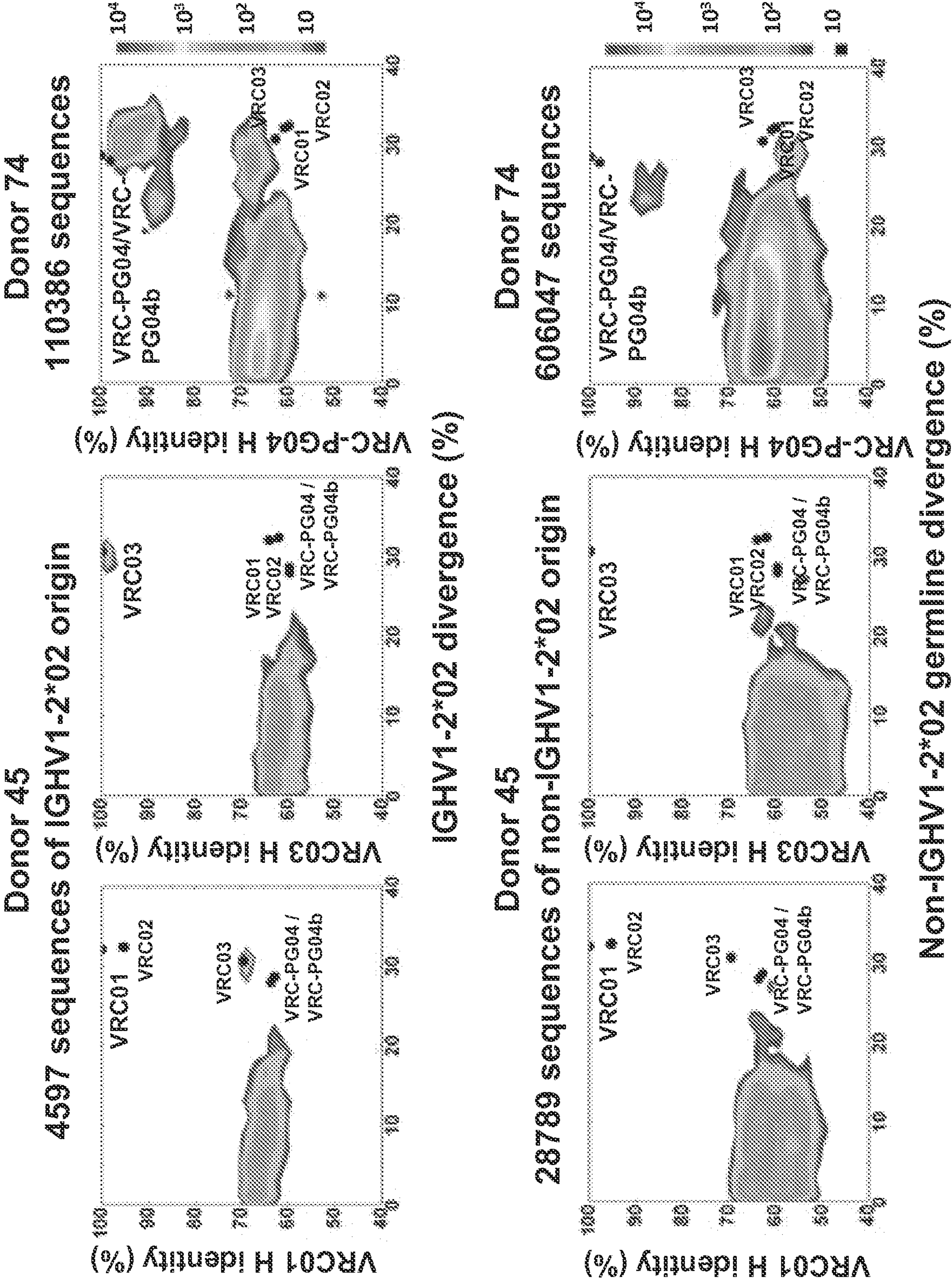
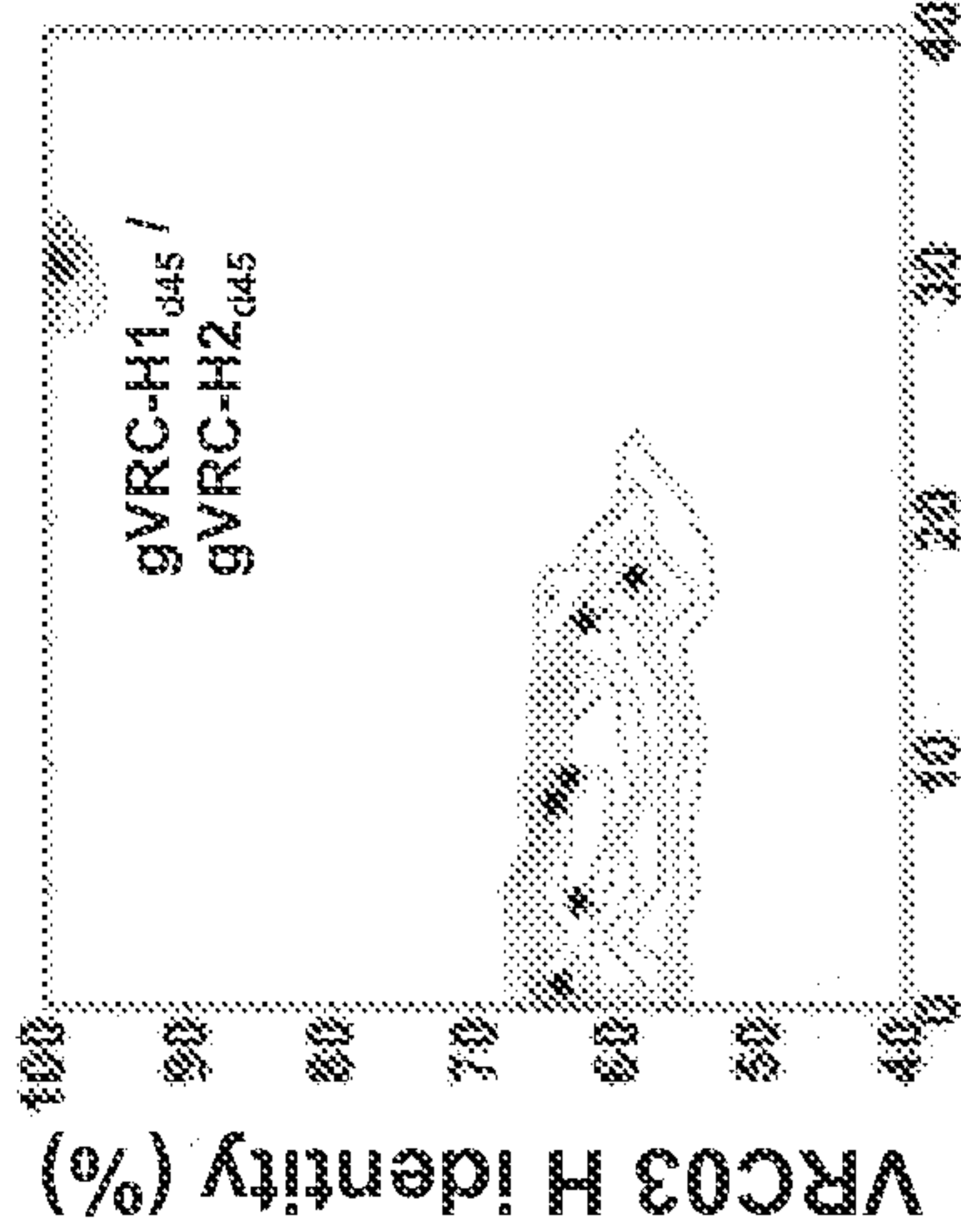
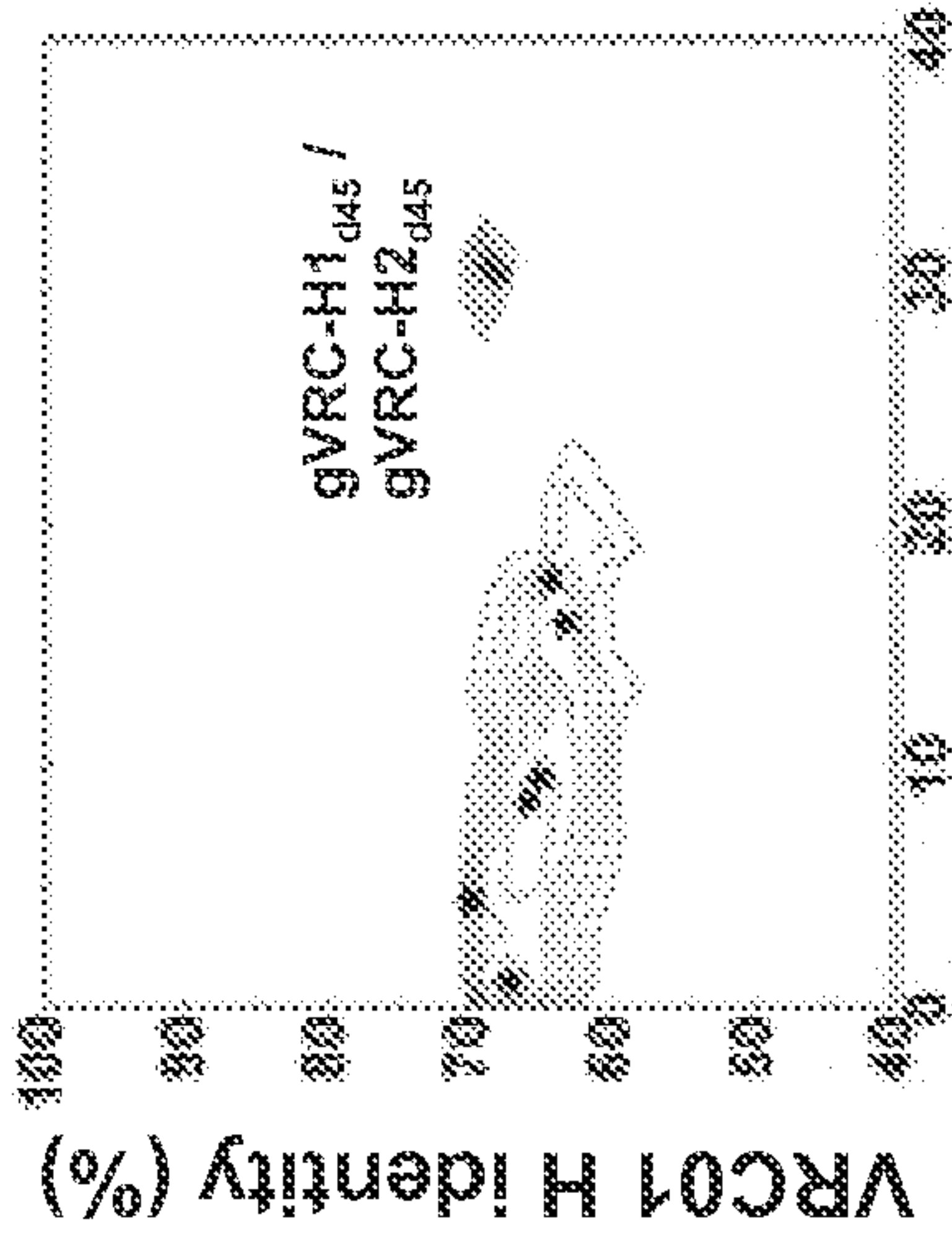


Figure 4

E Heavy-chain neutralization

Donor 45

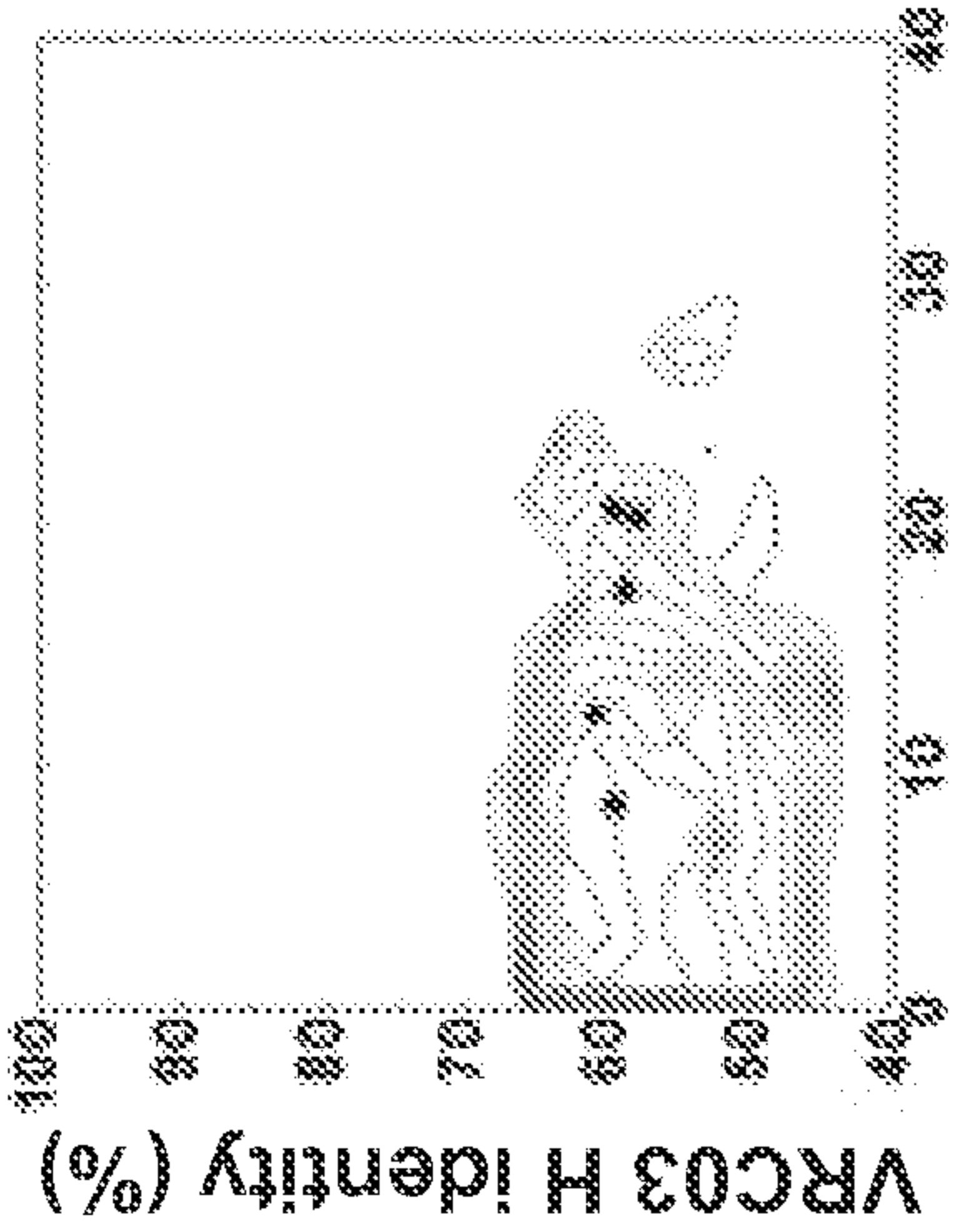
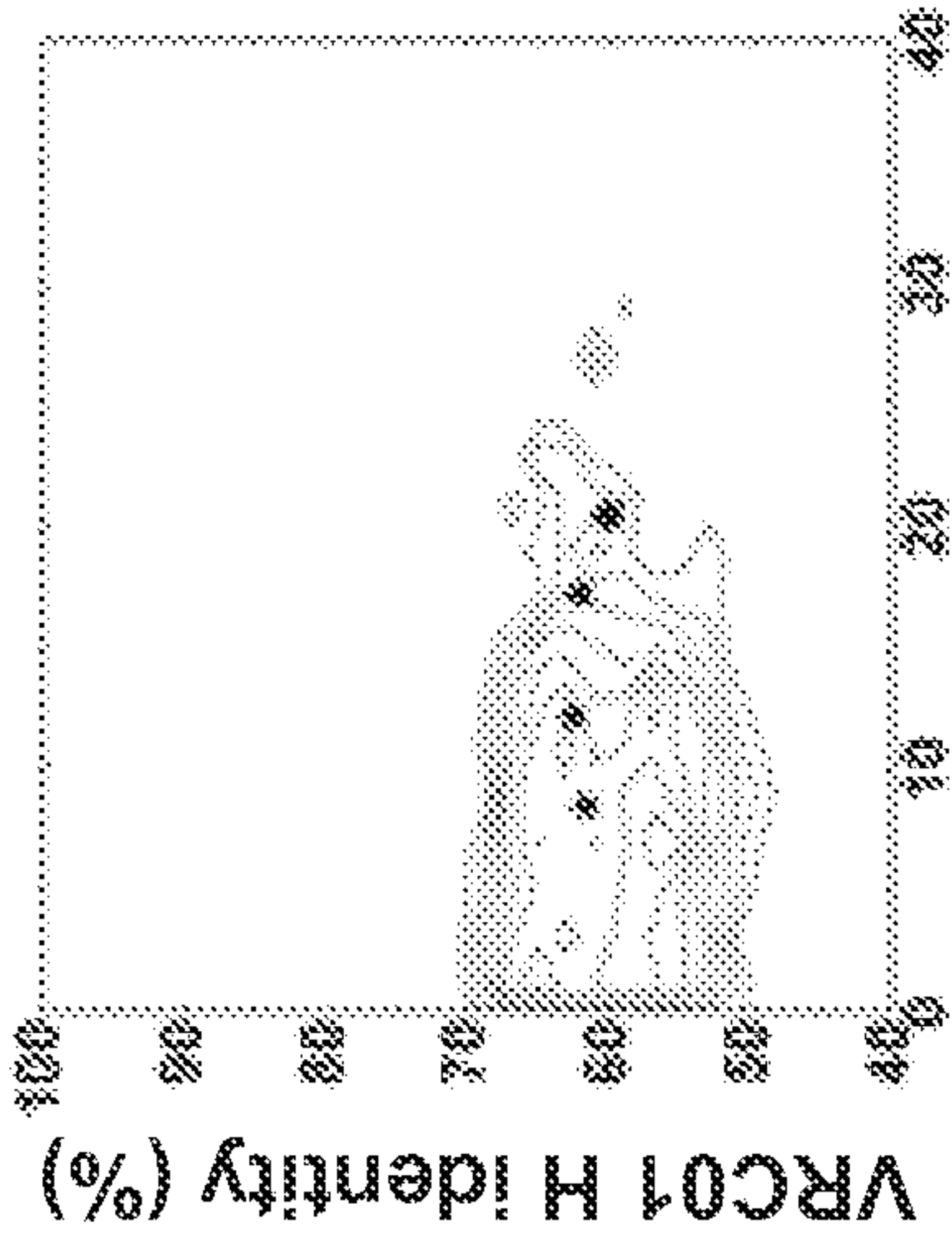
4597 sequences of IGHV1-2*02 origin



IGHV1-2*02 divergence (%)

Donor 45

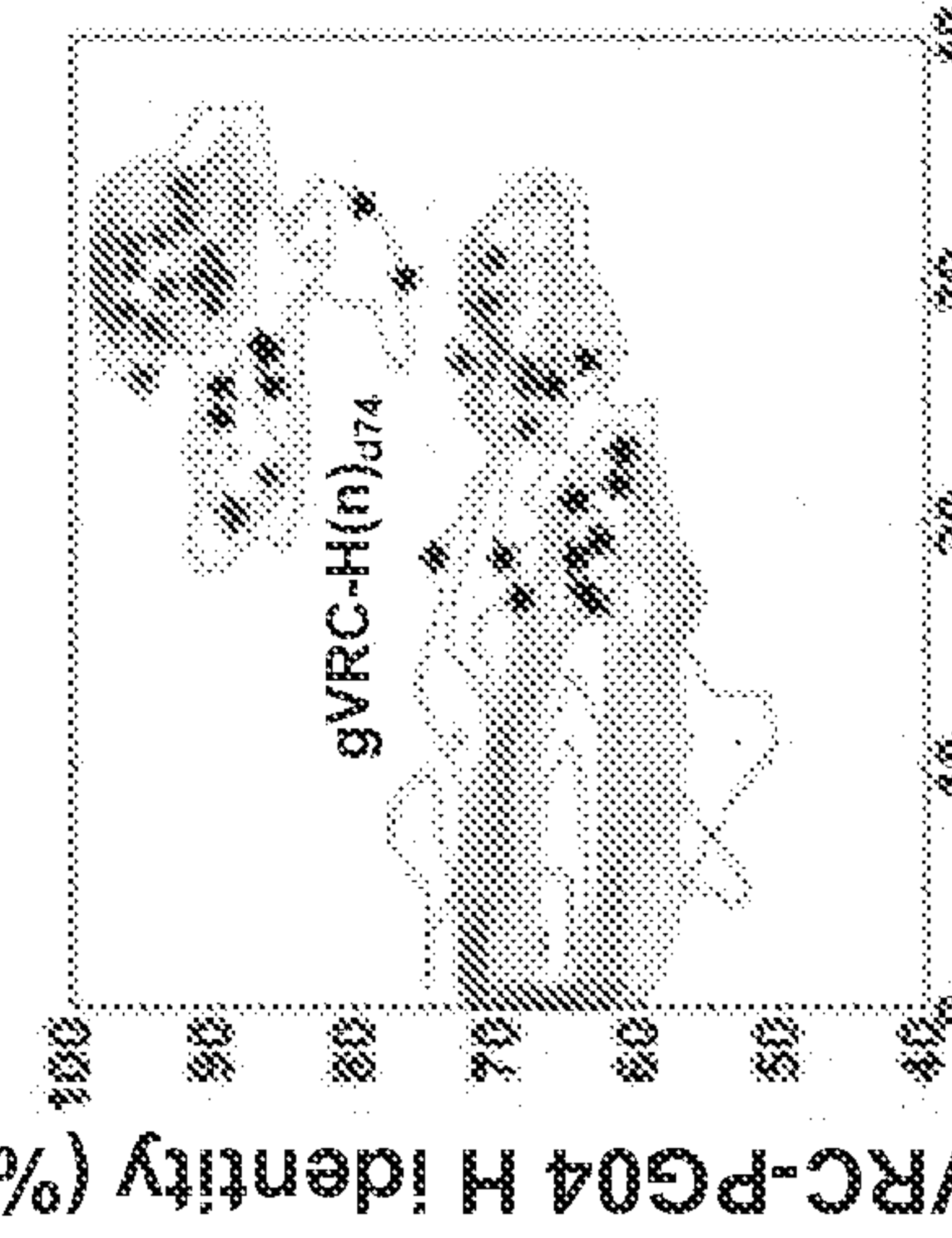
28789 sequences of non-IGHV1-2*02 origin



Non-IGHV1-2*02 germline divergence (%)

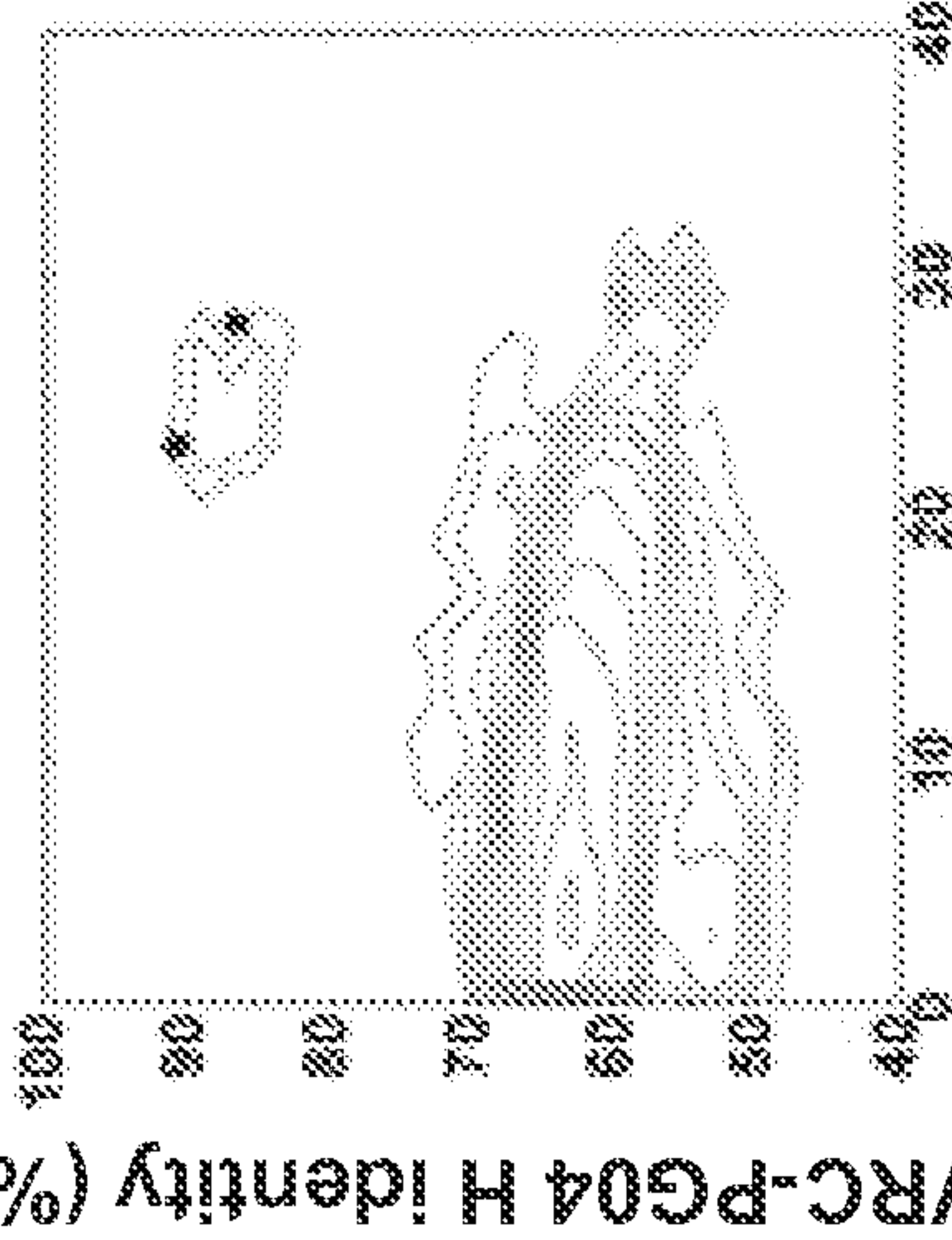
Donor 74

110386 sequences



Donor 74

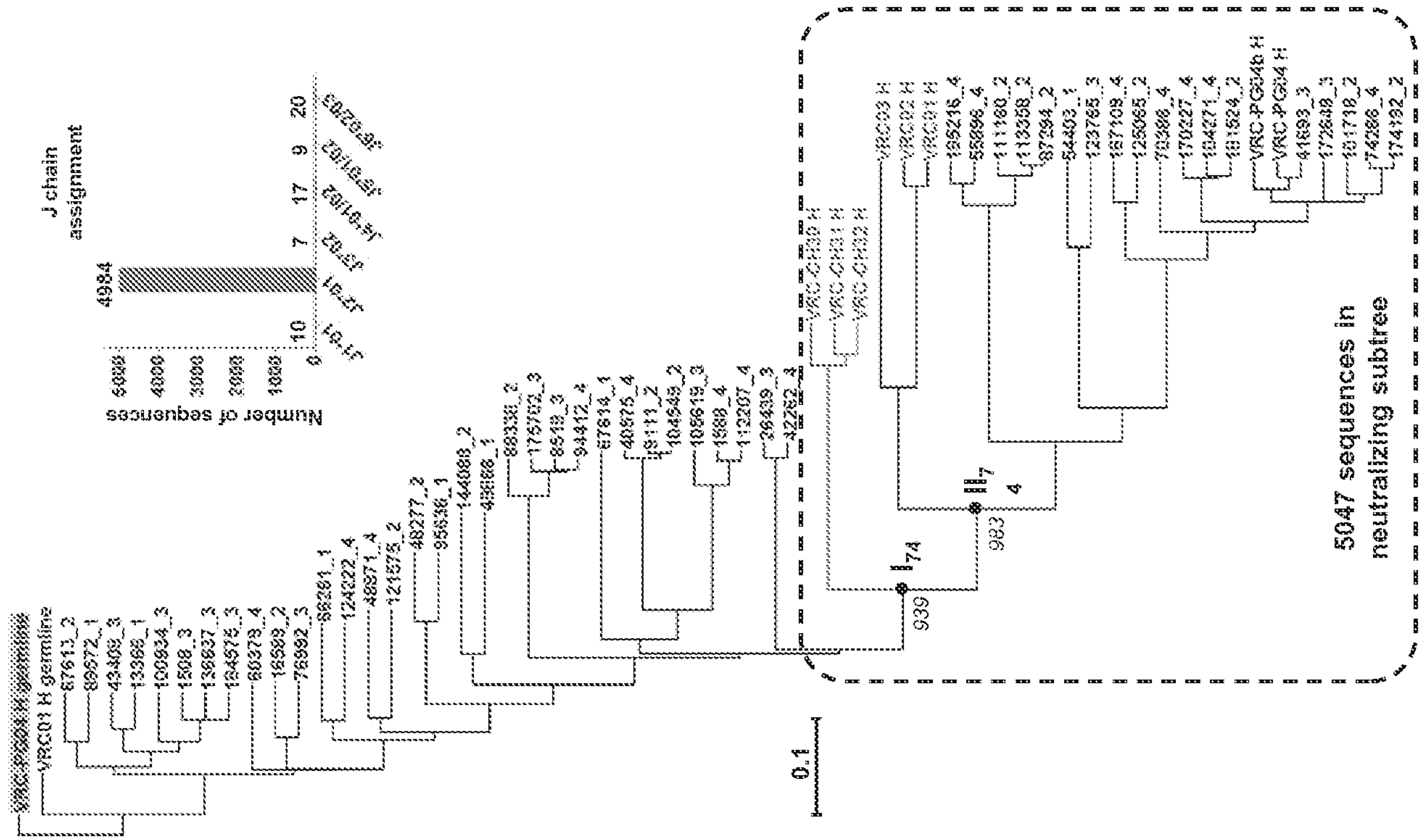
606047 sequences



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Figure 5

Donor 74 H 2008 - 110,0386 sequences of IGHV1-2*02 origin



Donor 45 H 2008 - 4597 sequences of IGHV1-2*02 origin

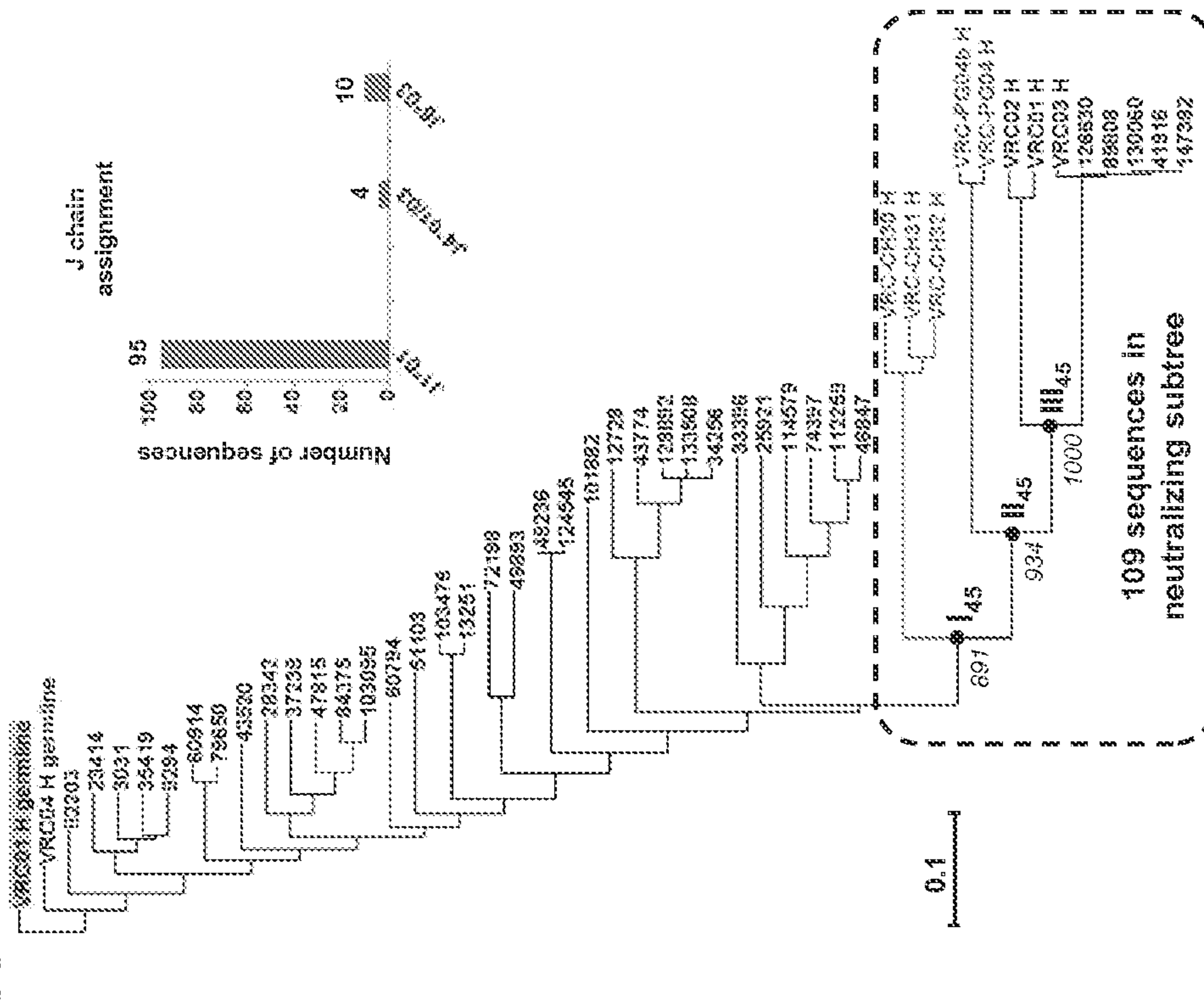
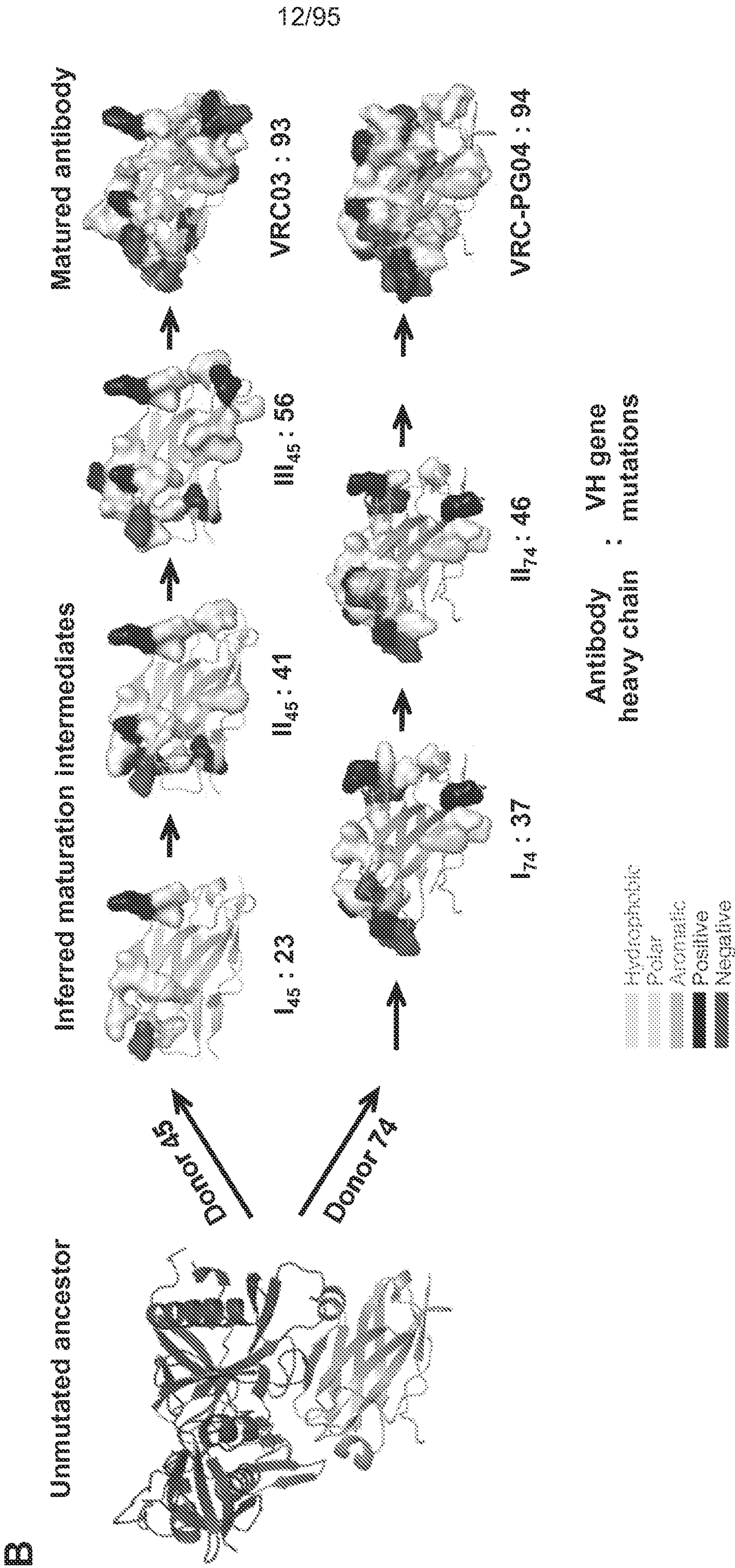
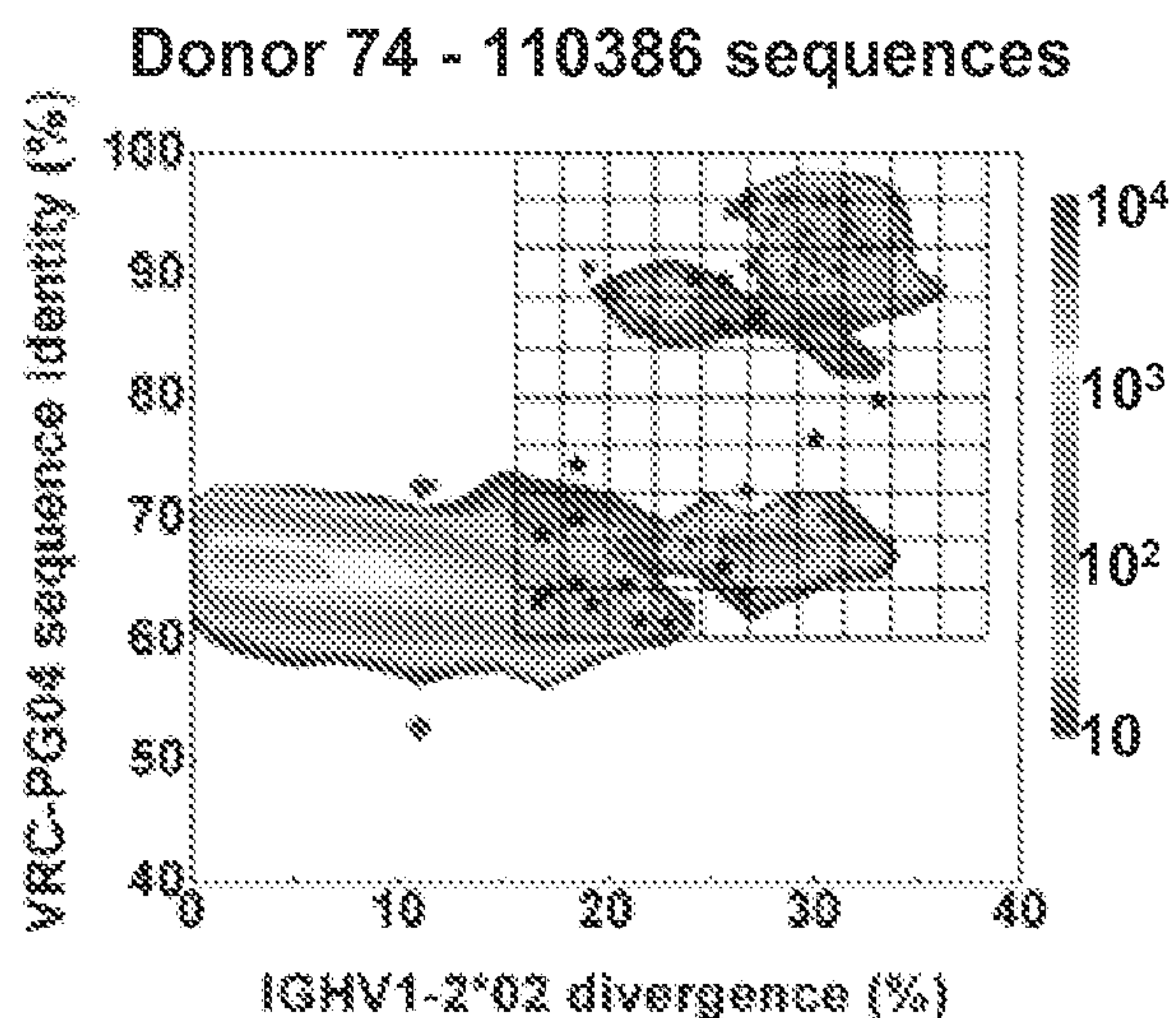
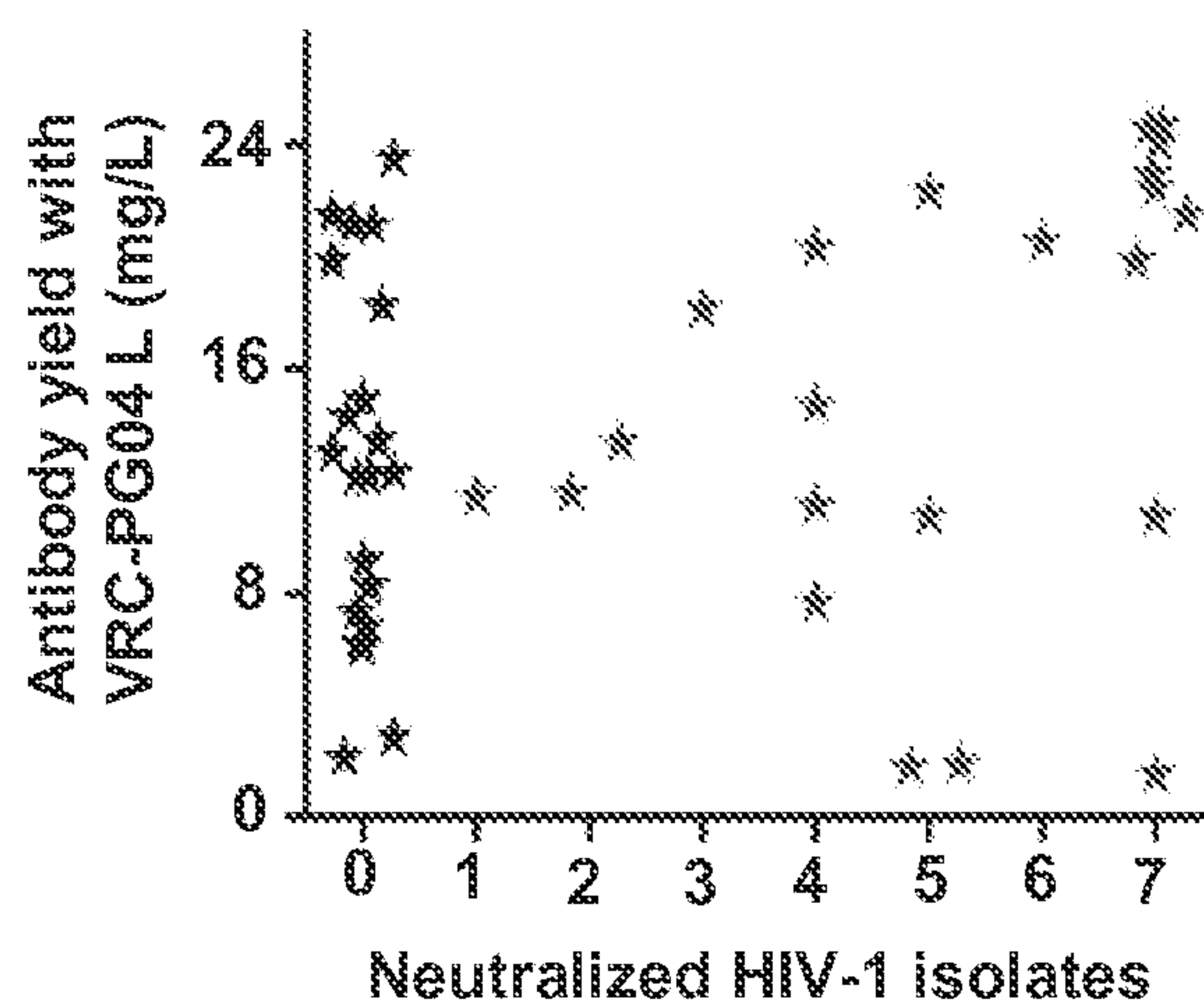
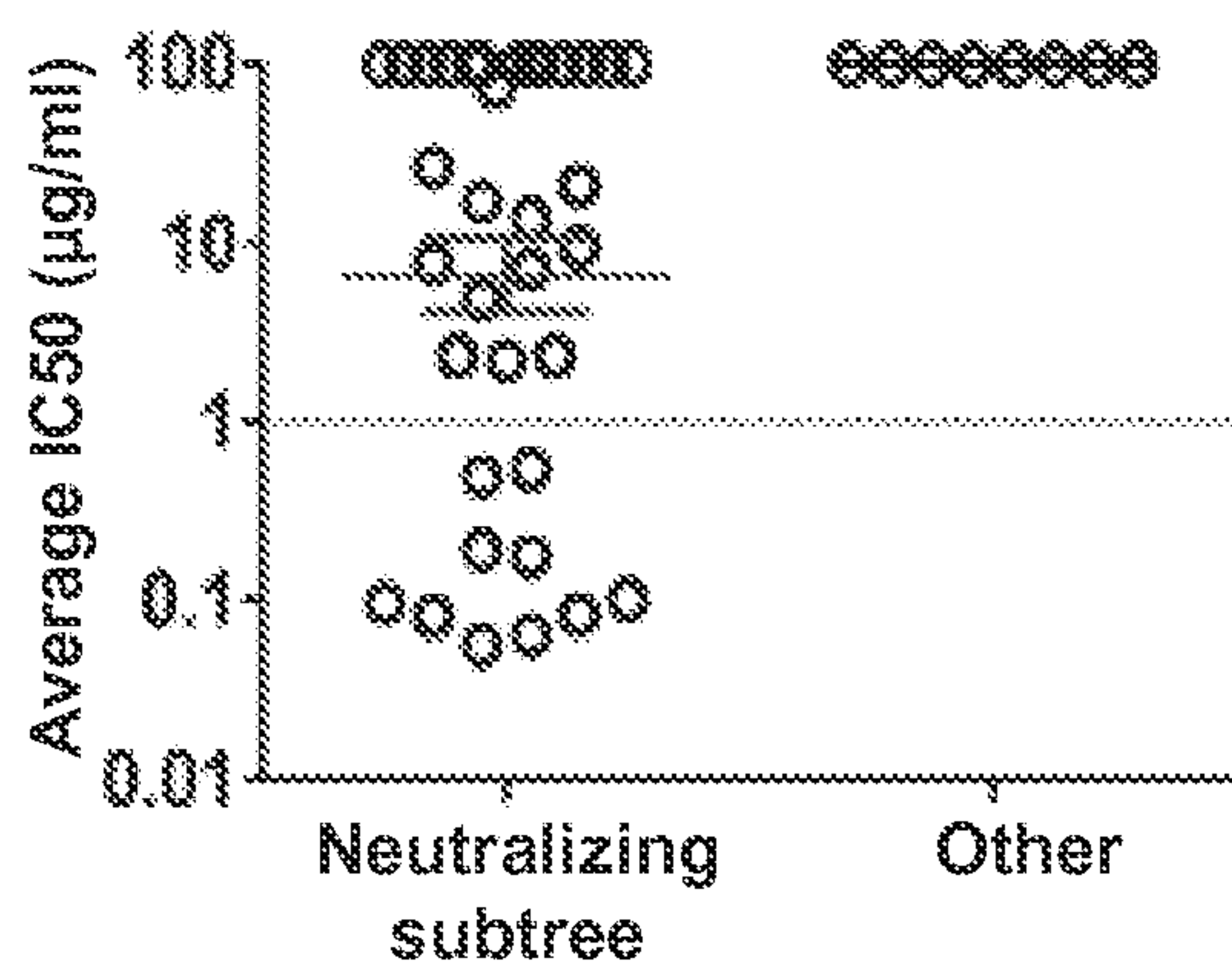
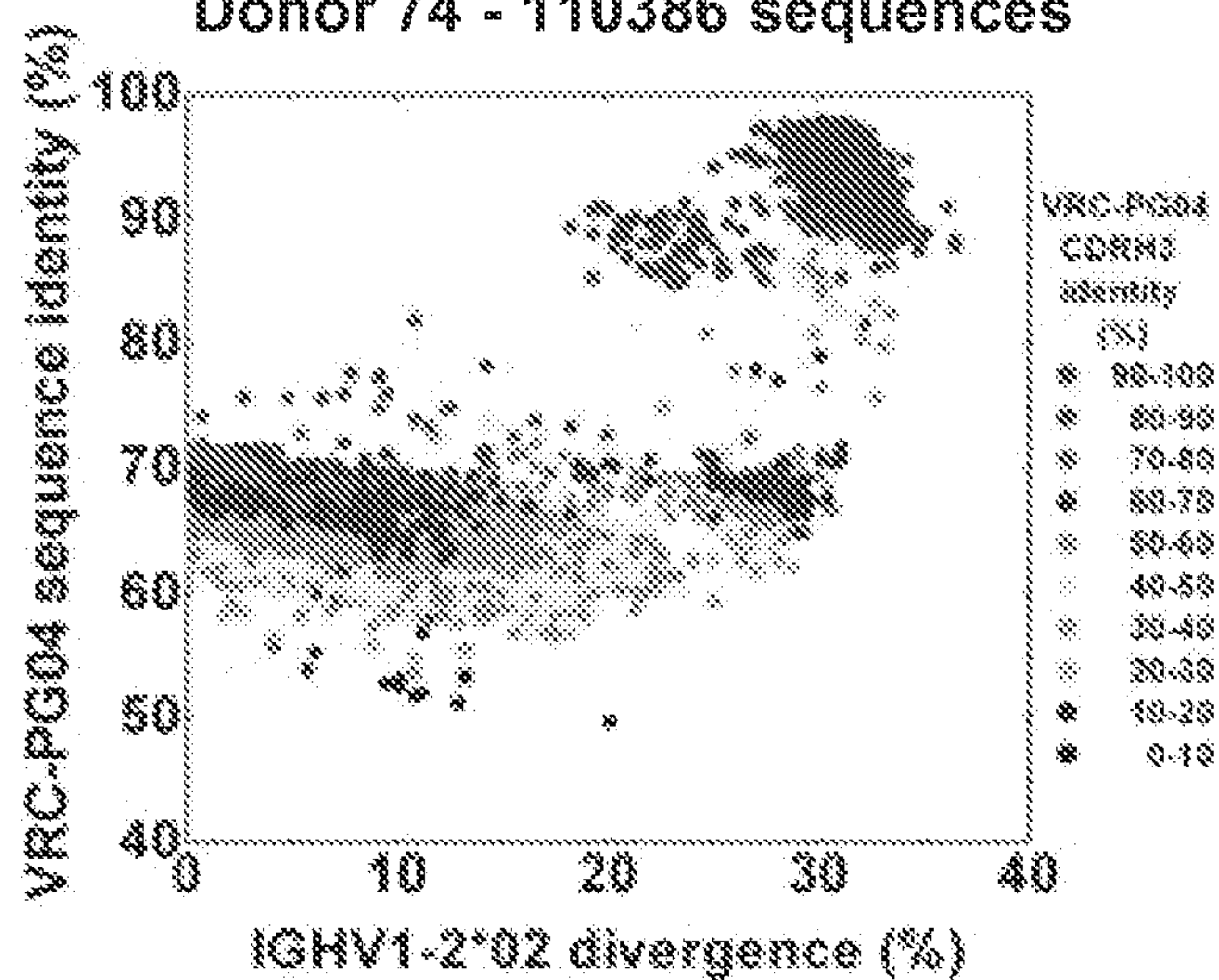


Figure 5



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Figure 6

A IGHV1-2*02 heavy chain antibodyome with grid neutralization assessment**C** Expression of grid-selected chimeric antibodies**D** Phylogeny-predicted neutralization
 P -value = 0.0085**E** IGHV1-2*02 heavy chain CDR H3 usage
Donor 74 - 110386 sequences

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Figure 6

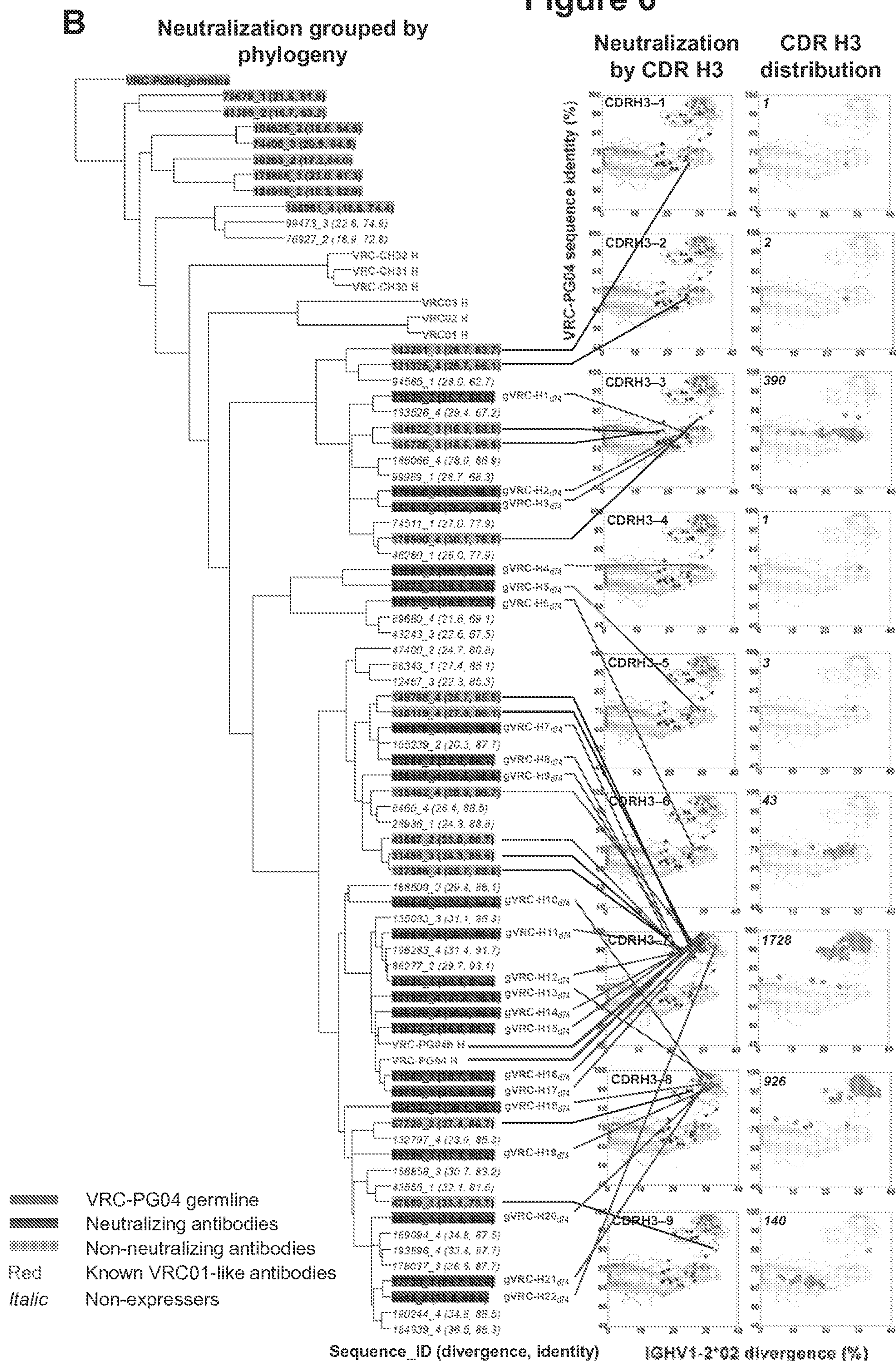


Figure 7

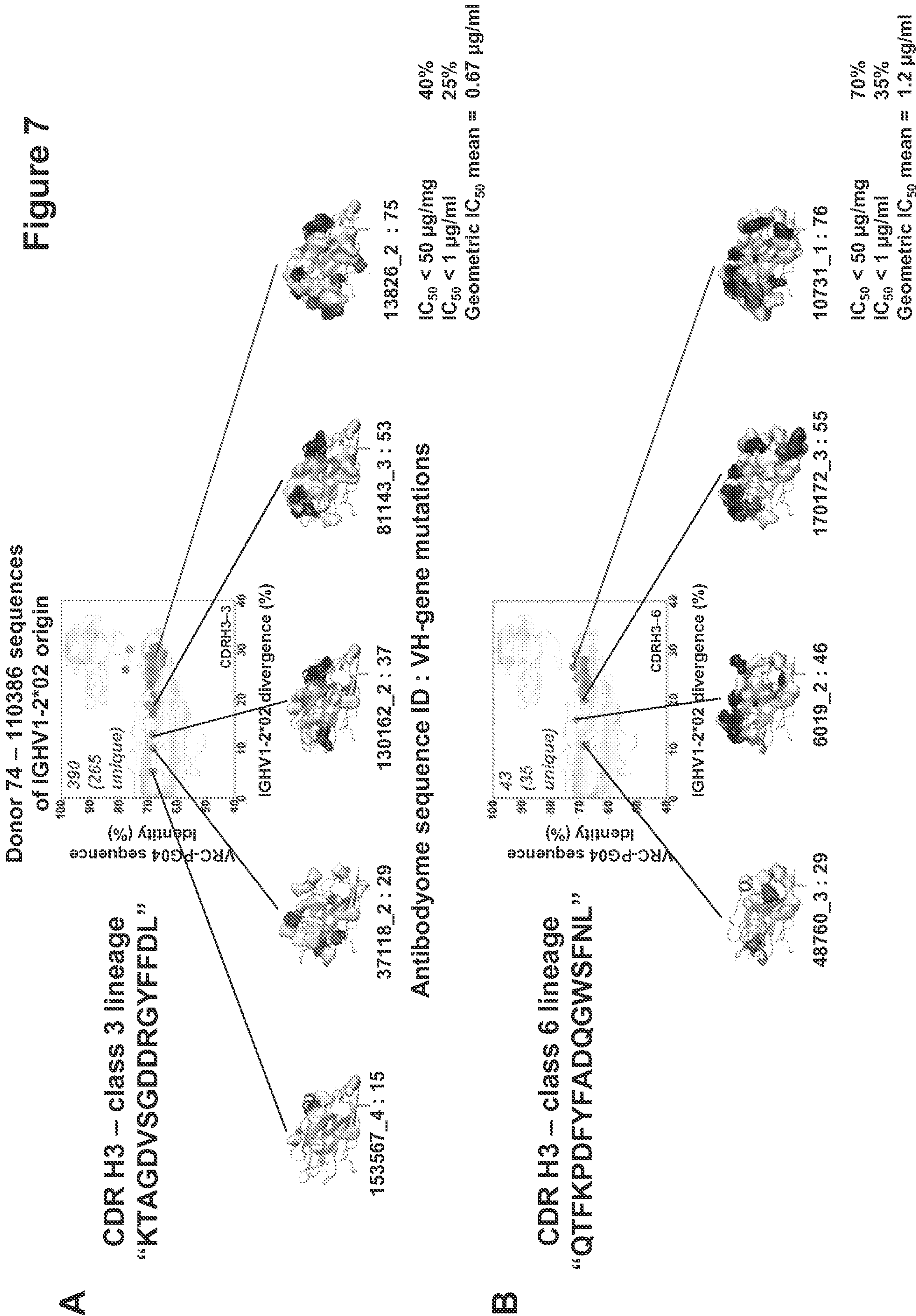
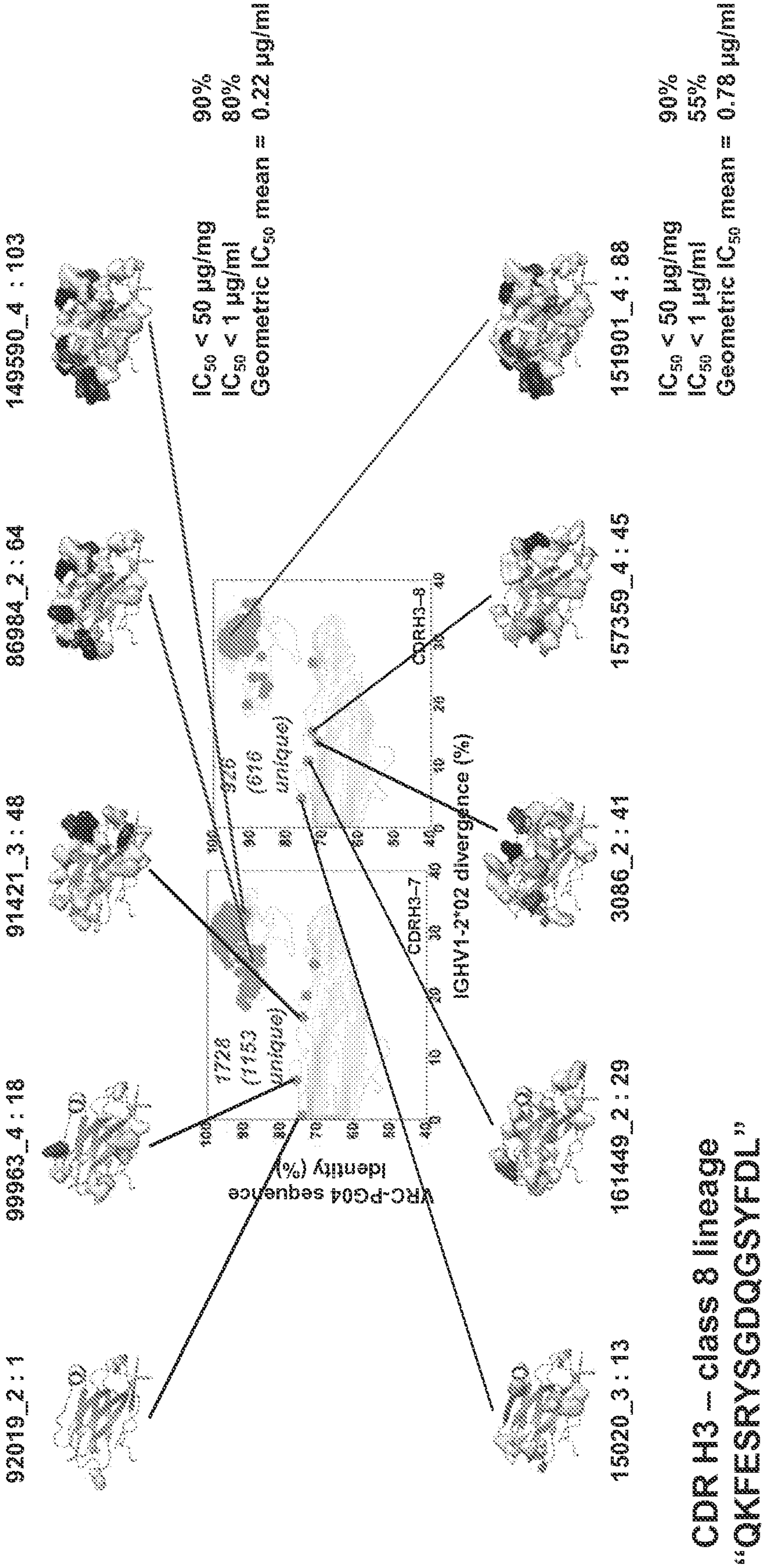


Figure 7

C

CDR H3 – class 7 lineage
“QKFYRGQGQWYFDL”



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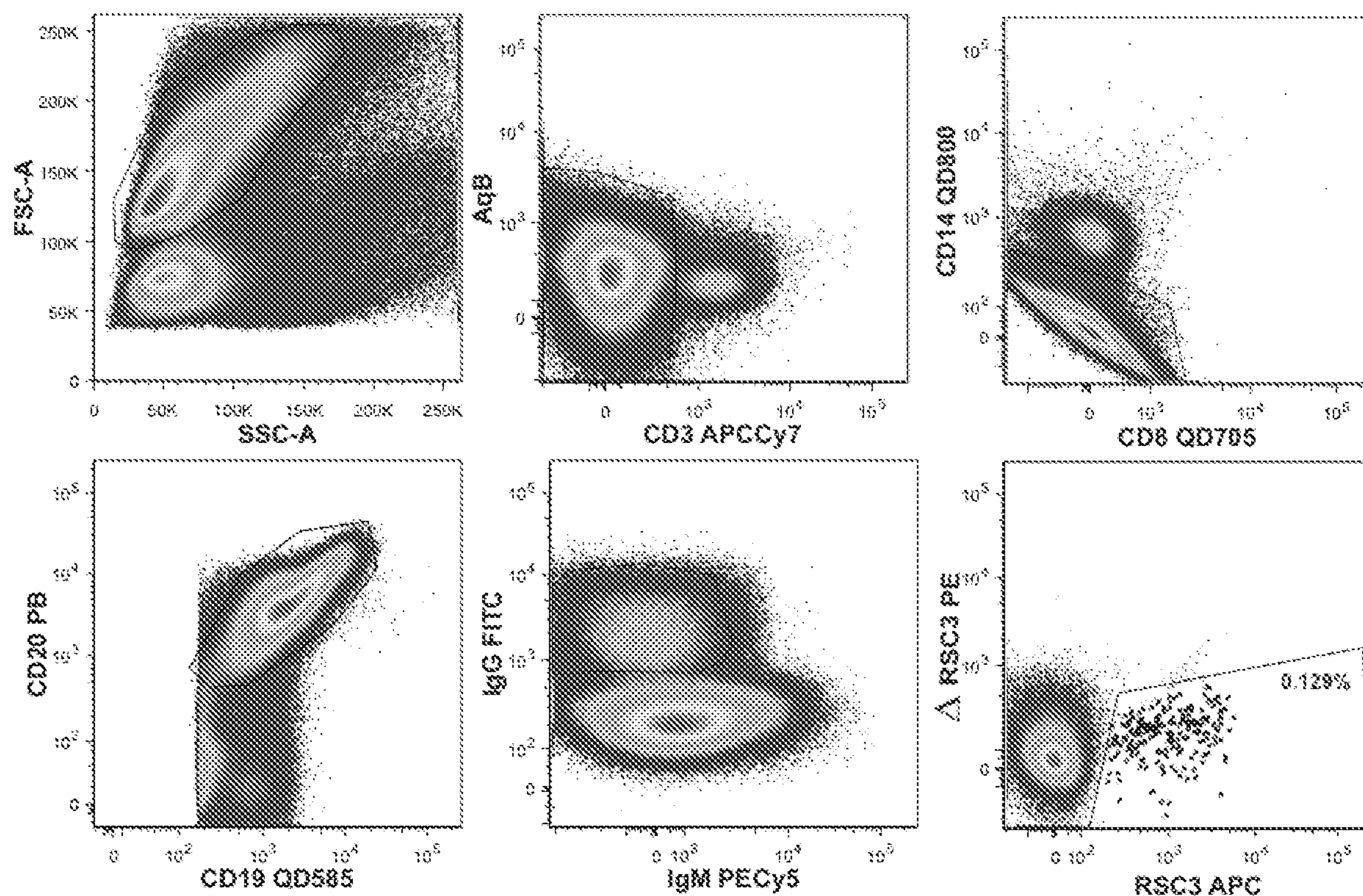
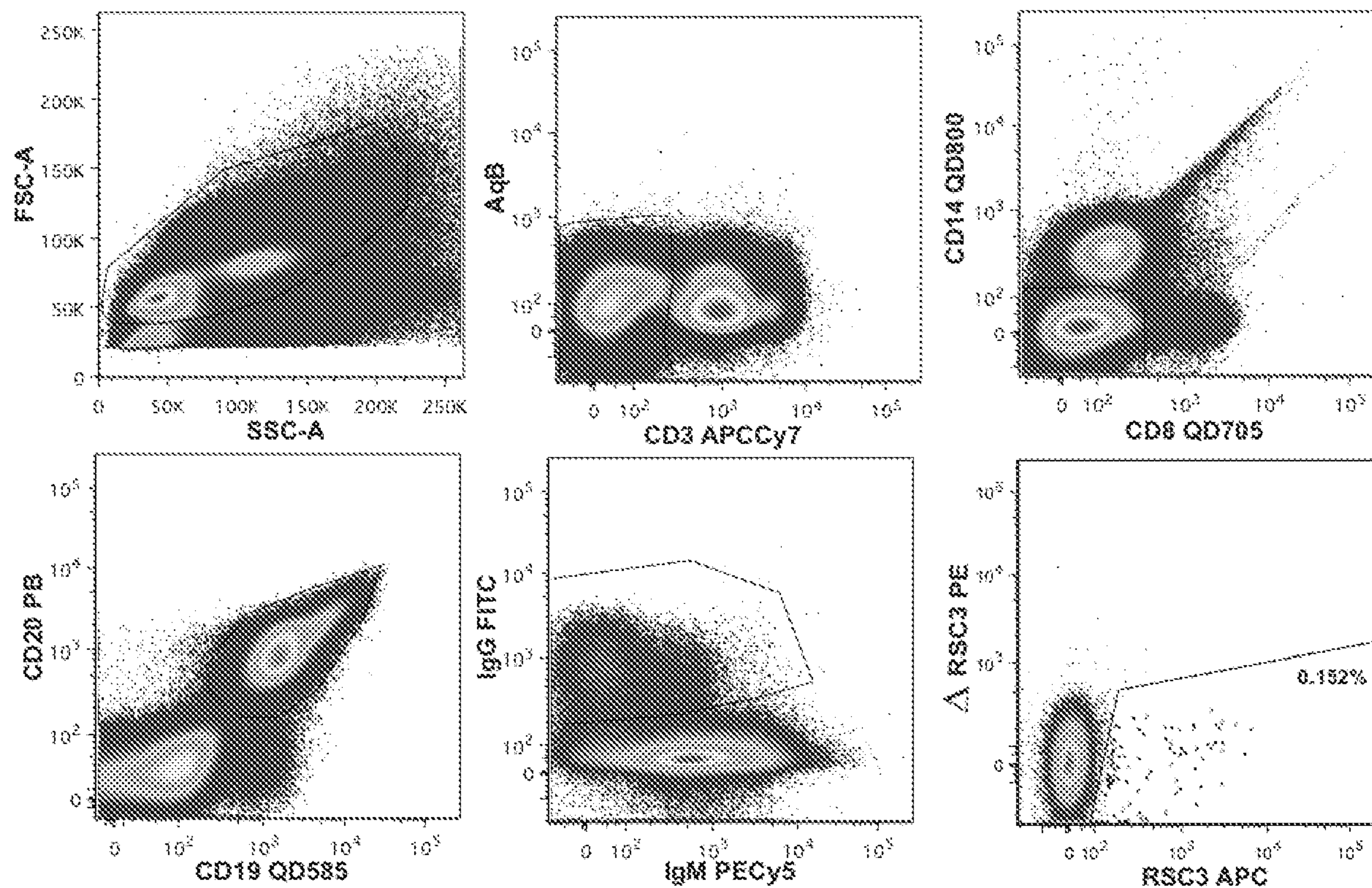
Donor 74**Donor 0219**

Figure S1. Single RSC3-specific B cell sorting. About 20 million PBMC from donors 74 and 0219 were incubated with APC and PE labeled RSC3 and RSC3, respectively. Memory B cells were selected on the basis of the presented gating strategy. The percentages of B cells that reacted with RSC3 and not RSC3 within IgG+ B cells are indicated. The actually sorted single B cells were 40 from donor 74 and 26 from donor 0219. The sorter configurations are indicated in the bottom panel.

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LSR and Aria Detector Configuration

Laser (nm / mW)	Detector Name	Fluorochrome Detected	Dichroic LP Filter	BP Filter
Blue 488 / 50	FSC	Forward Scatter		
Blue 488 / 50	B710	Cy55PerCP ⁺ , PerCP ⁺ **	685LP	710/50
Blue 488 / 50	B515	Fluorochrome, CFSE, GFP, Alexa 488, GrViD, Cy2	505LP	515/20
Blue 488 / 50	SSC	Side scatter	488LP	
Green 532 / 150	G780	Cy7PE, Alexa 750PE	740LP	780/40
Green 532 / 150	G710	Cy55PE, Alexa 700PE	690LP	710/50
Green 532 / 150	G660	Cy5PE, Alexa 647PE, 7AAD	640LP	660/40
Green 532 / 150	G610	TRPE, TR, GrViD, RFP, Alexa 594, PI, EMA	600LP	610/20
Green 532 / 150	G560	PE, Cy3, Alexa 532	empty	575/25
Green 532 / 150	Empty	na		
Green 532 / 150	Empty	na		
Green 532 / 150	Empty	na		
Red 628 / 200	R780	Cy7APC, Alexa 750APC	740LP	780/60
Red 628 / 200	R710	Cy55APC, Alexa 660, 680, 700	685LP	710/50
Red 628 / 200	R660	APC, Alexa 647	empty	660/20
Violet 408 / 100	V800	QD800	740LP	780/60
Violet 408 / 100	V705	QD705	670LP	705/70
Violet 408 / 100	V655	QD655	630LP	660/40
Violet 408 / 100	V605	QD605	595LP	605/30
Violet 408 / 100	V585	QD585	570LP	585/42
Violet 408 / 100	V565	QD565	557LP	560/40
Violet 408 / 100	V545	QD545, Pacific Orange, Aqua Blue ^{***}	535 LP	560/40
Violet 408 / 100	V450	CBlue, ViViD, PacBlue	empty	450/50

* Conjugate is incompatible with QD705

** Required filter change: 640LP + 660/50 and is incompatible with QD705

*** Required filter change: 505LP + 515/20

Complete reference list: <http://www3.niaid.nih.gov/labs/aboutlabs/VRC/PDF/table1commonfluorochromes1.pdf>

Figure S1 Cont'd-1. Single RSC3-specific B cell sorting. About 20 million PBMC from donors 74 and 0219 were incubated with APC and PE labeled RSC3 and RSC3, respectively. Memory B cells were selected on the basis of the presented gating strategy. The percentages of B cells that reacted with RSC3 and not RSC3 within IgG+ B cells are indicated. The actually sorted single B cells were 40 from donor 74 and 26 from donor 0219. The sorter configurations are indicated in the bottom panel.

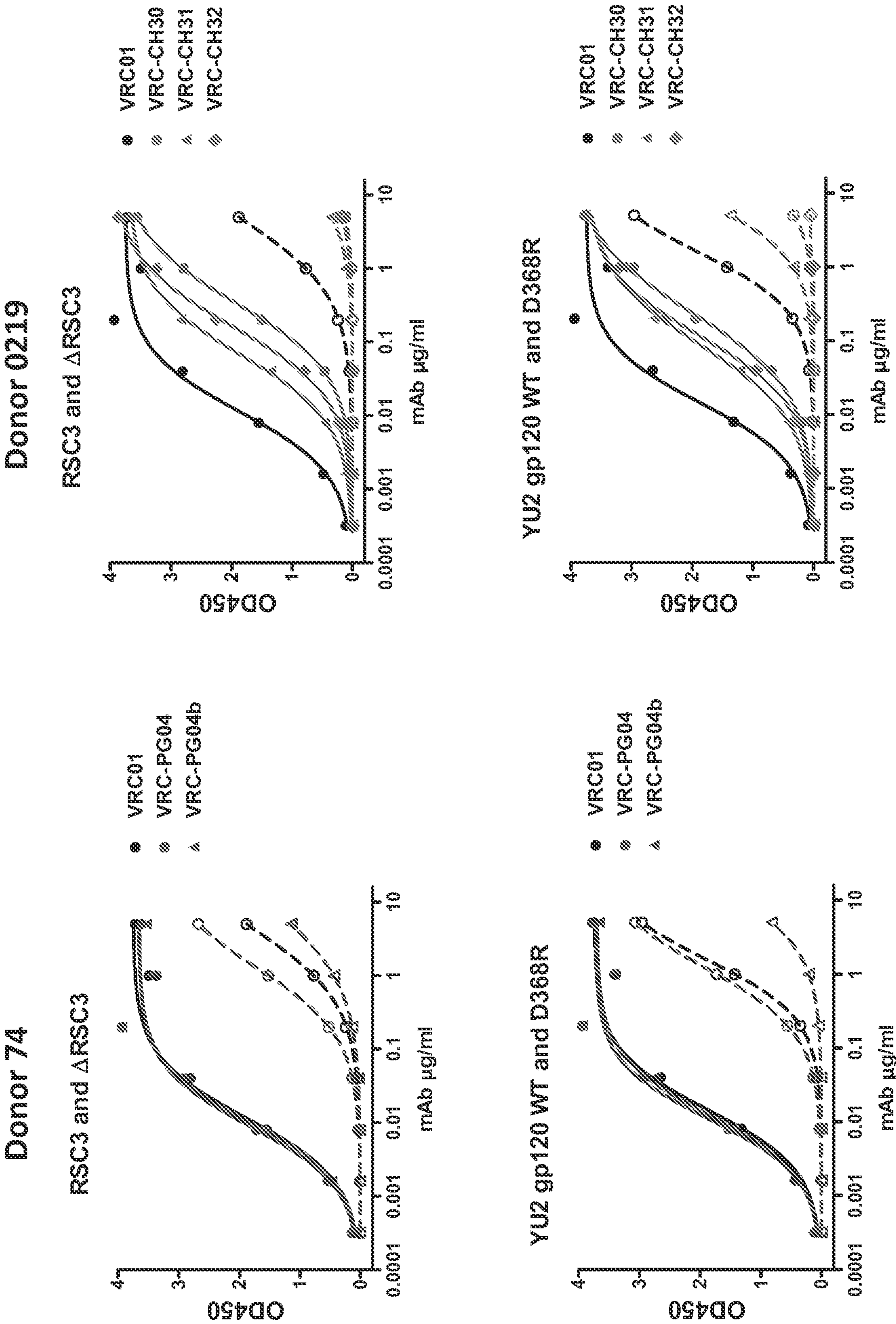


Figure S2. Antigen binding profiles of five newly isolated mAbs, VRC-PG04, VRC-PG04b, VRC-CH30, VRC-CH31 and VRC-CH32, measured by ELISA. Solid symbols show mAb binding to RSC3 (top) and YU2 gp120 (bottom). Open symbols indicate mAb binding to Δ RSC3 or to the CD4bs knockout mutant of gp120, D368R.

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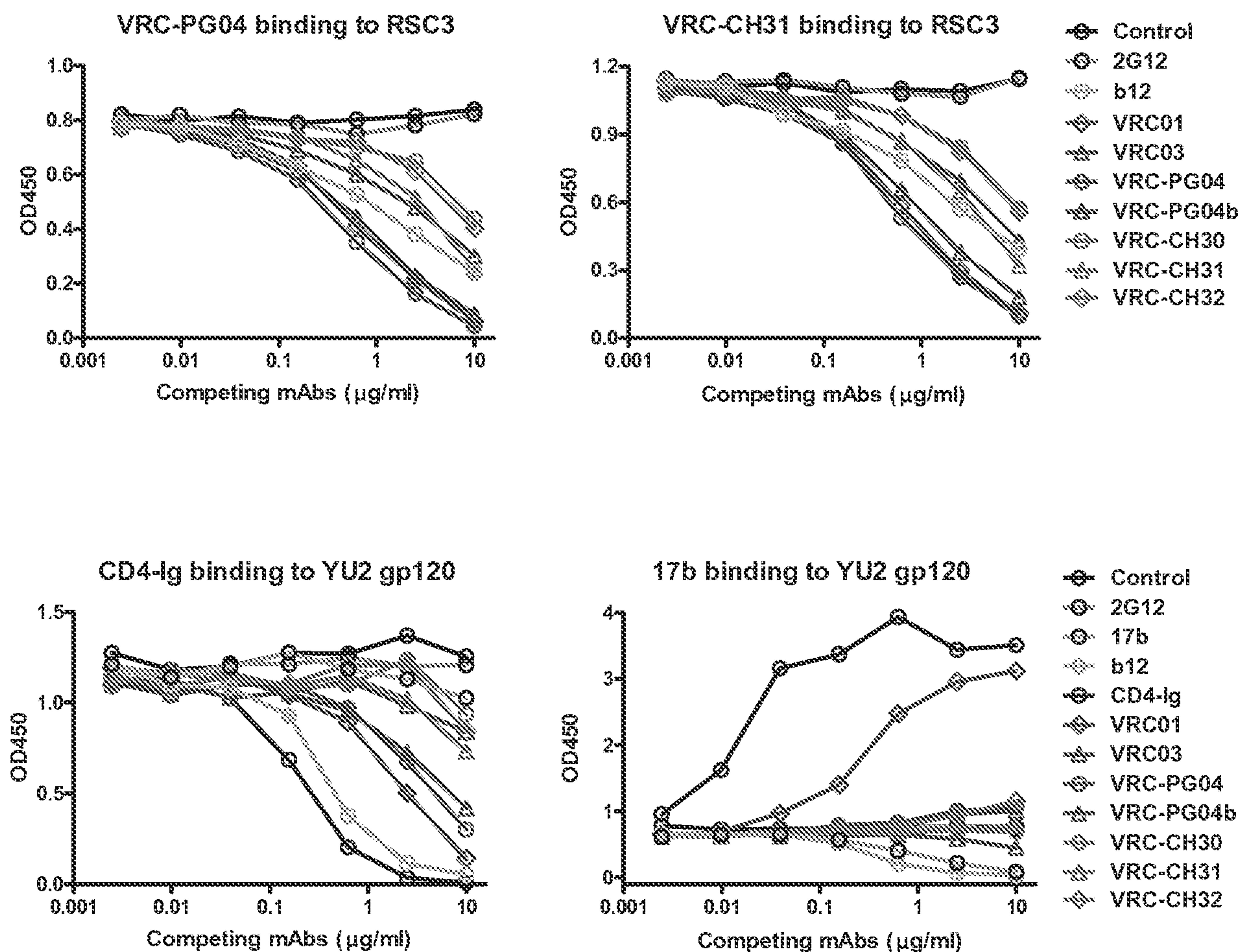


Figure S3. Competition ELISAs show that mAbs VRC-PG04 and VRC-CH31 are directed to the CD4bs of HIV-1 gp120. The competition ELISAs were performed with a single concentration of biotin-labeled VRC-PG04 or VRC-CH31. Unlabeled mAbs were titrated into the ELISA at increasing concentrations to evaluate the effect on VRC-PG04 or VRC-CH31 binding to RSC3 (top). The competition ELISAs were also performed with a single concentration of biotin-labeled CD4-Ig or the co-receptor binding site mAb 17b. Unlabeled mAbs were titrated into the ELISA at increasing concentrations to evaluate the effect on CD4-Ig or 17b binding to YU2 gp120 (bottom). CD4-Ig is a fusion protein of the N-terminal two domains of CD4 fused with IgG1 Fc to served as a CD4 surrogate.

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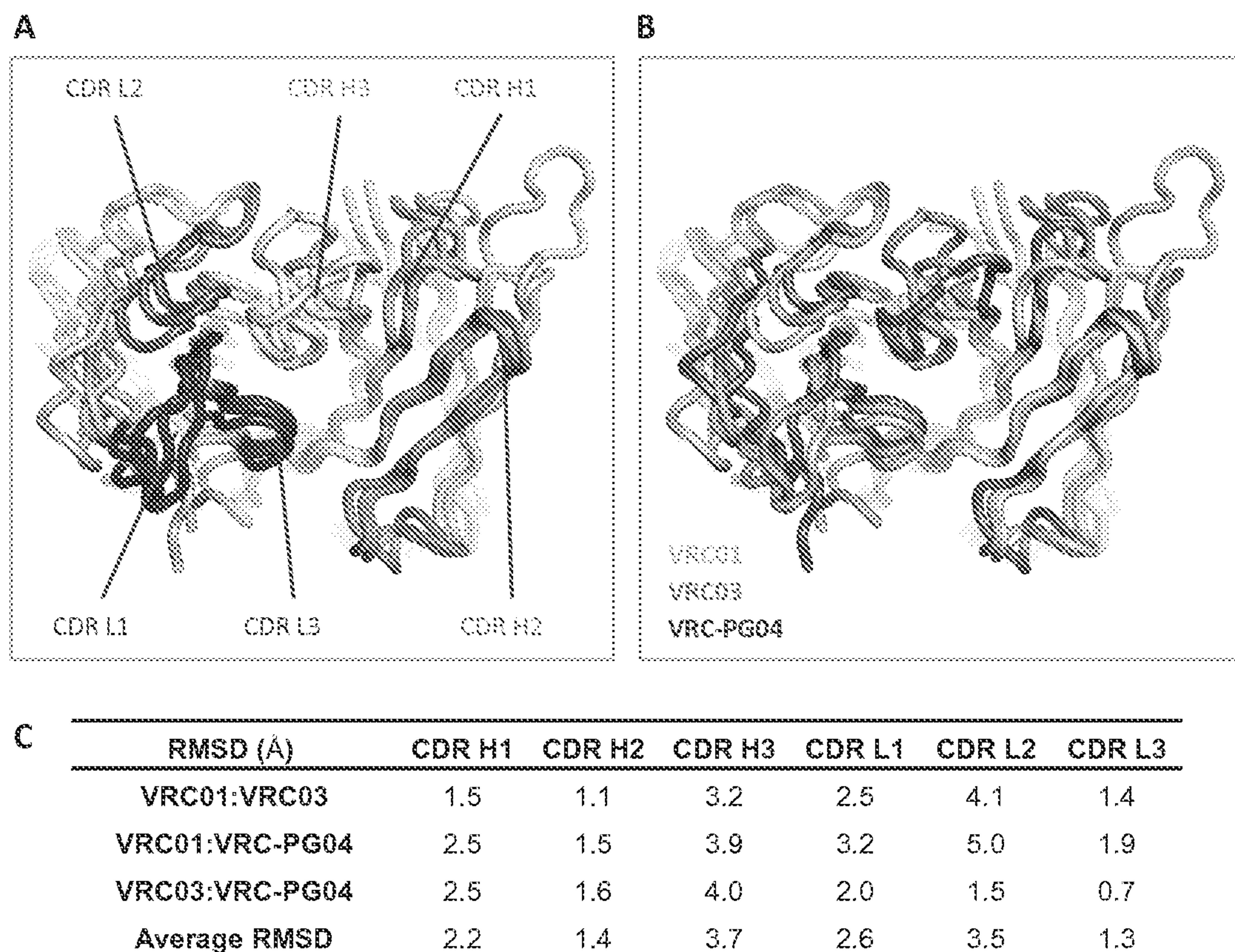


Figure S4. CDR H2 and CDR L3 regions of VRC01-like antibodies showed high degree of similarity in recognition. (A) When gp120 were superimposed, orientations of the antibodies in the gp120:antibody complexes were compared. CDR H2 and CDR L3 regions of VRC01-like antibodies showed high precision alignment. **(B)** Ribbon representation of VRC01, VRC03 and VRC-PG04 in the same orientation as panel A. **(C)** Pairwise root-mean-square deviation (RMSD) of CDR loops between VRC01, VRC03 and VRC-PG04.

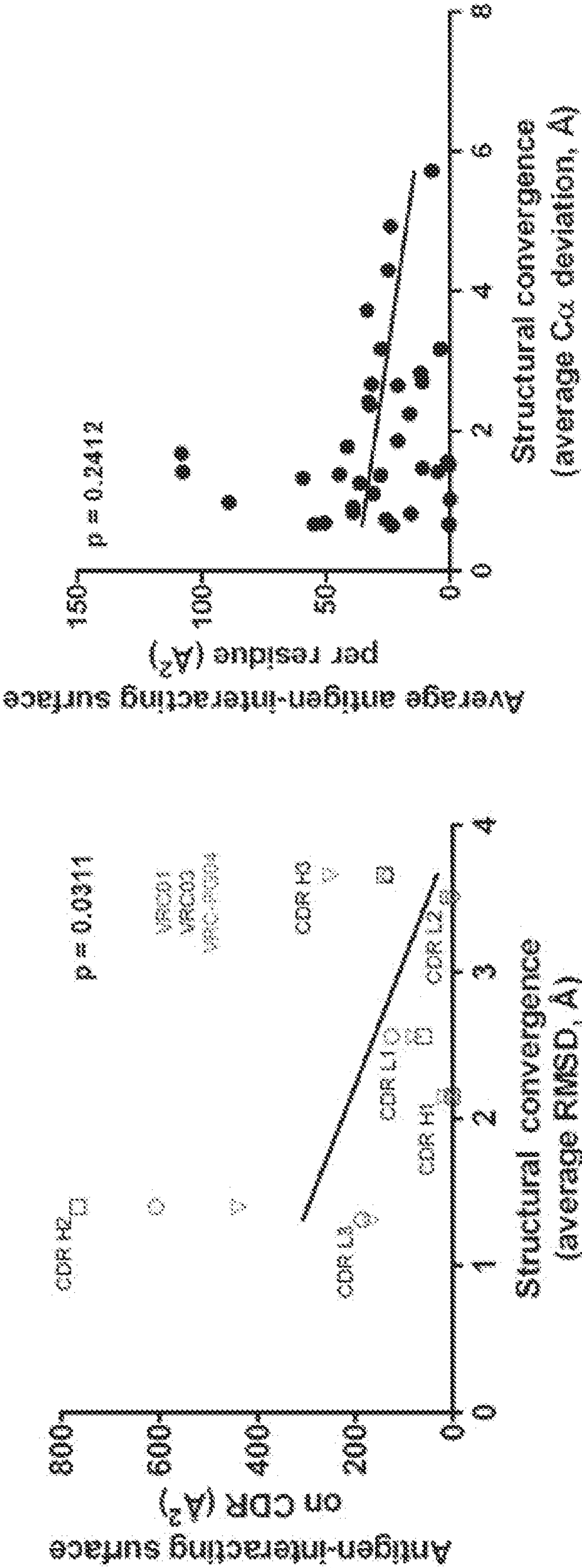


Figure S5. Correlations between structural convergence and antigen-interacting surface areas of antibody. (left) A significant correlation was found between antigen-interacting surface on CDR and average RMSD for the six CDR regions in the three available structures (VRC01, VRC03, and VRC-PG04). The point for CDR L3 of VRC03 overlaps almost perfectly with the point for CDR L3 of VRC01 and is not visible. (right) While no correlation was found between average antigen-interacting surface and Cα deviation for each interface residue, residues with large interface surface were observed to have low Cα deviations.

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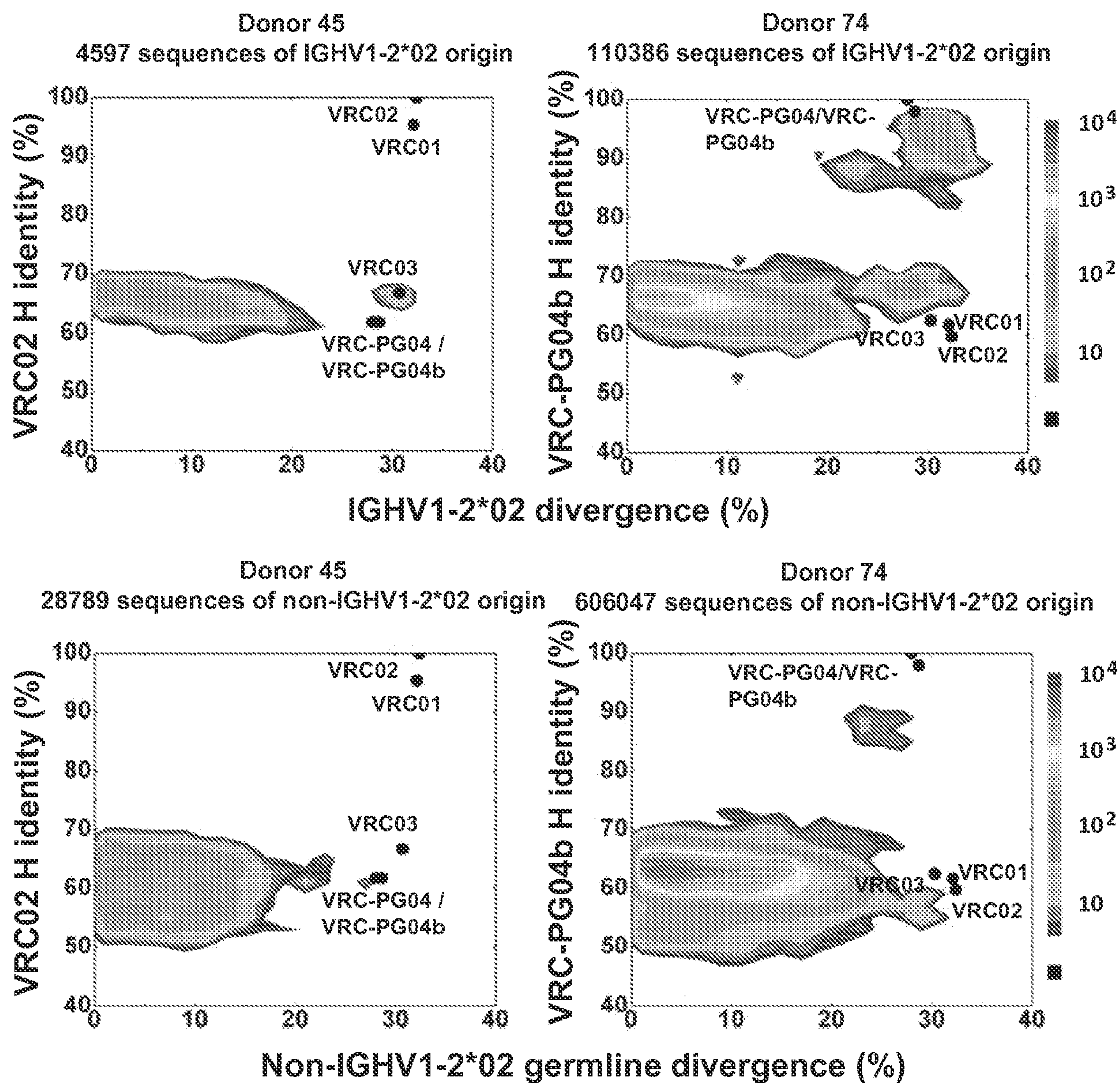


Figure S6. The 454 sequence distribution of donor 45 and donor 74 heavy-chain antibodyomes plotted as a function of sequence identity to VRC02 and VRC-PG04b and sequence divergence from respective germlines. Row one plots sequences of IGHV1-2*02 origin and row two plots sequences of other origins.

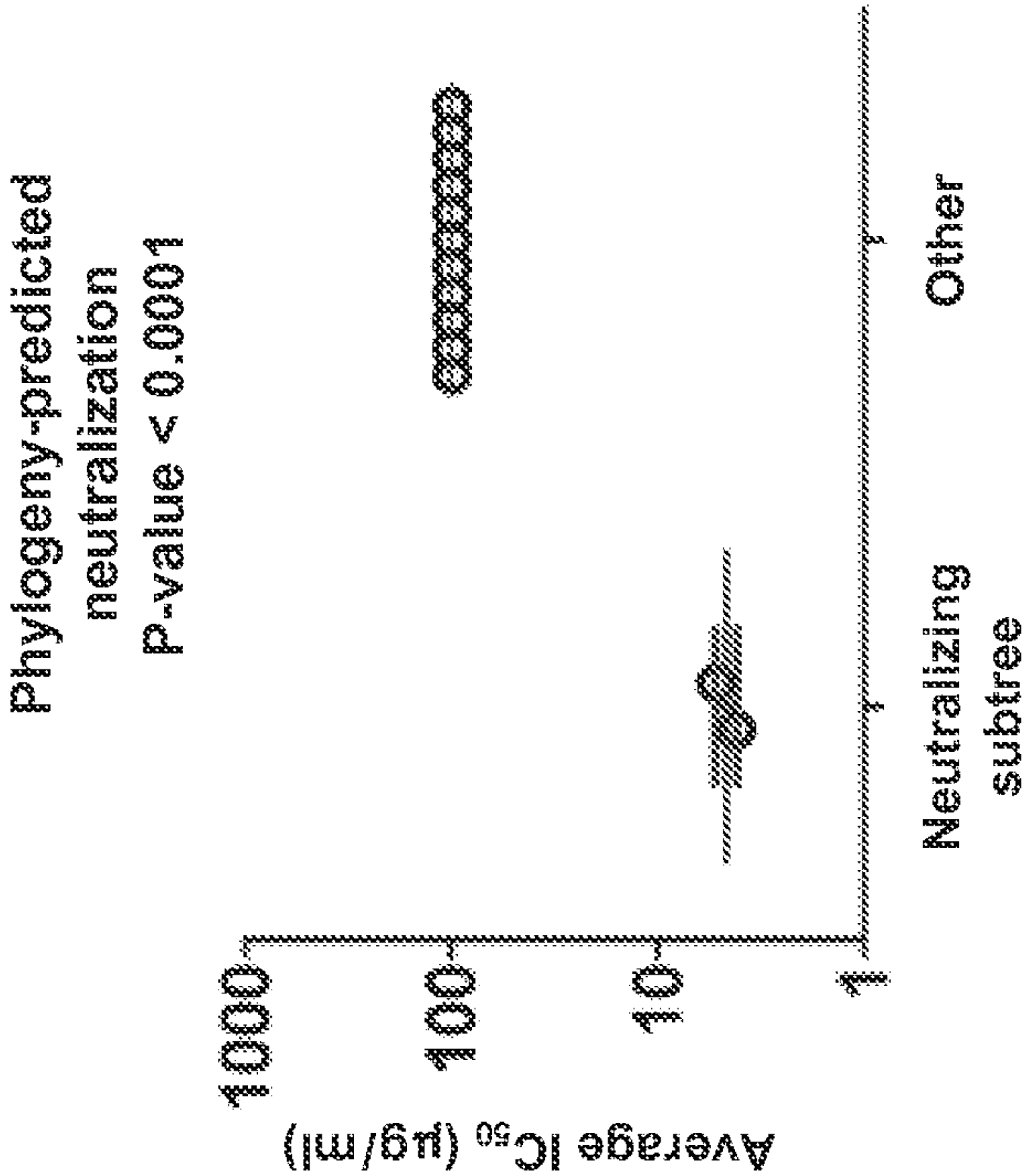


Figure S7. Neutralization of expressed phylogeny-segregated sequences and sequences selected by other criteria from donor 45 2008 heavy-chain antibodyome. Specifically, the two neutralizing sequences were selected from the phylogenetic subtree of IGHV1-2*02 sequences (see Fig. 5) where they segregate with VRC01, VRC02, VRC03, VRC-PG04 and VRC-PG04b, whereas the 11 non-neutralizing sequences were selected either from different divergence bins of IGHV1-2*02 family with high predicted structural compatibility with known VRC01-like antibody-gp120 structure complexes or from other germline families with high divergence and large family size (see Tables S11 and S12).

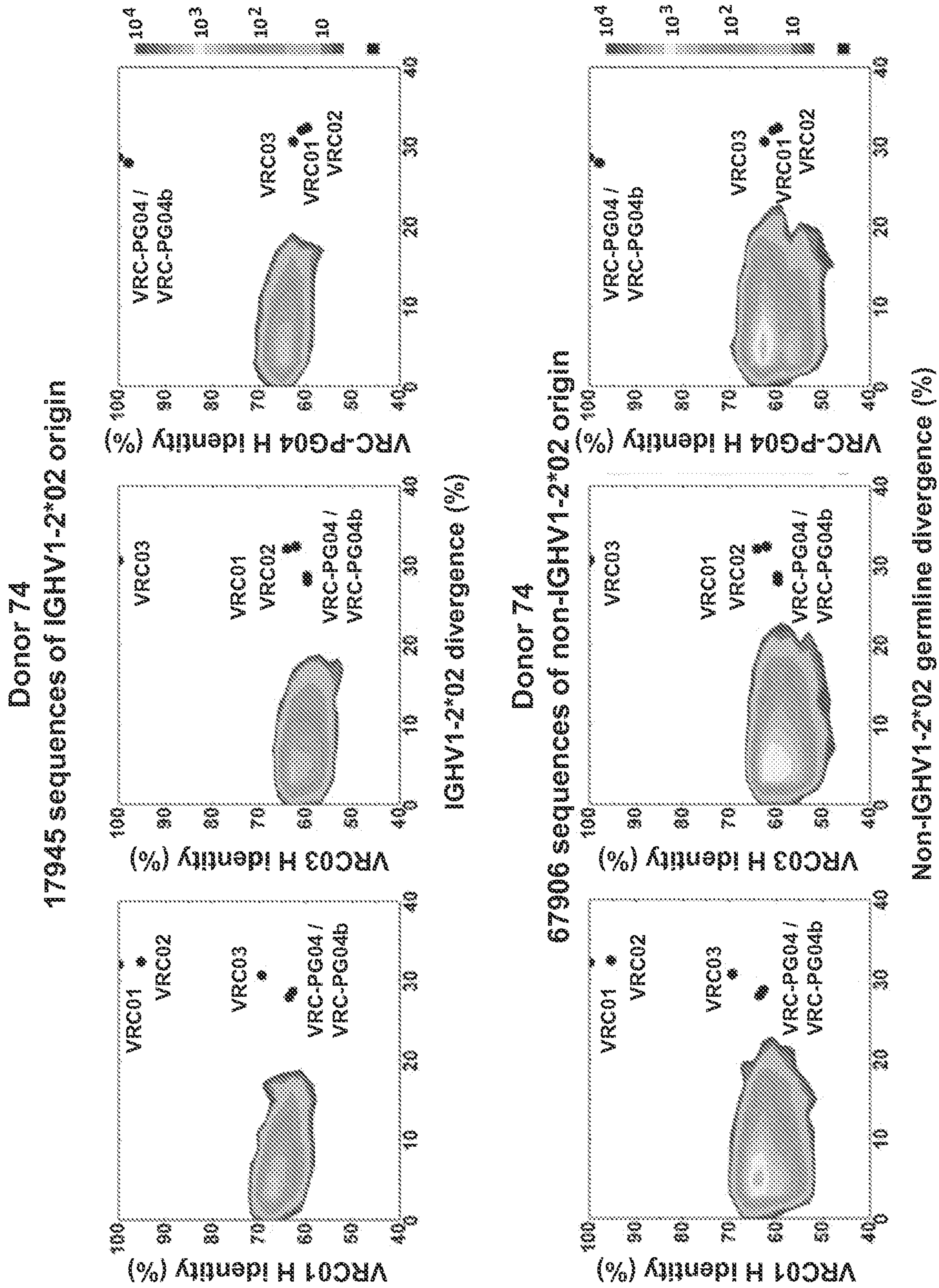


Figure S8. The sequence distribution of 454-pyrosequencing-determined donor 74 heavy-chain antibodyome (obtained from Beckman Coulter Genomics) plotted as a function of sequence identity to VRC01, VRC03 and VRC-PG04 and sequence divergence from respective germlines. Row one plots sequences of IGHV1-2*02 origin and row two plots sequences of non-IGHV1-2*02 origin.

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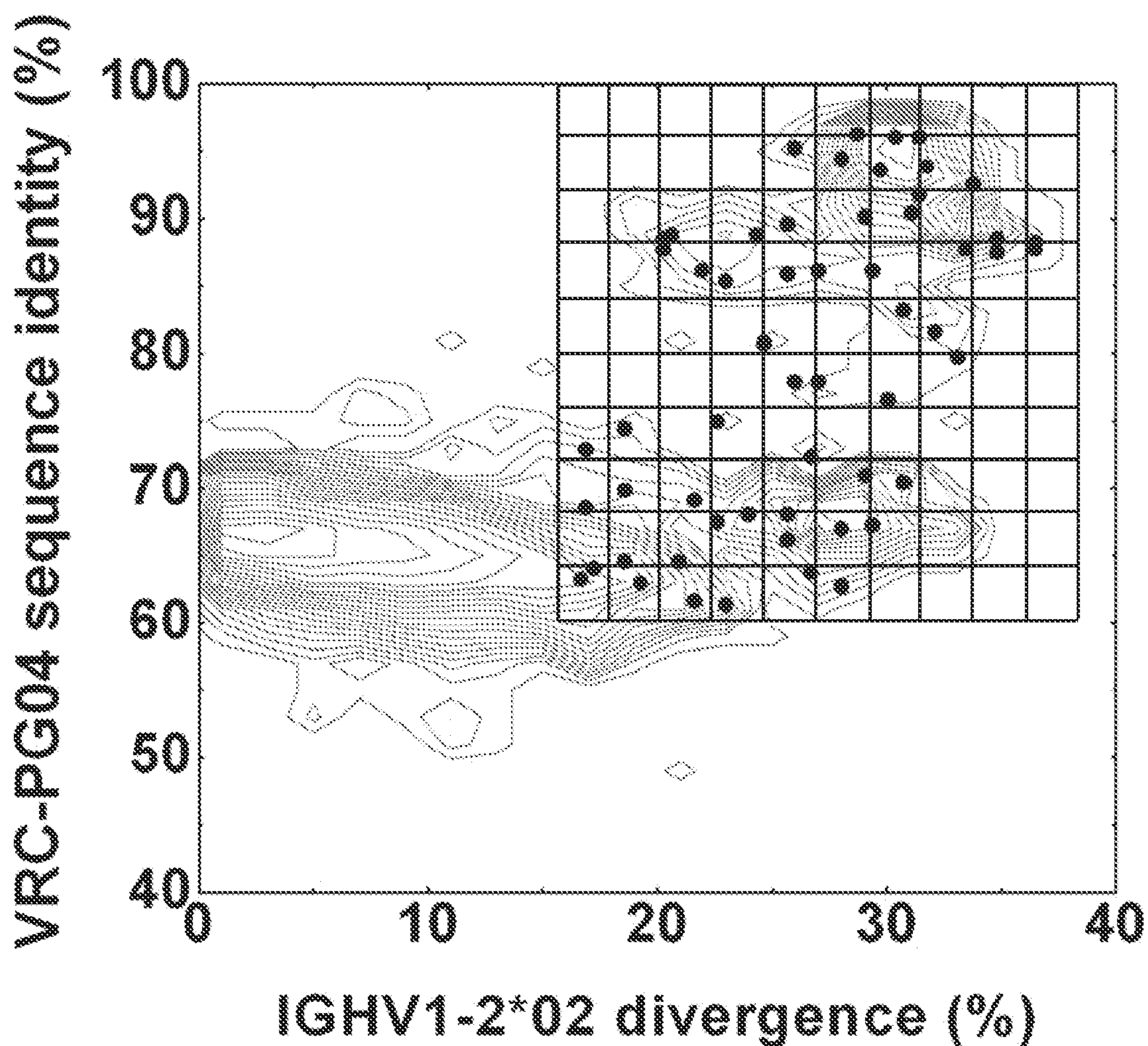


Figure S9. Identity/divergence-grid assessment of donor 74 heavy-chain 2008 antibodyome. A 10x10 grid was placed over the quadrant defined by high divergence and high sequence identity to VRC-PG04. The sequences within each square of the grid were subjected to a clustering procedure with a sequence identity cutoff of 90%. A sequence was then randomly selected from the largest cluster as candidate. An initial set of 57 sequences was obtained using this approach. Sequences with a identity of 95% or greater to others or containing uncorrected sequencing errors were replaced by new ones selected from the grid. Note that every time a new sequence was selected, the possibility of overlapping with sequences of neighboring squares was examined using sequence clustering. A total of 56 grid-selected sequences were synthesized to assess the function of 454-pyrosequencing-determined heavy-chain sequences.

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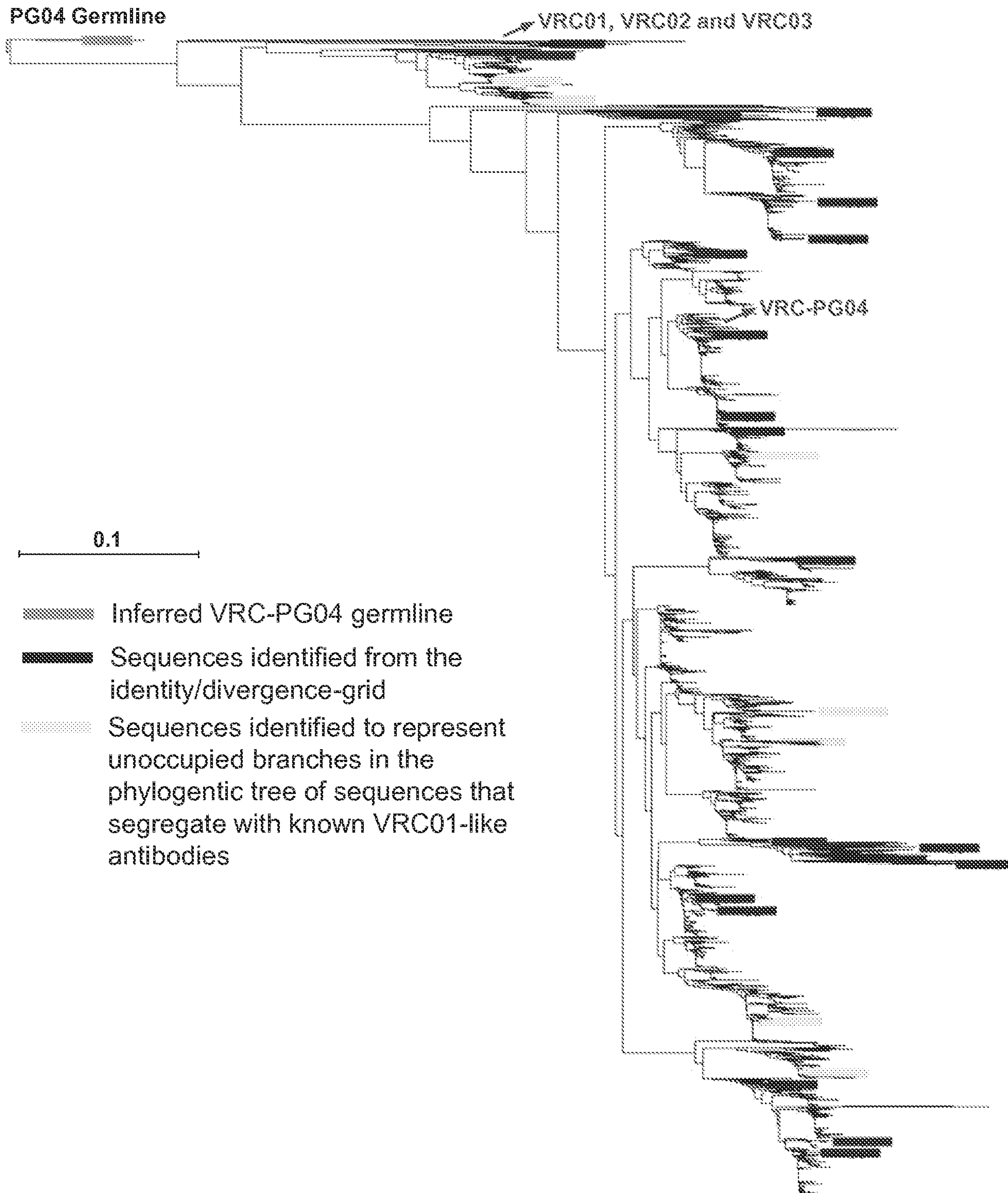
Inferred VRC-
PG04 Germline

Figure S10. Additional sequences selected to enhance the coverage of phylogeny-segregated sequences. In the iterative phylogenetic analysis of IGHV1-2*02 family of donor 74 2008 heavy-chain antibodyome, 5047 sequences were found to segregate with VRC01, VRC02, VRC03 and VRC-PG04 on a distinct branch. A neighbor-joining (NJ) tree of these 5047 sequences, rooted at the inferred VRC-PG04 germline, is shown in this figure. 38 out of the 57 identity/divergence-grid-derived sequences were found within these sequences and are labeled by blue rectangles. 7 additional sequences were selected to represent unoccupied branches and are labeled by yellow rectangles.

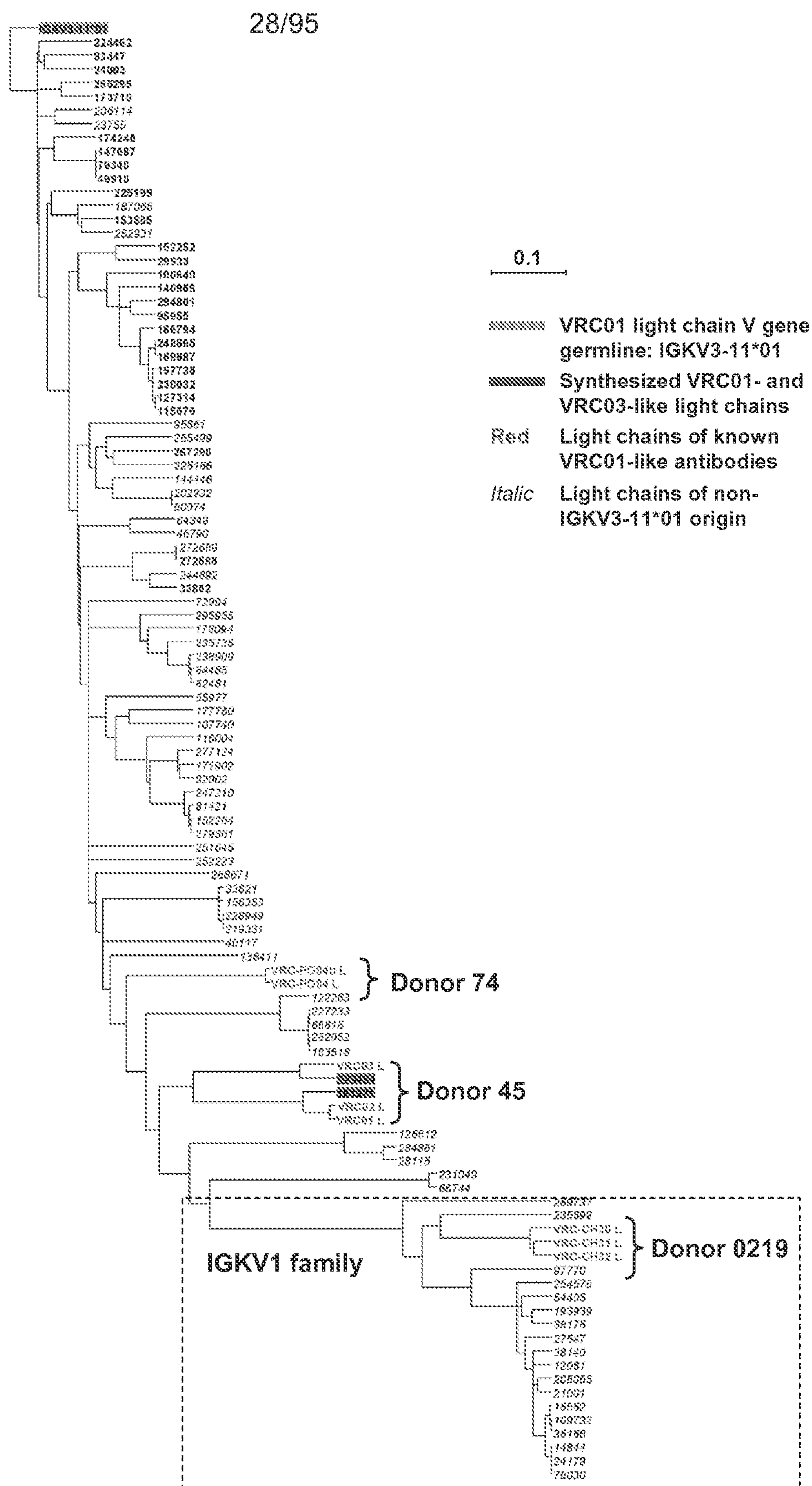


Figure S11. Phylogenetic tree of 98 sequences from donor 45 light-chain 2001 antibodyome that have the same VRC01-like and VRC03-like deletions. The maximum likelihood (ML) tree is rooted at the IGKV3-11*01, VRC01 light-chain V-gene germline, which is highlighted in green. The known VRC01-like antibody light-chain sequences are colored in red and the two synthesized sequences that show functional complementation with VRC01-like heavy chains are highlighted in red.

Heavy chain	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
IGHV1-2*02	QVQLVQSGAEVKKPGASVKVSKASGYTFTG	...YMHWVRQAPGQGLEWMGWINPNSGGTNY	AQKFQGRVMTMRDTSISTAYMELSLRSDDTAVYYCAR				
CDRH3-1 (J4*02)							
143251_3	QVQLVQAGGSGVKKPGASVTL	SCKTADDEVED	...AAYMHWVRQAPGQTEEWL	GWMPVTVGAVSY	ARKKFQGRVSEFYMTRELLG	WAYMDLRNLRFDDTAVYYCAR	VGGAAADD..SGYTEPPS..DYWGQGT
CDRH3-2 (J6*02)							
121325_4	QVQLVAGRGSGVKKPGASVTL	SCKTADDEVED	...AAYMHWVRQAPGQTEEWL	GWMPVTVGAVNY	ARKKFQGRVSEFYRTRELLG	IAYMDLRNLRFDDTAVYYCAR	AGVWFGELLPHWSGVGGMDVWGQGT
CDRH3-3 (J2*01)							
13826_2	QVQLVQSGAEVKKPGASMTV	SCEIADEDED	...AAYMHWVRQAPGQTEEWL	GWMPVTVGAVNY	ARKKFQGRISFYRTRELLA	IAYMDLRDLRFDDTAVYFC	ARKKTAGDVSGD...NRGYFFDLWGRGSR
179400_4	QVQLVQSGAEVKKPGASVTL	SCKTADDEVED	...AAYMHWVRQAPGQTEEWL	GWMPVTVGAVSY	ARKKFQGRVSEFYMTRELLG	WAYMDLRNLRFDDTAVYFC	ARKKTAGDVSGD...NRGYFFDLWGRGSR
167612_4	QVQLVQAGGSGVKKPGASVTL	SCKTADDEVED	...AAYMHWVRQAPGQTEEWL	GWMPVTVGAVNY	ARKKFQGRVSEFYRTRELLG	IAYMDLRNLRFDDTAVYFC	ARKKTAGDVSGD...KRGEFFDLWGRGSR
164922_3	QVQLVQSGAAVKKPGSSVK	VSCKASGYIFTG	...YTHHWIRQAPGQGLEWMG	WINPSTGDTKF	ARQFQGRVSEFYRTRELLG	IAYMDLRDLKFDDTAVYFC	ARKKTGADVSGD...DRGEFFDLWGRGTR
166726_3	QVQLVQSGAEVKKPGASVK	VSCKASGYTFTG	...YMHWVRQAPGQTEEWL	GWMPVTVGAVNY	ARQFQGRVSEFYRTRELLG	IAYMDLRDLKFDDTAVYFC	ARKKTADVSGD...DRGEFFDLWGRGTR
179500_4	QVQLVQSGSAMKKPGASV	RVSCWTSIEDIFDT	...TELLYHWVRQAPGQGLEW	I	GWYKIVSGTVNE	ARQFQGRVSEFYRTRELLG	IAYMDLRDLKFDDTAVYFC
CDRH3-4 (J2*01)							
95589_2	QVQLVQSGGSGVKKPGASAS	FSCTSDPFDN	EFFQSEEMHWVRLLTFQGR	PEWMGWINPNSGGVNY	AGQFRPRMSMRDDELSTAY	MELRLDTADTGLYFC	ARRKEDD...YDWYYDLWGRGAHI
CDRH3-5 (J2*01)							
24972_2	QVQLVQSGGSGVKKPGTS	ASFSCKTSDDIYDN	EFFDSAFMHWVRLLIPGQR	PEWMGWINPNSGAVNYA	RQLQFRVSMIRDKDLSTAY	MEFKSLTSADTGT	YFCARKKKRGDG...FNLYEDLWGRG
CDRH3-6 (J2*01)							
10731_1	QVQLVQSGGSGVKKPGASV	RVSCPASEDLFQDEI	LYDDEVTHWLRQVFGQR	PEWMGWINPNSGARNQA	RQFQPRISLIRDEALSTAY	LDLNSLTSADSGTYFC	ARRQTKPDPFYF...ADQGWSENLWGRGAH

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Figure S12. CDR H3 classification of 35 expressed and experimentally tested heavy-chain sequences in the neutralization tree shown in Fig. 6, with the J gene of each CDR H3 class listed in parentheses. The Germline sequence, IGHV1-2*02, is used as reference in sequence alignment and VRC-PG04 heavy-chain sequence is included for comparison. Amino acids in the variable region that are different from IGHV1-2*02 are highlighted in red. Note that of the 35 sequences 22 showed neutralizing activity, as highlighted in red in Fig. 6.

CDRH3-7 (J2*01)		
149768_4	QVQLVQSGAEVKKPKGSSVKVSKASGCTFRM.....SPISWVRQAPQGQLEWIGWVKAVSGAVNYGSLDFFRHRVSLTRDRDLSTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
120119_4	QVQLVQSGAEVKKPKGSSVKVSKVSGGVFTS.....YAVTVVRQAPQGQLEWIGWVKAVSGAVNYGSLDFFRHRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
186275_2	QVQLVQSGAEVKKPKGASVKVSKASGYTFTG.....YYMHWVRQAPQGQLEWIGWVKIVSGAVNFGSLNFRHRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
86984_2	QVQLVQSGAEVKKPKGASVQVSKASGYFTK.....YYMHWVRQAPQGQLEWIGWVKAVSGAVNYGSLDFFRHRVSLTRDRDLSTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
196147_4	QVQLVQSGAEVKKPKGASVKVSKASGYTFTS.....YYMHWVRQAPQGQLEWIGWVKVETGAVNFGSPNFRHRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
195462_4	QVQLVQVRDQEVKKPKGSSMKVSCVTSASGTFS.....YALSWVRQAPQGQLEWIGWVKIVTGAVNFGSAYFRHRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
43567_2	QVQLVQSGAEVKKPKGASVKVSKASGYTFTS.....YDINWVRQATQGQLEWIGWVKIVTGAVNFGSSDFRQVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
31458_3	QVQLVQSGAEVKKPKGASVKVSKASGYTFTT.....YGISWVRQAPQGQLEWIGWVKIVTGAVNFGSSDFRQVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
127586_4	QVQLVQSGAEVKKPKGASVKVSKASGYTFTT.....YGISWVRQAPQGQLEWIGWVKIVTGAVNFG.....HQISDVRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
149590_4	QVQLVQSGSGVKKVKGASVVRVSCVTSSEDIFFR.....TELIHWVRQAPQGQLEWIGWVKAVSGAVNYGSLDFFRHRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
53821_1	QVQLVQSGSAMKKPKGASVVRVSCVTSSEDIFFT.....TELIHWVRQAPQGQLEWIGWVKAVSGAVNYGSLDFFRHRVSLTRDRDLSTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
165478_2	QVQLVQSGSGVKKPKGASVVRVSCVTSSEDIFFG.....SELIHWVRQAPQGQLEWIGWVKIVTGAVNFGSAYFRHRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
71632_2	QVQLVQSGAEVKKPKGASVVRVSCVTSSEDIFFR.....TELIHWVRQAPQGQLEWIGWVKIVSGAVNFGSDFRHRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
VRC-EG04	QVQLVQSGSGVKKPKGASVVRVSCVTSSEDIFFR.....TELIHWVRQAPQGQLEWIGWVKIVTGAVNFGSPDFRQVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
61048_1	QVQLVQSGAEVKKPKGASVVRVSCVTSSEDIFFK.....SELIHWVRQAPQGQLEWIGWVKIVTGAVNFGSSDFRQVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
69713_1	QVQLVQSGSGVKKPKGASVVRVSCVTSSEGIFFK.....SELIHWVRQAPQGQLEWIGWVKIVTGAVNFG.....HQISDRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
9815_2	QVQLVQSGSAMKKPKGASVVRVSCVTSSEDIFFT.....TELIYWVRQAPQGQLEWIGWVKIVSGVYNFASLDFFRHRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWFFDLWGRGTLIVVSS	
CDRH3-8 (J2*01)		
186640_2	QVQLVQSGAEVKKPKGSSVVRVSCVTSSEDIFFR.....TELIHWVRQAPQGQLEWIGWVKIVTGAVNFGSDFRHRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGSYFDLWGRGTLIVVSS	30/95
151901_4	QVQLVPSGSGVKKPKGASVVRVSCVTSSEDIFFR.....TELIHWVRQAPQGQLEWIGWVKIVTGAVNFGSLDFFRHRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWYFDLWGRGTHIVVSS	
164202_3	QVQLVQSAAGVRRPKGASVVRVSCVTSSEDIFFR.....SELIYWVRQAPGRGLEWIGWIKLVSGAVNFGSVDFRHRVSLTRDRDLFTAHMDIRGLTQDDTATYFCARQKFA.....GDQGSYFDLWGRGTLIVVSS	
96298_1	QVQLVQSGLEVKKPKGASVVRVSCVTSSEDIFFT.....TELIYWVRQAPQGQLEWIGWVKIVSGVYNFASLDFFRHRVSLTRDRDLSTAHMDIRGLTQDDTATYFCARQKFA.....GDQGSYFDLWGRGTLIVVSS	
57729_2	QVQLVQSGAEVKKPKGSSVVRVSCVTSSEDIFFR.....YAINWVRQAPQGQLEWIGWVKIVSGVYNFASLDFFRHRVSLTRDRDLSTAHMDIRGLTQDDTATYFCARQKFA.....GDQGSYFDLWGRGTLIVVSS	
18278_1	QVQLVQSGSAMKKPKGASVVRVSCVTSSEDIFFR.....TELIYWVRQAPGRGLEWIGWVKIVSGVYNFGSSDFRHRVSLTRDRDLSTAHMDIRGLTQDDTATYFCARQKFA.....DEQGSYFDLWGRGTLIVVSS	
17720_4	QVQLVQSGAEVKKPKGASVVRVSCVTSSEDIFFT.....TELIYWVRQAPQGQLEWIGWVKIVSGVYNFASLDFFRHRVSLTRDRDLSTAHMDIRGLTQDDTATYFCARQKFA.....GDQGWYFDLWGRGTHIVVSS	
CDRH3-9 (J4*01)		
47890_1	QVQLVQSGSAMKKPKGASVVRVSCVTSSEDIFFT.....TELIYWVRQAPQGQLEWIGWVKIVSGVYNFASLDFFRHRVSLTRDRDLSTAHMDIRGLTDDTGLIYCARGPMGG.....SHVYWGQGSIVTVSS	

Figure S12 Cont'd-1. CDR H3 classification of 35 expressed and experimentally tested heavy chain sequence in the neutralization tree shown in Fig. 6, with the J gene of each CDR H3 class listed in parentheses. The Germline sequence, IGHV1-2*02, is used as reference in sequence alignment and VRC-PG04 heavy-chain sequence is included for comparison. Amino acids in the variable region that are different from IGHV1-2*02 are highlighted in red. Note that of the 35 sequences 22 showed neutralizing activity, as highlighted in red in Fig. 6.

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CDRH3 class I

	HV1-2*02	HD3-22*01	HJ4*02
TGTGCGAGAGA	CTATTACTATGATAGTAGTGTTATTAGTAC	AGTACGTTGACTACTGGGCCAGGAACCC	TGTCACCGTCTCCTCA
C A R	Y Y D S S G Y Y Y	Y F D Y W G Q G T L V	T V S S
TGTGCGAGAGTGGGCTGATGATAGTGTTATACAGAGCCCCCTCTGACTACTGGGCCAGGAACCC	TGTCACCGTCTCCTCA		
C A R	V C A A D D S G Y Z E P P S D Y W G Q G T L V	T V S S	

143251_3 (27%)

[illegible]

Figure S13a. CDRH3 analysis of expressed heavy chain sequences from donor 74. CDRH3 and HJ alignments of nucleotide and amino acid for CDRH3 classes 1-6 sequences, aligned to the putative V, D and J germline genes. Putative nucleotide excisions are indicated with strikethrough lines. In blue are the putative TdT N additions in V-D and D-J junctions. In red are mutations from the putative germline genes and the TdT N additions. The non-neutralizing sequences are shown in *italic*.

CORTEX CLASS 3

CDRH3 class 3

	HV1-2*01	HD4-17*01	HJ2*01
164922__3 (17%)	TGTGCGAGAGA	TGACTACGGGTGACTAC	GTACGCGTACTTCGATCTCTGGGCGCGTGGCACCCCTGGTCACTGTCTCCCTCA
	C A R	D Y G D Y	Y W Y F D L W G R G T L V T V S S
	TGTGCGGAGAAAGGACAAAGGGGCGGATGTGCACCGGTGACACCGGGGCGCTTCTTCTTCGATCTCTGGGGCGCGGGCACCCCGGTCAATTGTTTCGTCA		
	C A R K T K G G D V S G D D R G F F D L W G R G T R V I V S S		
	TGTGCGGAGAAAGGACAAAGGCGCGGATGTGCACCGGTGACACCGGGGCGCTTCTTCTTCGATCTCTGGGGCGCGGGCACCCCGGTCAATTGTTTCGTCA		
	C A R K T K A D V S G D D R G F F D L W G R G T R V I V S S		
	TGTGCGGAGAAAGGACAAAGGGGCGGATGTGCACCGGTGACACCGGGGCGCTTCTTCTTCGATCTCTGGGGCGCGGGCACCCCGGTCAATTGTTTCGTCA		
	C A R K T K G D V S G D D R G F F D L W G R G T R V I V S S		
V74-08-179400__4 (24%)	TGTGCGGAGAAAGGACAGCGCGGCGGACACCGGGGCGGACCGCGGTGACACCGGGGCGCTTCTTCTTCGATCTCTGGGGCGCGGGTCCCGGTCAATTGTTTCGTCA		
	C A R K T A G D V S G D N R G Y F F D L W G R G S R V I V S S		
	TGTGCGGAGAAAGGACAGCGCGGCGGACACCGGGGCGGACCGCGGTGACACCGGGGCGCTTCTTCTTCGATCTCTGGGGCGCGGGTCCCGGTCAATTGTTTCGTCA		
	C A R K T A G D V S G D N R G Y F F D L W G R G S R V I V S S		
V74-08-13826__2 (26%)	TGTGCGGAGAAAGGACAGCGCGGCGGACACCGGGGCGGACCGCGGTGACACCGGGGCGCTTCTTCTTCGATCTCTGGGGCGCGGGTCCCGGTCAATTGTTTCGTCA		
	C A R K T A G D V S G D N R G Y F F D L W G R G S R V I V S S		
	TGTGCGGAGAAAGGACAGCGCGGCGGACACCGGGGCGGACCGCGGTGACACCGGGGCGCTTCTTCTTCGATCTCTGGGGCGCGGGTCCCGGTCAATTGTTTCGTCA		
	C A R K T A G D V S G D N R G Y F F D L W G R G S R V I V S S		

CDRHS 3 cases 4

[illegible]

Figure S13a Cont'd-1. CDRH3 analysis of expressed heavy chain sequences from donor 74. CDRH3 and HJ alignments of nucleotide and amino acid for CDRH3 classes 1-6 sequences, aligned to the putative V, D and J germline genes. Putative nucleotide excisions are indicated with strikethrough lines. In blue are the putative TdT N additions in V-D and D-J junctions. In red are mutations from the putative germline genes and the TdT N additions. The non-neutralizing sequences are shown in *italic*.

33/95

[illegible][illegible]

Figure S13a Cont'd-2. CDRH3 analysis of expressed heavy chain sequences from donor 74. CDRH3 and HJ alignments of nucleotide and amino acid for CDRH3 classes 1-6 sequences, aligned to the putative V, D and J germline genes. Putative nucleotide excisions are indicated with strikethrough lines. In blue are the putative TdT N additions in V-D and D-J junctions. In red are mutations from the putative germline genes and the TdT N additions. The non-neutralizing sequences are shown in *italic*.

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CLASS 7

Figure S13b. CDRH3 analysis of expressed heavy chain sequences from donor 74. CDRH3 and HJ alignments of nucleotide and amino acid for VRC-PG04, 04b and their clonally related sequences, aligned to the putative V, D and J germline genes. Putative nucleotide excisions are indicated with strikethrough lines. In blue are the putative TdT N additions in V-D and D-J junctions. In red are mutations from the putative germline genes and the TdT N additions. The alignment analysis suggested that the CDRH3 classes 7 and 8 might be clonally related, as indicated by conserved V-D and D-J junctions, despite that a deletion “ ” occurred in the CDRH3 region. The non-neutralizing sequences are shown in *italic*.

Blue: junctions
Red: mutations
.: gaps

V74-08-53821_1 (30%)	TGTGGGAGACAGAGAAATTTGCGAG.....GGCGACCAAGGCTGGTCTTCGATCTCTGGGCCCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F A R G D Q G W F F D L W G R G T L I V S S
V74-08-71632_2 (26%)	TGCGGGAGACAGAGAAATTTGAGAG.....GGCGGCCCAAGGCTGGTACTTCGATCTCTGGGCCCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F E R G G Q G W Y F D L W G R G T L I V S S
V74-08-61048_1 (29%)	tGGCGGAGACAGAGAAATTTGACAG.....GGCGGCCCAAGGCTGGTATTTCGATCTCTGGGCCCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F E R G G Q G W Y F D L W G R G T L I V S S
VRC-PG04b (30%)	TGCGGGAGACAGAGAAATTTGAGAG.....GGCGGCCCAAGGCTGGTATTTCGATCTCTGGGCCCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F E R G G Q G W Y F D L W G R G T L I V S S
V74-08-69713_1 (32%)	TGCGGGAGACAGAGAAATTTGAGAG.....GGCGGCCCAAGGCTGGTACTTCGATCTCTGGGCCCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F E R G G Q G W I F D L W G R G T L I A V S S
99963_4 (6%)	TGTGGGAGACAGAGAAATTTTATGC.....GGCGGCCCAAGGCTGGTACTTCGATCTCTGGGCGCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F Y A G G Q G W Y F D L W G R G T L I V S S
43567_2 (23%)	TGCGGGAGACAGAGAAATTTTATGC.....GGCGGCCCAAGGCTGGTACTTCGATCTCTGGGCCCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F Y A G G Q G W Y F D L W G R G T L I V S S
31458_3 (24%)	TGCGGGAGACAGAGAAATTTTATGC.....GGCGGCCCAAGGCTGGTACTTCGATCTCTGGGCCCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F Y A G G Q G W Y F D L W G R G T L I V S S
127568_4 (26%)	TGCGGGAGACAGAGAAATTTTATGC.....GGCGGCCCAAGGCTGGTACTTCGATCTCTGGGCCCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F Y A G G Q G W Y F D L W G R G T L I V S S
195462_4 (28%)	TGCGGGAGACAGAGAGTTCTATAA.....GGCGGCCCAAGGCTGGTACTTCGATCTCTGGGCCCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F Y K G G Q G W Y F D L W G R G T L I V S S
V74-08-196147_4 (21%)	TGCGGGAGACAGAGAAATTTATGC.....GGCGGCCCAAGGCTGGTATTTCGATCTCTGGGCGCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K Y Y A G G Q G W Y F D L W G R G T L I V S S
VRC-PG04 (30%)	TGCGGGAGACAGAGAAATTTATAC.....GGCGGCCCAAGGCTGGTACTTCGATCTCTGGGCCCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F Y T G G Q G W Y F D L W G R G T L I V S S
V74-08-149590_4 (30%)	TGCGGGAGACAGAGAAATTTTATGC.....GGCGGCCCAAGGCTGGTACTTCGATCTCTGGGCCCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F Y A G G Q G W Y F D L W G R G T L I V S S
V74-08-165478_2 (30%)	TGCGGGAGACAGAGAAATTTATGTC.....GGCGGCCCAAGGCTGGTACTTCGATCTCTGGGACGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F M S G G Q G W Y F D L W G R G T V I V S S
V74-08-9815_2 (34%)	TGCGGGAGACAGAGAAATTTTATGC.....GGCGGCCCAAGGCTGGTACTTCGATCTCTGGGCCCGTGGAAACCCCTCATTTGTTGTTCTCGTCA
	C A R Q Q K F Y A G G Q G W Y F D L W G R G T L I V S S

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Figure S13b Cont'd-1. CDRH3 analysis of expressed heavy chain sequences from donor 74. CDRH3 and HJ alignments of nucleotide and amino acid for VRC-PG04, 04b and their clonally related sequences, aligned to the putative V, D and J germline genes. Putative nucleotide excisions are indicated with strikethrough lines. In blue are the putative TdT N additions in V-D and D-J junctions. In red are mutations from the putative germline genes and the TdT N additions. The alignment analysis suggested that the CDRH3 classes 7 and 8 might be clonally related, as indicated by conserved V-D and D-J junctions, despite that a deletion “.” occurred in the CDRH3 region. The non-neutralizing sequences are shown in italic.

Blue: junctions
Red: mutations
.: gaps

CDRH3 class 9

	HV1-2*02		
	TGTGCGAGAGA		
	C A R	HD3-16*01	
	GTATTATGATTACGTTTGGGGAGTTATGCTTATAGG		
	Y Y D Y V W G S Y A Y T		
		HJ4*01	
		AGTACTTGGACTACTGGGGCCAAAGGAACCTGGTCACCGTCTCCTCA	
		Y F D Y W G Q G T L V T V S S	
47890_1 (33%)	TGTGCGGGGGGACCATGGGAGGAGTCATGCTACTGGGGCCAAAGGATCCCTGGTCACCGTCTCCTCA		
	C A K G E M G G S H V Y W G Q G S L V T V S S		

Figure S13b Cont'd-2. CDRH3 analysis of expressed heavy chain sequences from donor 74. CDRH3 and HJ alignments of nucleotide and amino acid for VRC-PG04, 04b and their clonally related sequences, aligned to the putative V, D and J germline genes. Putative nucleotide excisions are indicated with strikethrough lines. In blue are the putative TdT N additions in V-D and D-J junctions. In red are mutations from the putative germline genes and the TdT N additions. The alignment analysis suggested that the CDRH3 classes 7 and 8 might be clonally related, as indicated by conserved V-D and D-J junctions, despite that a deletion "." occurred in the CDRH3 region. The non-neutralizing sequences are shown in italic.

15020	3	(4.7%)	QVQLVQSGAEVKKPGASVKVSCKASGYTFETFA.YYIMHWLRQAPGQGLEWMGWINPHSGGHNFT.AQKFQGRVMTTRDTSITTAAYMELSKRSEDATYFCAKQKFASRYSGDQGSYFDIMGRGCTLIIVVSS
161449	2	(10.8)	QVQLVQSGAEVKKPGASVKVSCKGSGTFEN.YYIHWLRQAPGQGSLEWMGWINPDTGGSNS.AQKFLGRVTLTRDTSITTAAYMEMTRLTYYDDTAIYYCAQDRNLRLVAIVATKAHTSIWGRGCTLIIVVSP
3086	2	(13.9%)	QVQLVQSGTEVQKPGASVKVSCKAFCYSETD.YYVYVVRQAPGQGLEYYAVINPNSCYTKY.AQKFQDWVTLTRDSSINTAYLQLARVTSDDTAIYYCAQKQKFASRYSGDQGSYFDIMGRGCTLIIVVSS
157359	4	(15.5%)	QVQLVQSGAEKKKPGASVKLSCKMTSGKVETD.FYIHWVRQAPGQGLEWMGWINPDTGCTGIS.APRFLGRVTLTRDTSISAAIYIEINRVITDDTAIYFCAQKQKFASRYSGDQGSYFDIMGRGCTLIIVVSS
			QVQLVPSGSGVKKPGASVRSVCSWTSEDIFERTELIHWVRQAPGQGLEWIGWVKVITGAVNFGSLDFRRHRIISLTRDRDLFTAHMDIRGLTQGDATYFCAQKQKFESRYTGCQGWYFDIMGRGCTHIIVVSS

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Figure S15. Amino acid frequencies in the VH domains of VRC01-like neutralizing antibodies. Sequence alignment was generated for the VH domains of the twenty-two identified neutralizing sequences from donor 74, along with VRC01, VRC02, VRC03, VRC-PG04, and VRC-PG04b. The amino acid frequencies for each of the VH residue positions were plotted using WebLogo (542). The height of each letter is proportional to the frequency with which the respective amino acid type is observed for the given residue position. The IGHV1-2*02 germline sequence is shown for comparison; insertions with respect to IGHV1-2*02 were not included in this analysis. For each residue position, the amino acid identity of IGHV1-2*02 is shown in maroon, while all other amino acid types are shown in black. Residue positions for which the IGHV1-2*02 identity is of low or zero frequency could indicate affinity maturation changes of functional significance.

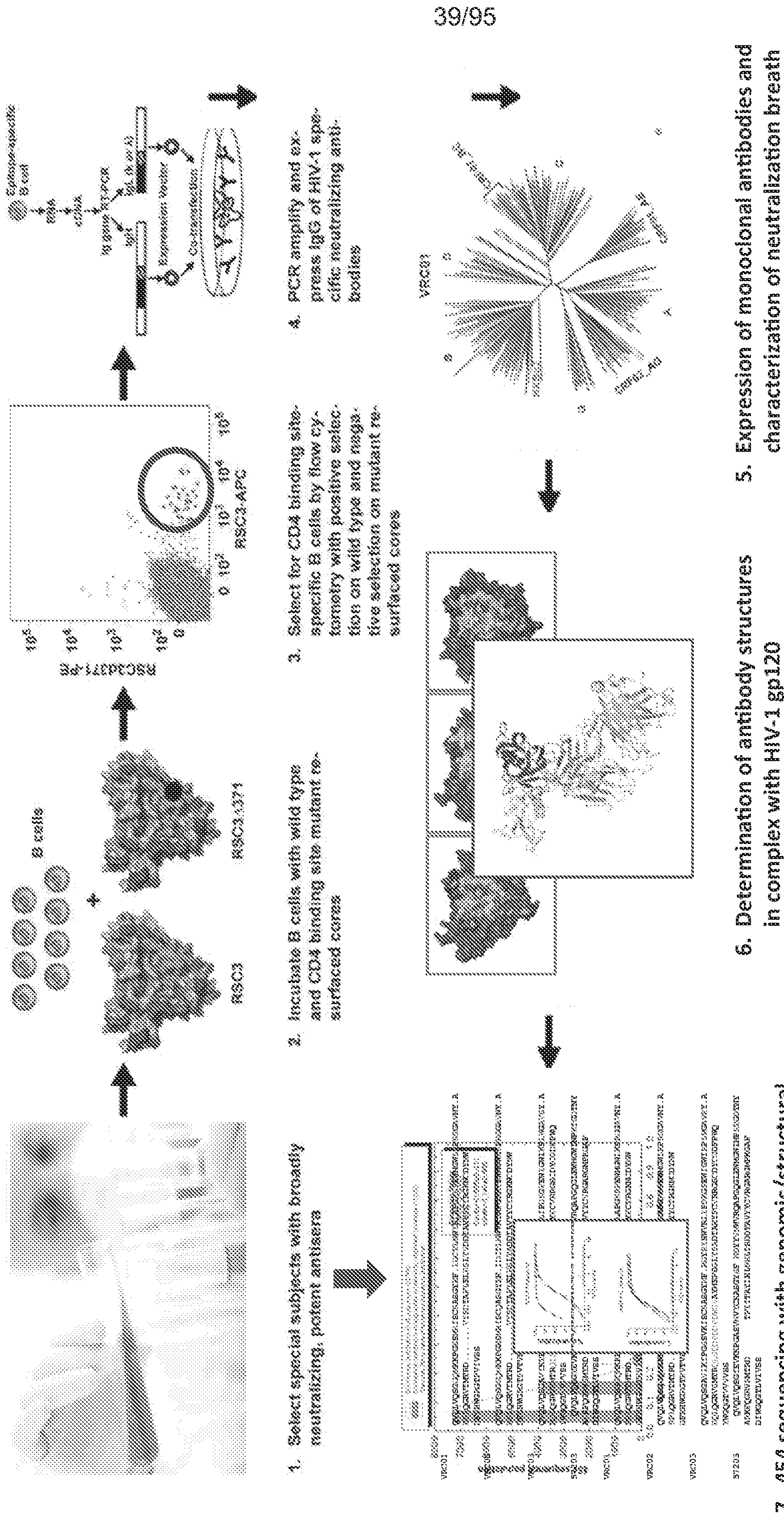
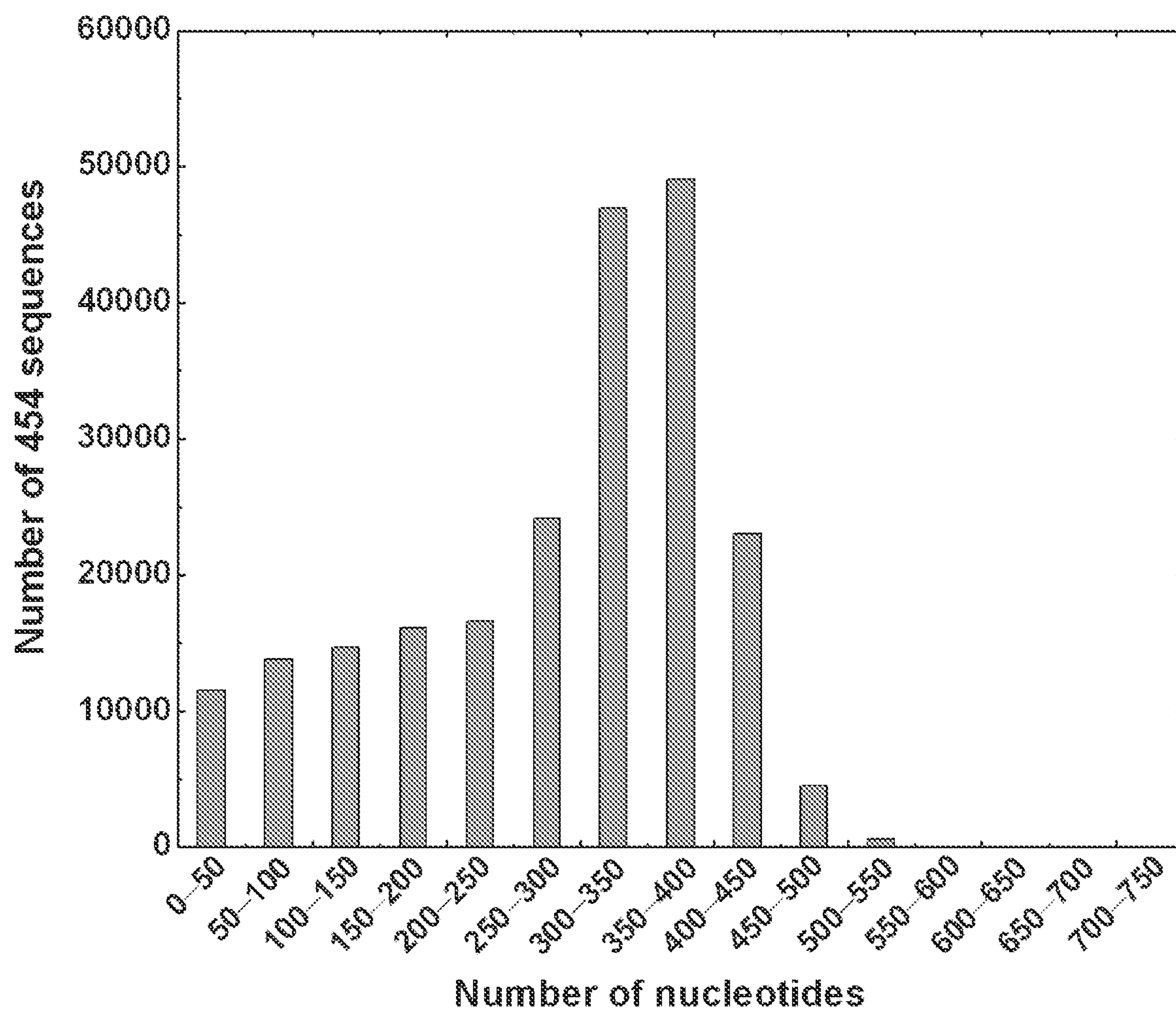


Figure S16. Deep sequencing and structural bioinformatics methodologies facilitate direct analysis of the human antibodyome from PBMCs. The initial process of antibody identification (steps 1-7) involved sorting, single cell sequencing, characterization of neutralization, crystallographic analyses, and 454pyrosequencing. The computational bioinformatic methods described here allow for identification of neutralizing antibodies directly from deep sequencing data (shortcuts shown by red arrow).

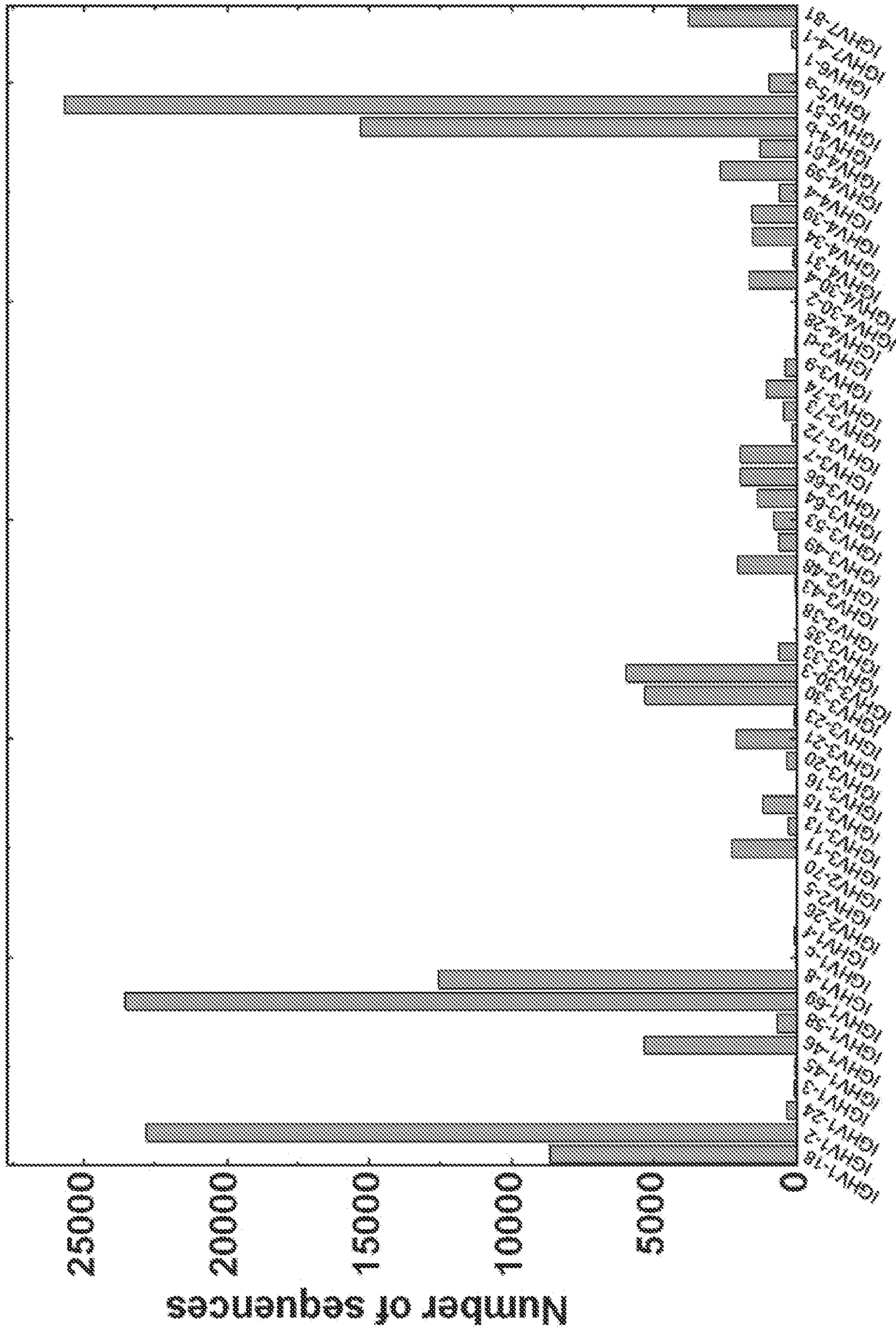
40/95



1. There are 221104 reads in the data set.
2. 78045 (or 35.3%) reads are longer than 350 nucleotides.
3. The average read length is 283.4.

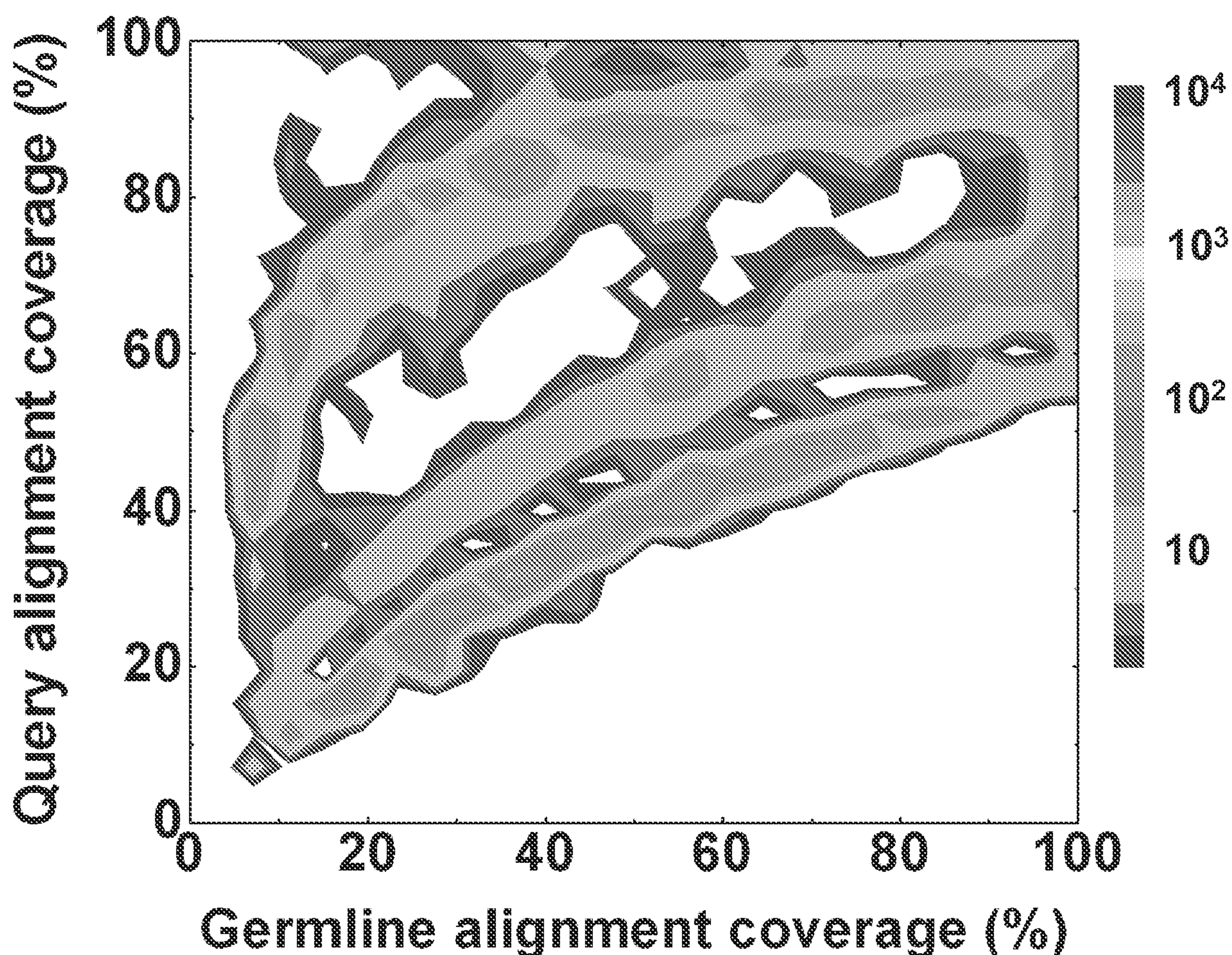
Figure A-1

Figure A-2



1. E-value from IGBlast was used to determine whether the assignment is reliable. A cutoff of 1.0E-3 was used in current analysis.
2. 158309 reads remained after removing the sequences with an E-value lower than 1.0E-3
3. 22790 sequences were assigned to IGHV1-2 family with four possible alleles, IGHV1-2*01, IGHV1-2*02, IGHV1-2*03 and IGHV1-2*04.

42/95



1. We defined a metric – alignment coverage –to characterize the effect of sequence length variation on the alignment of a 454 sequence to a germline gene.

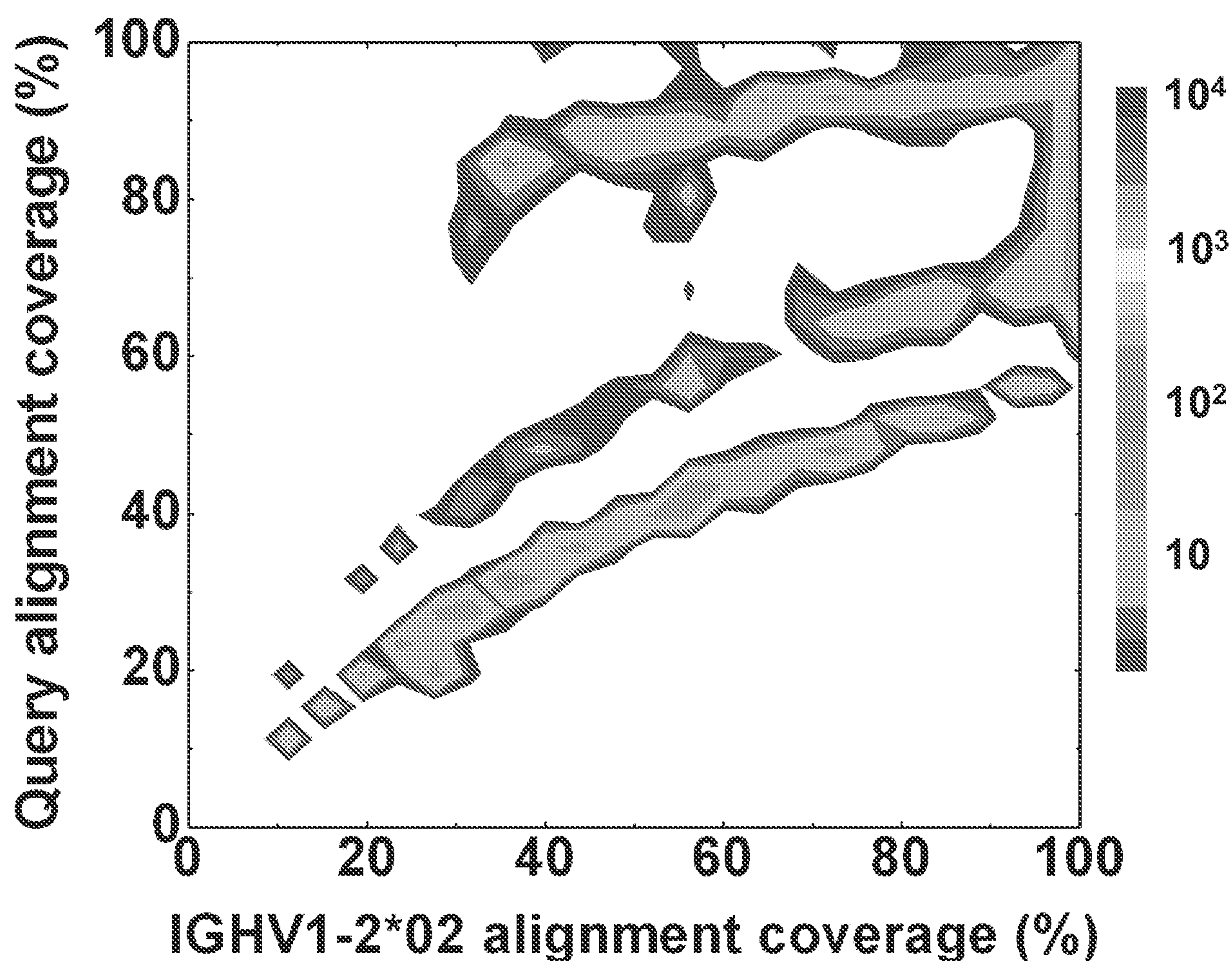
$$Coverage_{Germline} = \frac{Length(aligned\ region)}{Length(Germline\ gene)}$$

$$Coverage_{Query\ sequence\ from\ 454} = \frac{Length(aligned\ region)}{Length(Query\ sequence)}$$

2. When aligned to their respective germline genes, 61471 sequences (or 38.8%) could cover 95% of the germline sequence and thus were considered to contain the “complete” variable (V) gene.

Figure A-3

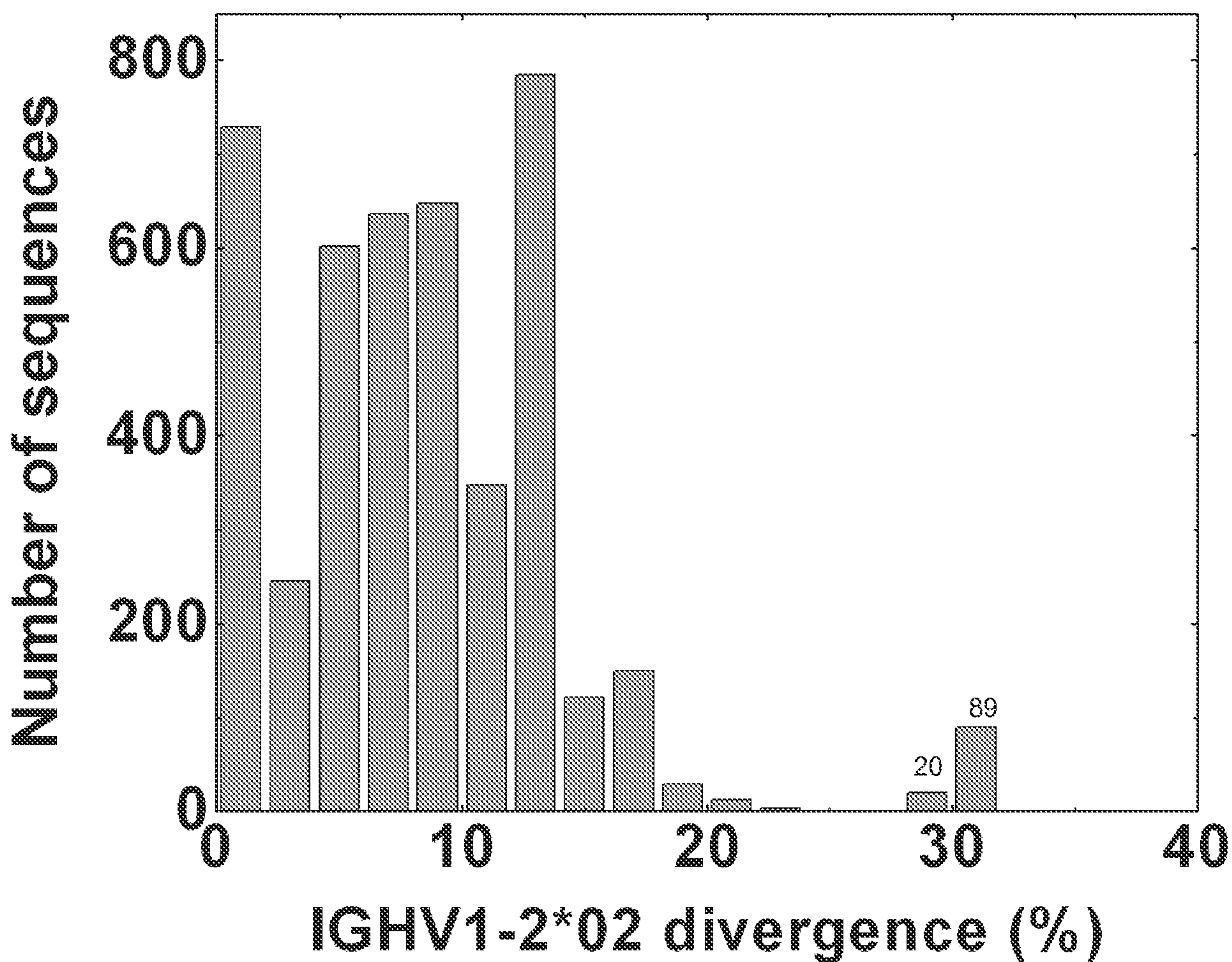
43/95



1. Alignment coverage was also used to characterize the sequences that were assigned to IGHV1-2*02 family. Note that alleles IGVH1-2*01, IGHV1-2*03 and IGHV1-2*04 were also considered in the calculation due to their high similarities to IGHV1-2*02.
2. When aligned to the germline genes, 9598 sequences (or 42.1%) of IGHV1-2*02 family could cover 95% of the germline sequence and thus were considered to contain the "complete" IGHV1-2*02 gene.

Figure A-4

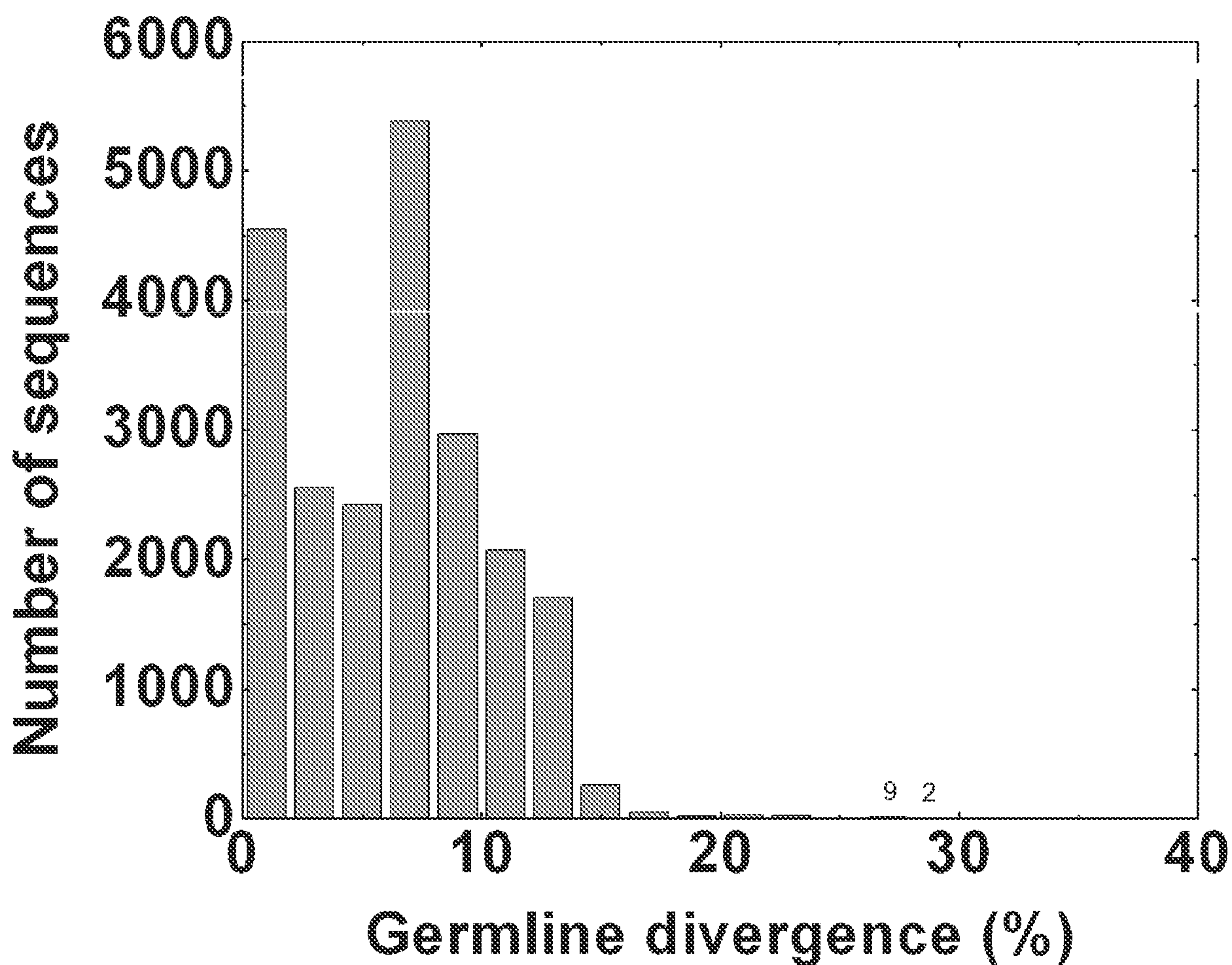
44/95



1. The full-length sequences were extracted from the data set using VRC01 H sequence as a template, resulting in a total of 26445 sequences with 4417 sequences assigned to IGHV1-2*02 family (IGHV1-2*01, IGHV1-2*03 and IGHV1-2*04 alleles included).
2. The divergence of full length IGHV1 2*02 sequences was calculated and plotted as a histogram.
3. A total of 109 highly divergent sequences were found, with 20 in the 28-30% bin and 89 in the 30-32% bin.

Figure A-5

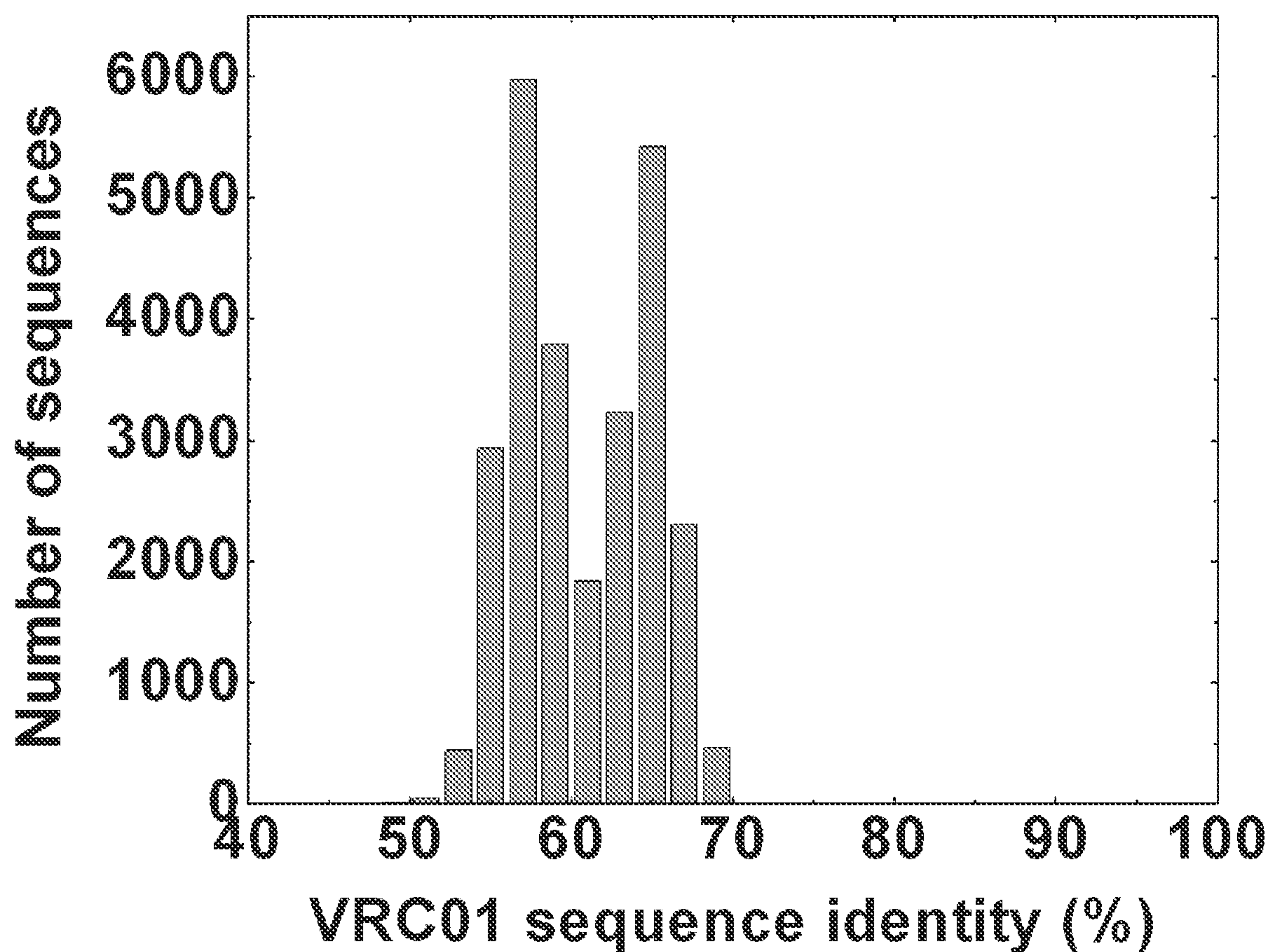
45/95



1. The divergence of full-length sequences that were assigned to non-IGHV1-2*02 germlines was calculated and plotted as a histogram.
2. A total of 11 highly divergent sequences were found, with 9 in the 26-28% bin and 2 in the 28-30% bin.
3. No sequences were found to be more divergent than 30%.

Figure A-6

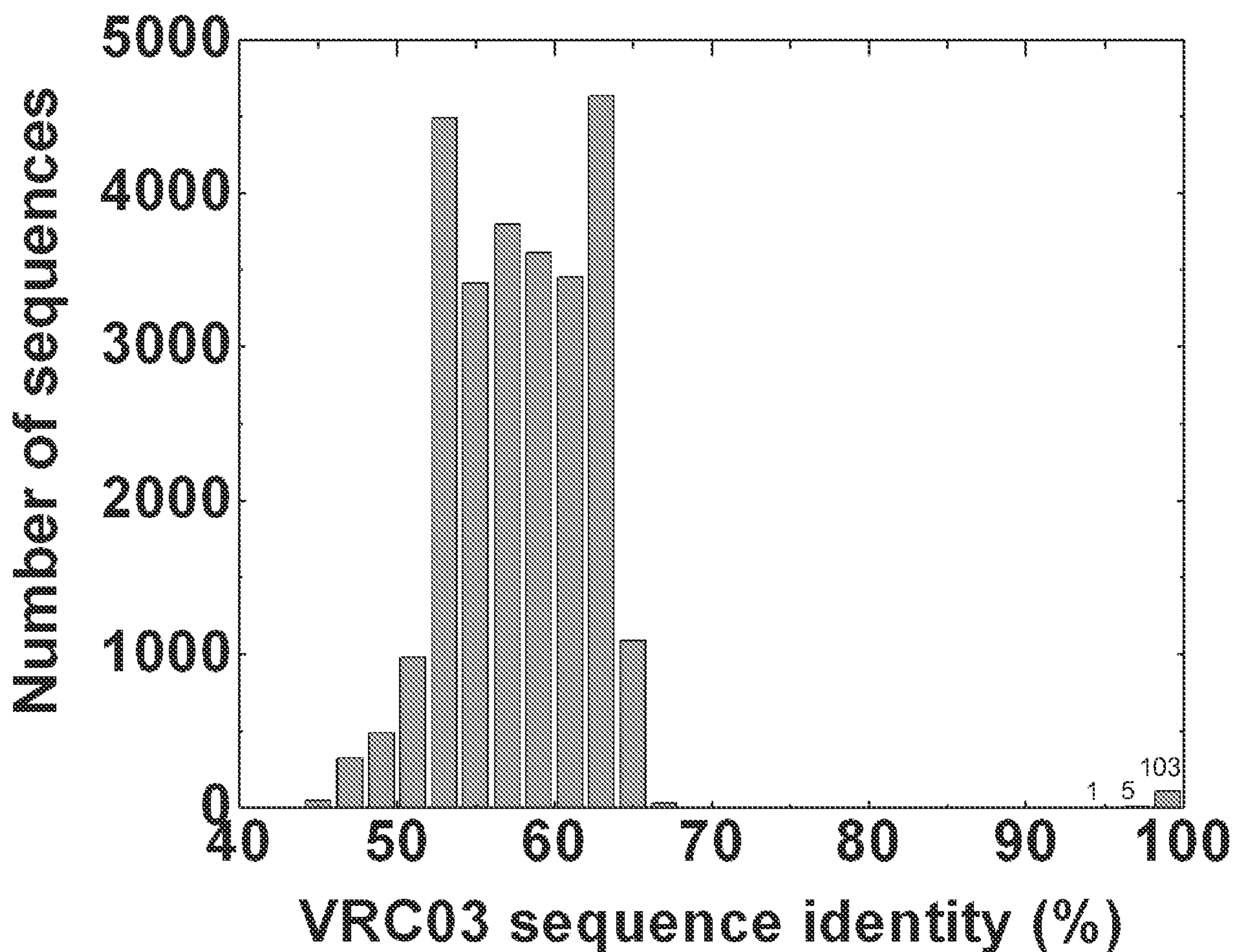
46/95



1. The sequence identity to VRC01 H (at the nucleotide level) was calculated for all full-length sequences and plotted as a histogram.
2. No sequences in the data set were found to be over 70% identical to the VRC01 H sequence.

Figure A-7

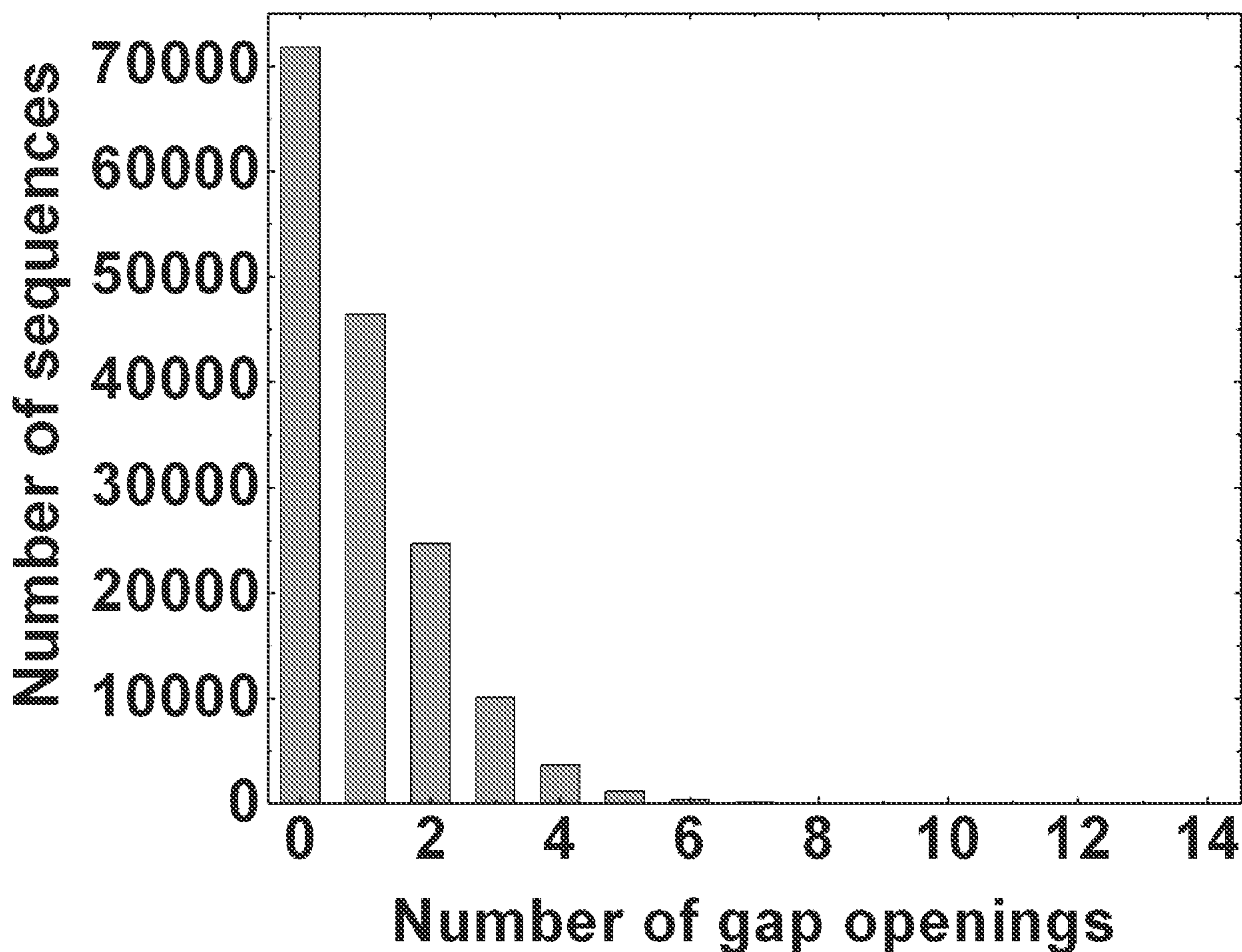
47/95



1. The sequence identity to VRC03 H (at the nucleotide level) was calculated for all full-length sequences and plotted as a histogram.
2. 109 sequences were found to be over 90% identical to the VRC03 H sequence, with 1 in the 92-94% bin, 5 in the 96-98% bin, and 103 in the 98-100% bin.

Figure A-8

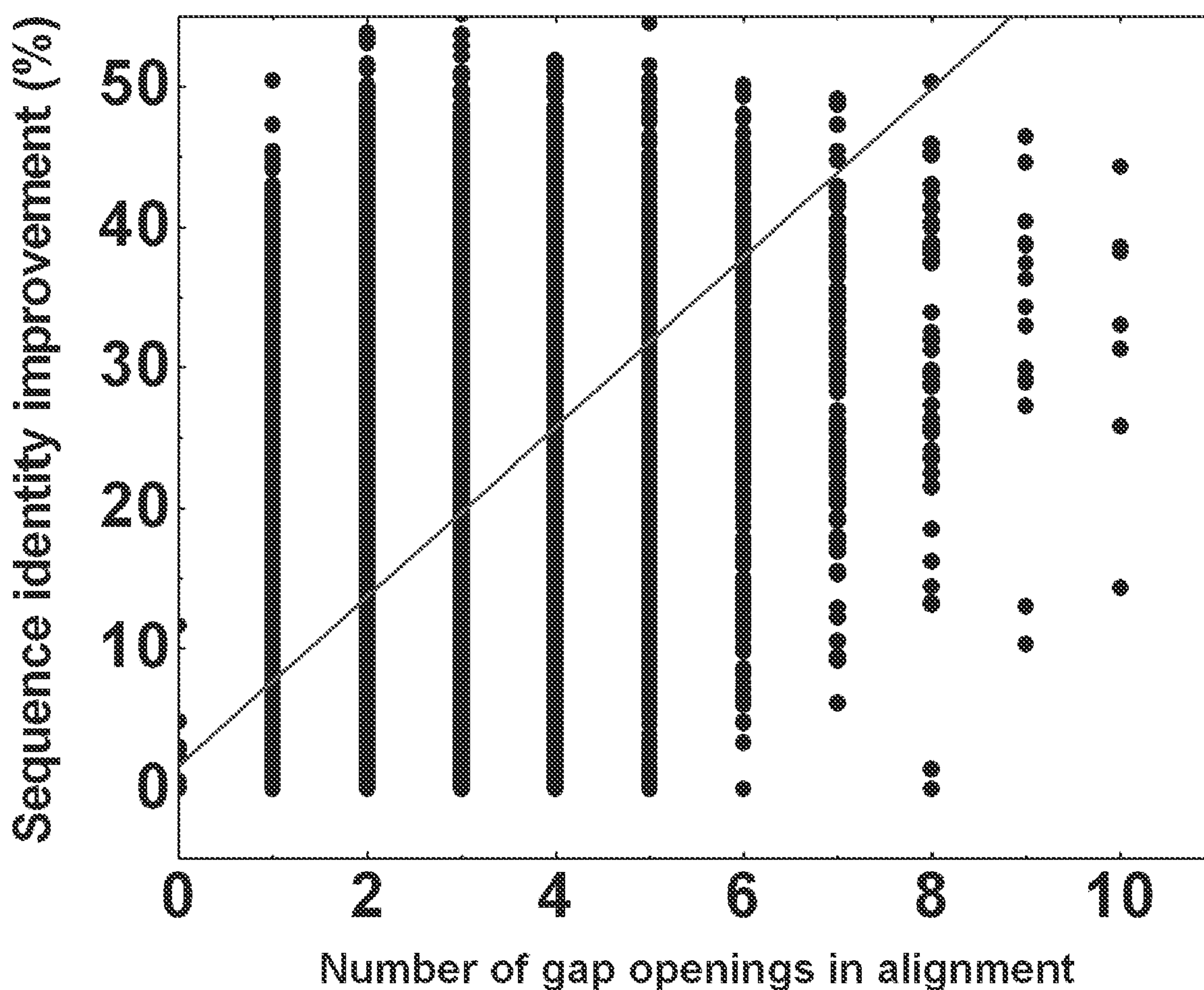
48/95



1. After aligned to the respective germlines, the number of gap openings in the variable region was calculated and plotted as a histogram for the whole data set.
2. 54.7% of the sequences were found to have at least one gap opening in the variable region alignment, which might be caused by 454 sequencing error or naturally occurring insertion/deletion.

Figure A-9

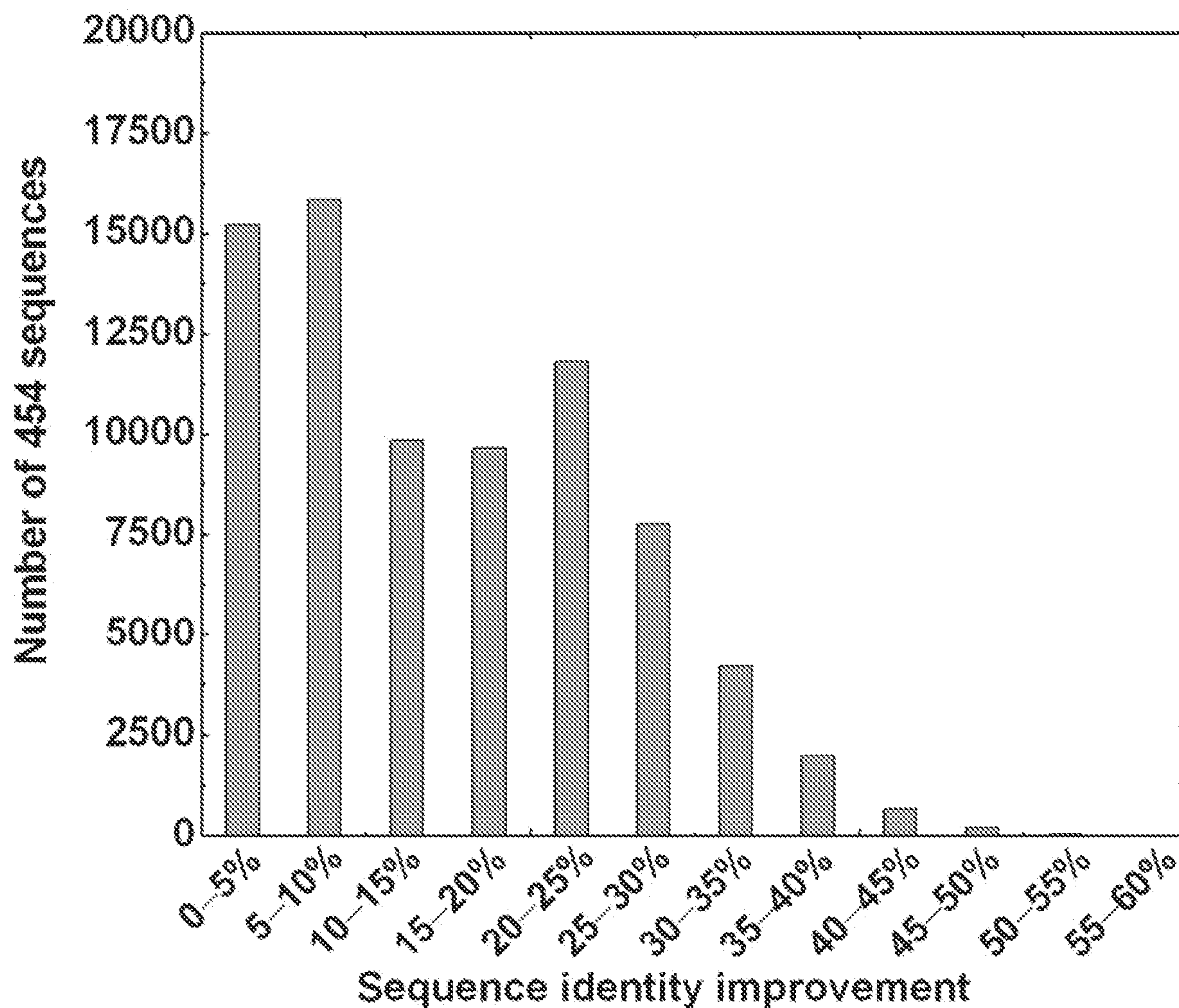
49/95



1. The sequencing errors in the variable region were corrected based on the respective germline and the improvement of sequence identity to the germline sequence was plotted as a function of number gap openings in the variable region.
2. The linear regression R value is 0.652 and the P-value is lower than 0.0001, suggesting that the correlation is significant.

Figure A-10

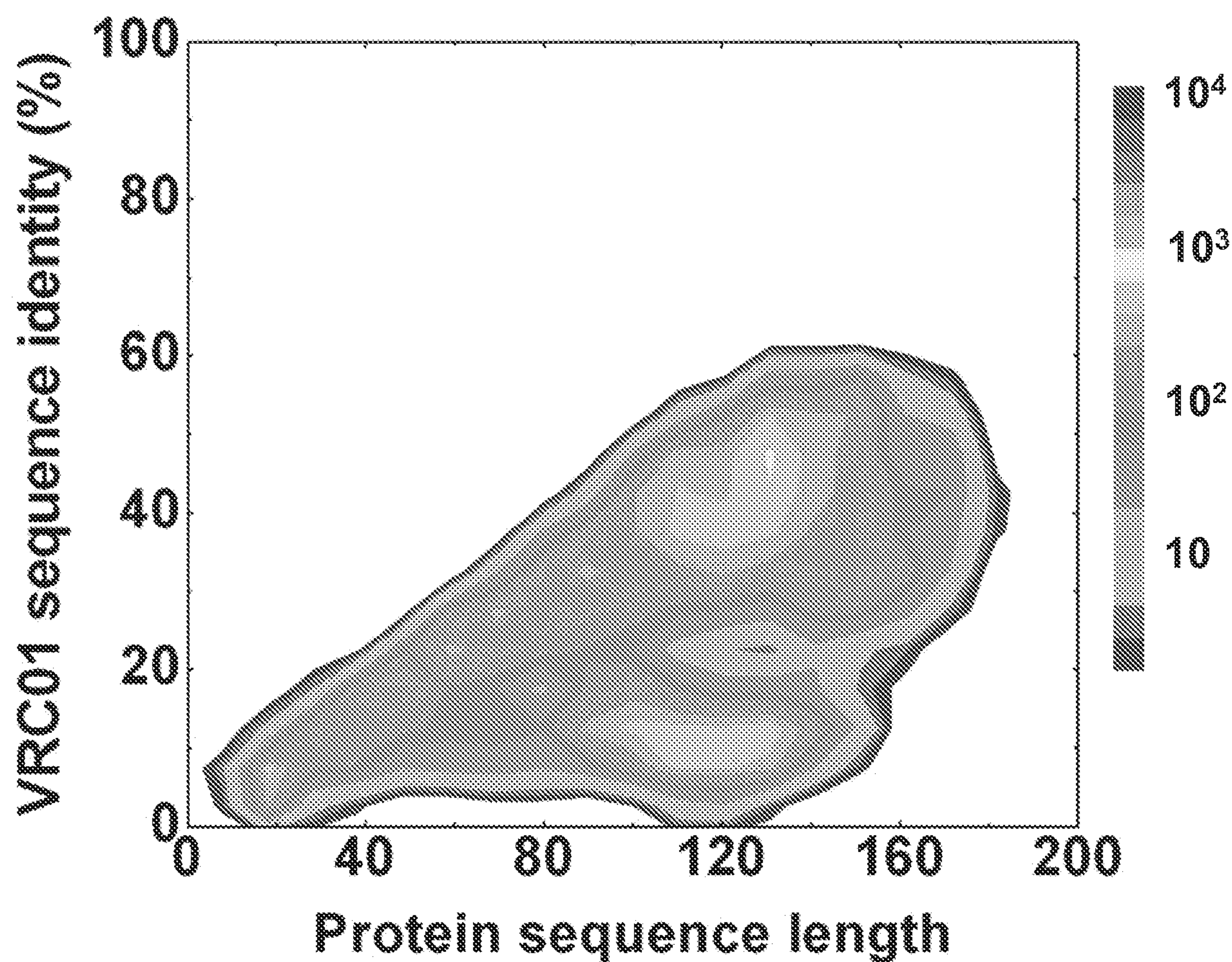
50/95



1. The improvement of sequence identity to the respective germline was calculated for the whole data set and plotted as a histogram.
2. The average improvement is 15.1%.

Figure A-11

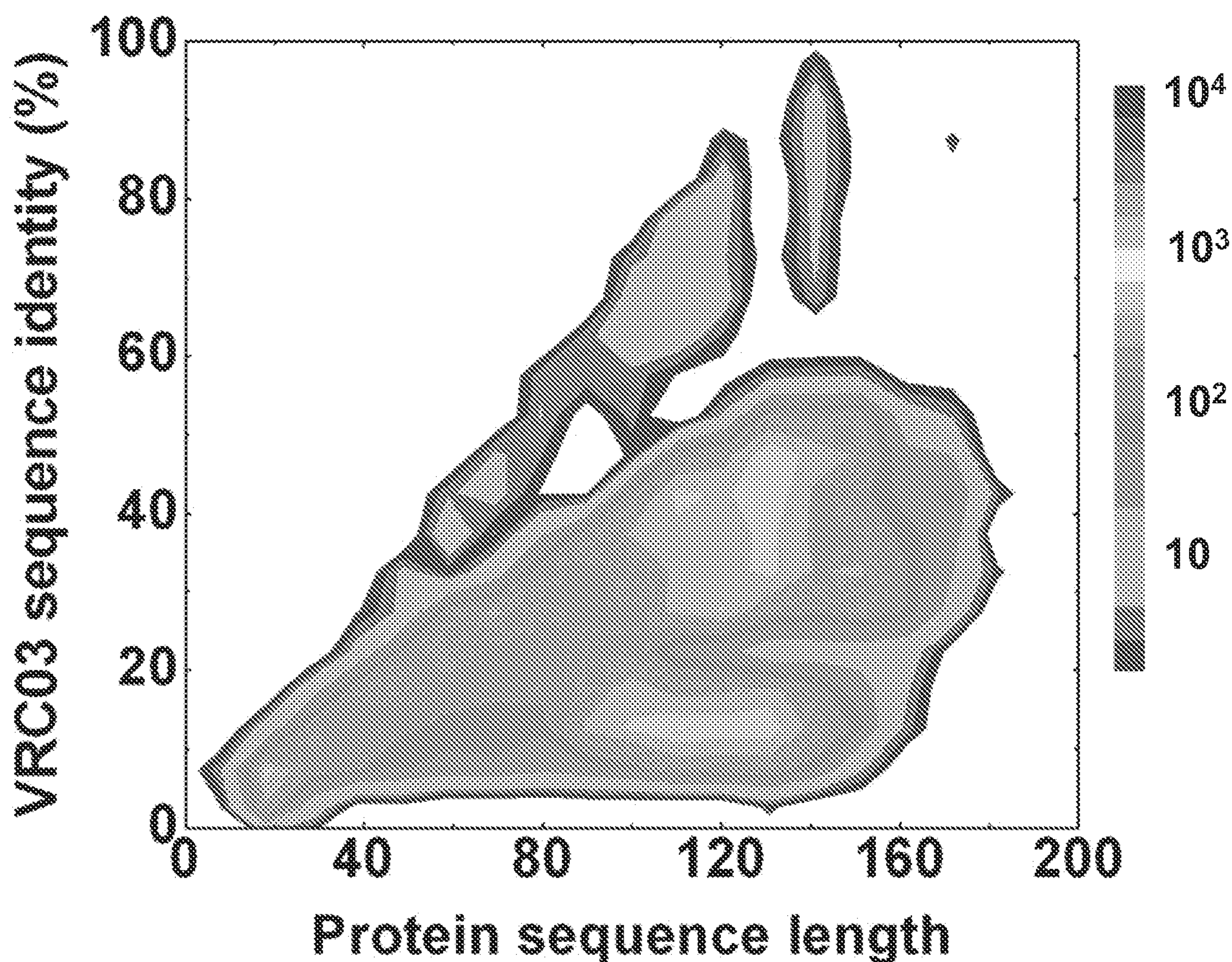
51/95



1. The protein sequences translated from corrected nucleotide sequences were plotted as a function of sequence length and sequence identity to VRC01 H.
2. No VRC01-like sequences were identified from this analysis.

Figure A-12

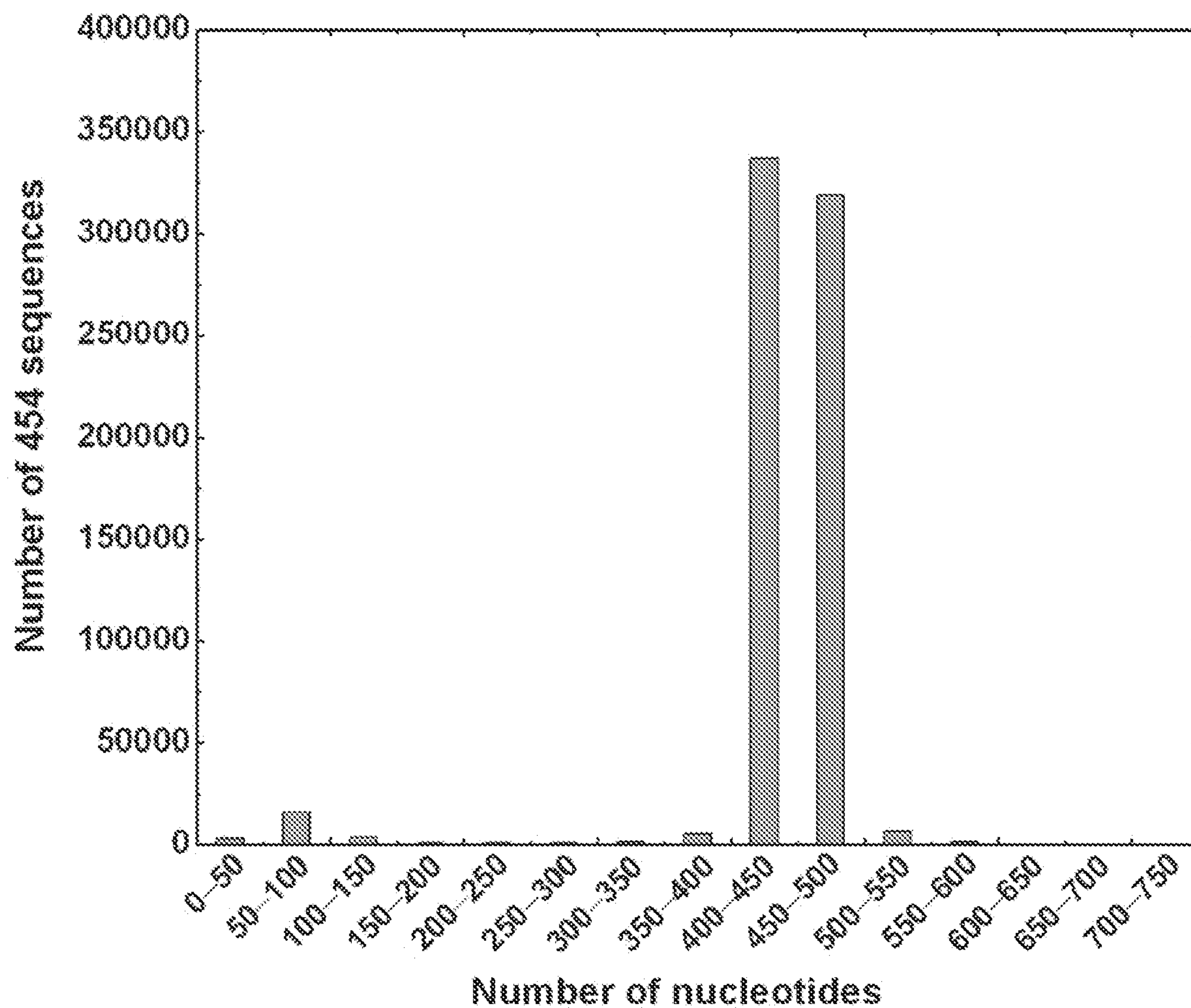
52/95



1. The protein sequences translated from corrected nucleotide sequences were plotted as a function of sequence length and sequence identity to VRC03 H.
2. A set of sequences that extend from the main population to the 100% identity to VRC03 H was identified from the analysis. Within this set, 109 sequences were full-length and identical to those identified by nucleotide-level divergence and sequence identity analyses.

Figure A-13

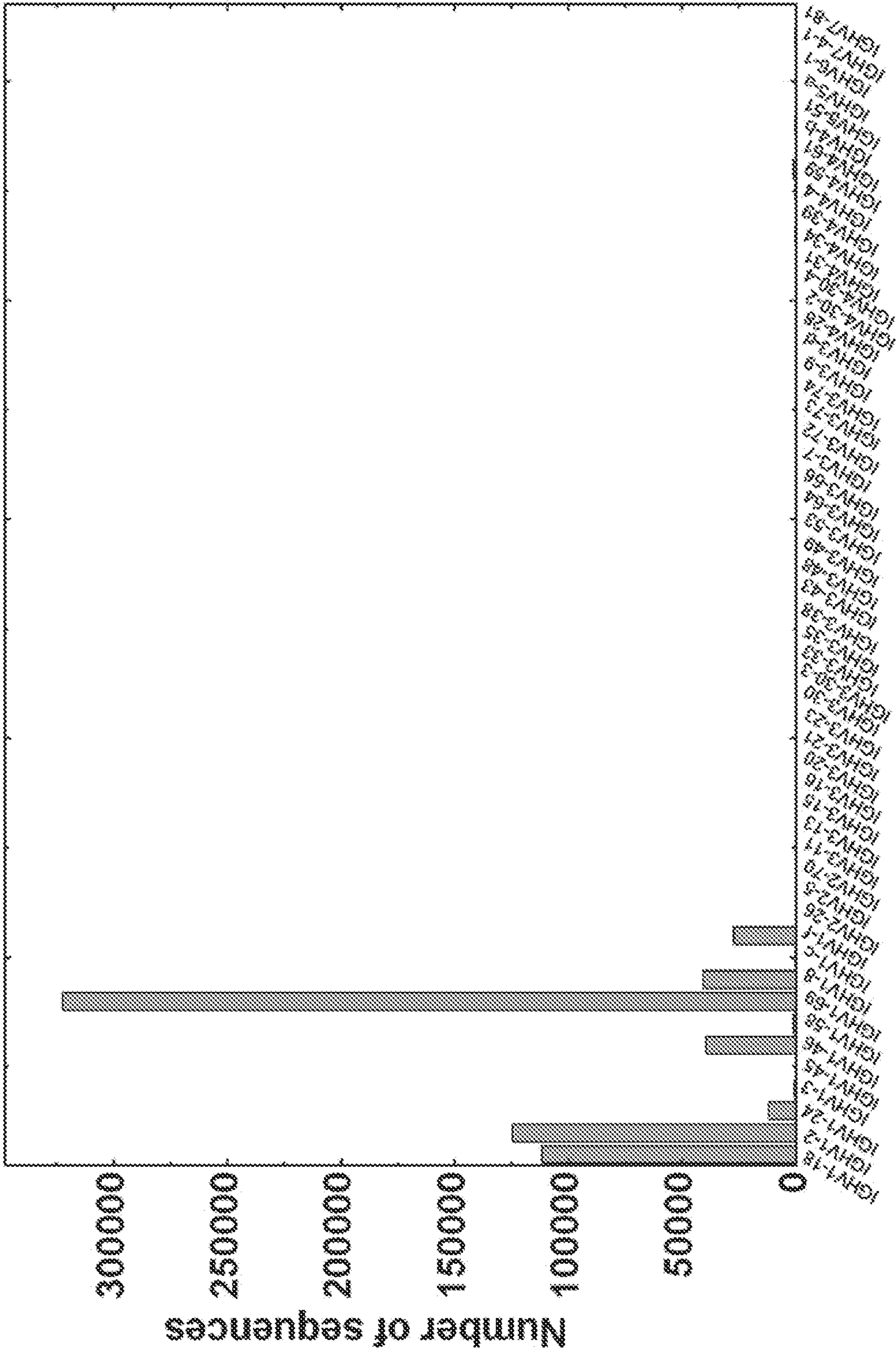
53/95



1. There are 697079 reads in the data set.
2. 669247(or 96.0%) reads are longer than 350 nucleotides.
3. The average read length is 437.5.

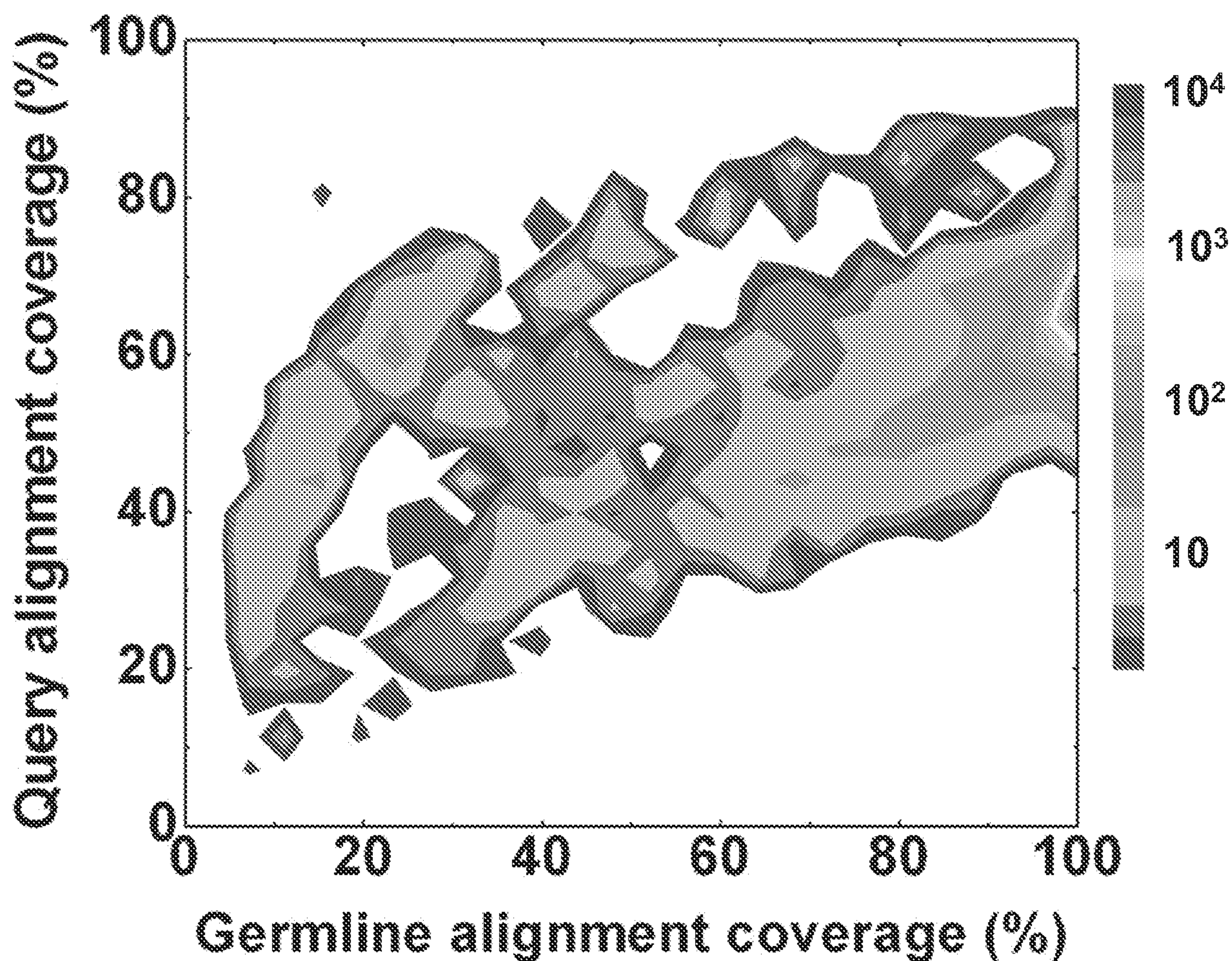
Figure A-14

Figure A-15



1. E-value from IGBlast was used to determine whether the assignment is reliable. A cutoff of 1.0E-3 was used in current analysis.
2. 680047 reads remained after removing the sequences with an E-value lower than 1.0E-3
3. 124109 sequences were assigned to IGHV1-2 family with four possible alleles, IGHV1-2*01, IGHV1-2*02, IGHV1-2*03 and IGHV1-2*04.

55/95



1. A metric – alignment coverage – was defined to characterize the effect of sequence length variation on the alignment of a 454 sequence to a germline gene.

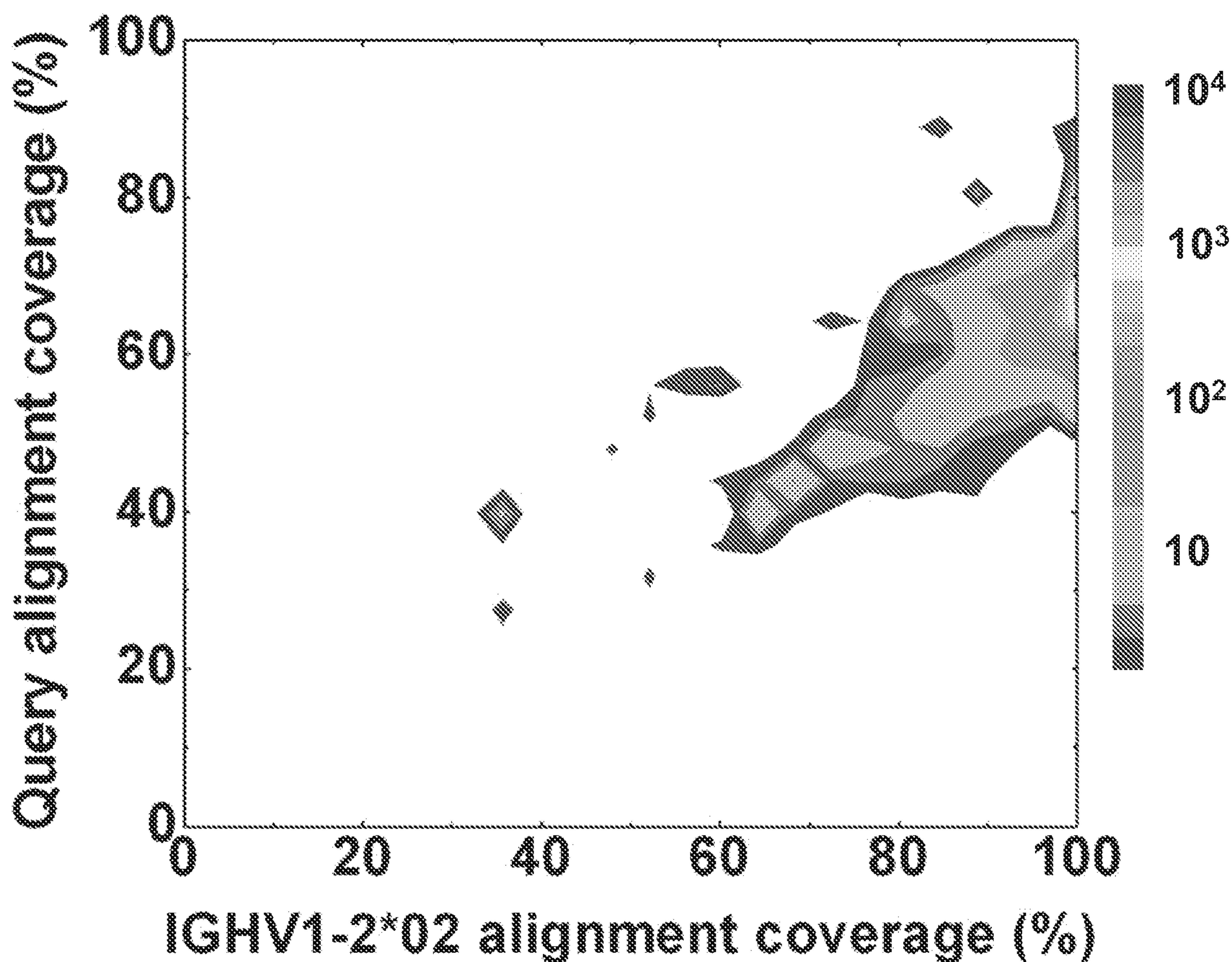
$$Coverage_{Germline} = \frac{Length(aligned\ region)}{Length(Germline\ gene)}$$

$$Coverage_{Query\ sequence\ from\ 454} = \frac{Length(aligned\ region)}{Length(Query\ sequence)}$$

2. When aligned to their respective germline genes, 642754 sequences (or 94.5%) could cover 95% of the germline sequence and thus were considered to contain the “complete” variable (V) gene.

Figure A-16

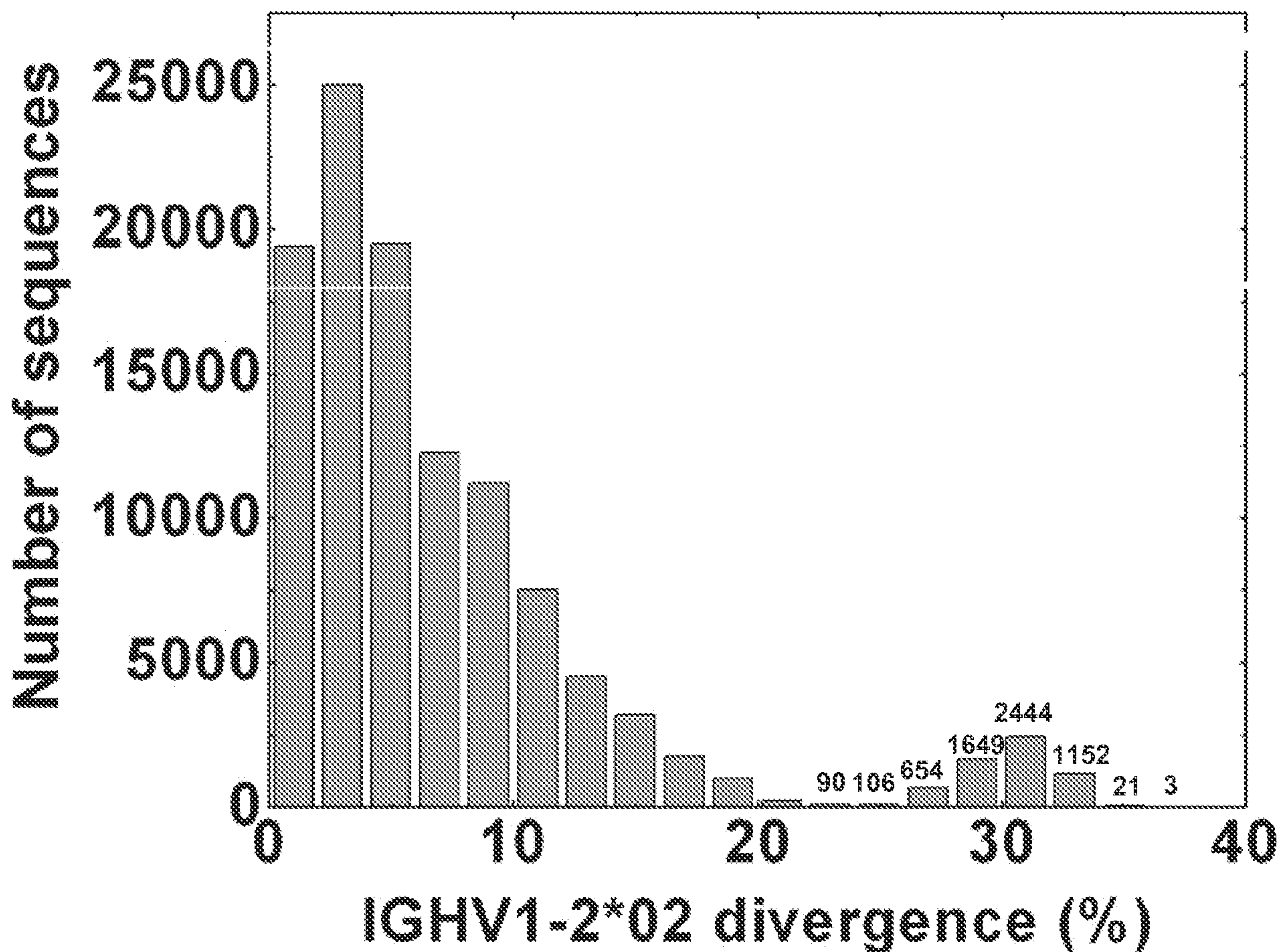
56/95



1. Alignment coverage was also used to characterize the sequences that were assigned to IGHV1-2*02 family. Note that alleles IGVH1-2*01, IGHV1-2*03 and IGHV1-2*04 were also considered in the calculation due to their high similarities to IGHV1-2*02.
2. When aligned to the germline genes, 116563 sequences (or 93.9%) of IGHV1-2*02 family could cover 95% of the germline sequence and thus were considered to contain the "complete" IGHV1-2*02 gene.

Figure A-17

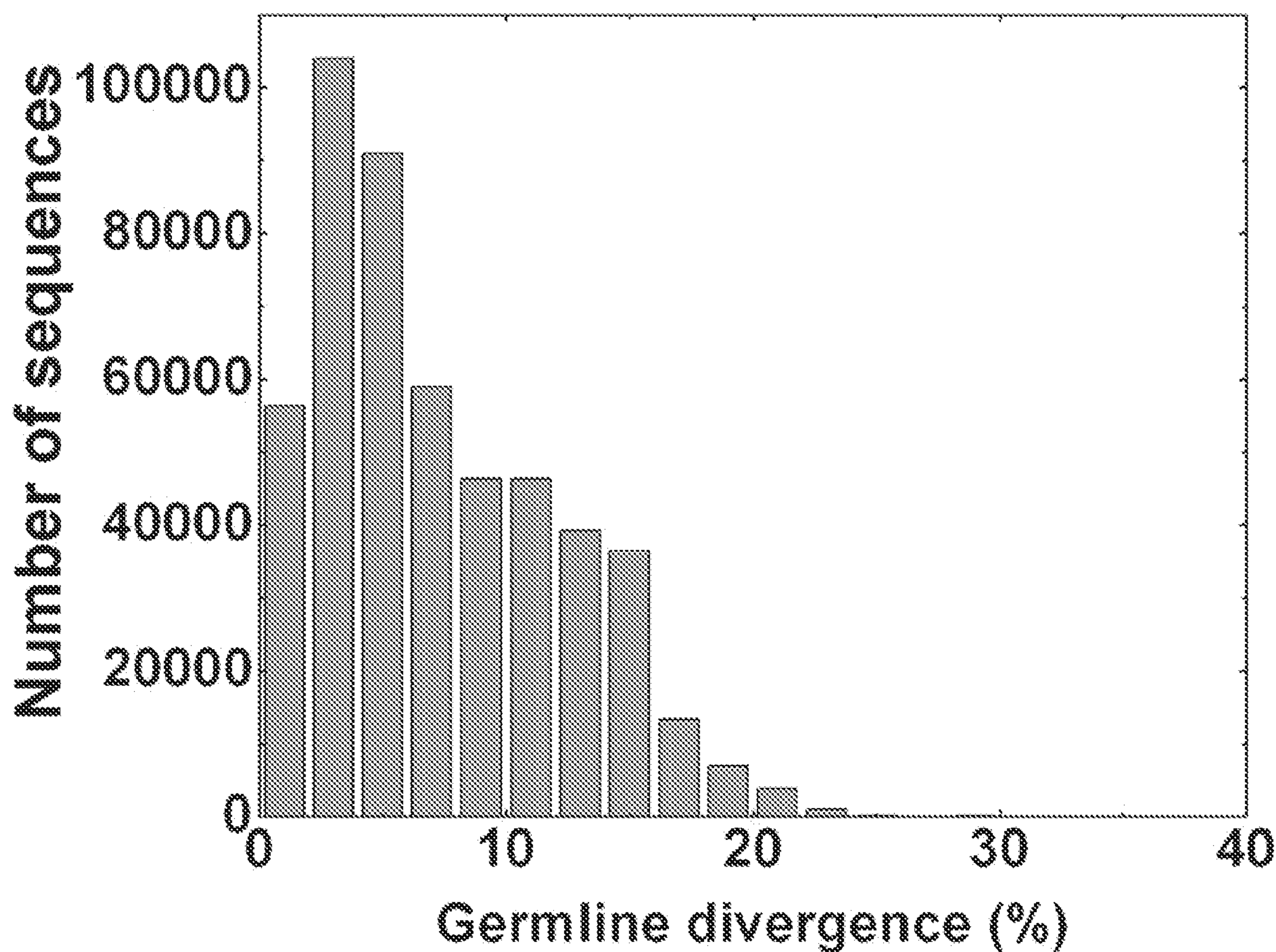
57/95



1. The full-length sequences were extracted from the data set using VRC01 H sequence as a template, resulting in a total of 615876 sequences with 111692 sequences assigned to IGHV1-2*02 family (IGHV1-2*01, IGHV1-2*03 and IGHV1-2*04 alleles included).
2. The divergence of full-length IGHV1-2*02 sequences was calculated and plotted as a histogram.
3. A population of highly divergent sequences were found to be centered at a divergence value of 31%. The number of sequences in each bin is labeled on the histogram.

Figure A-18

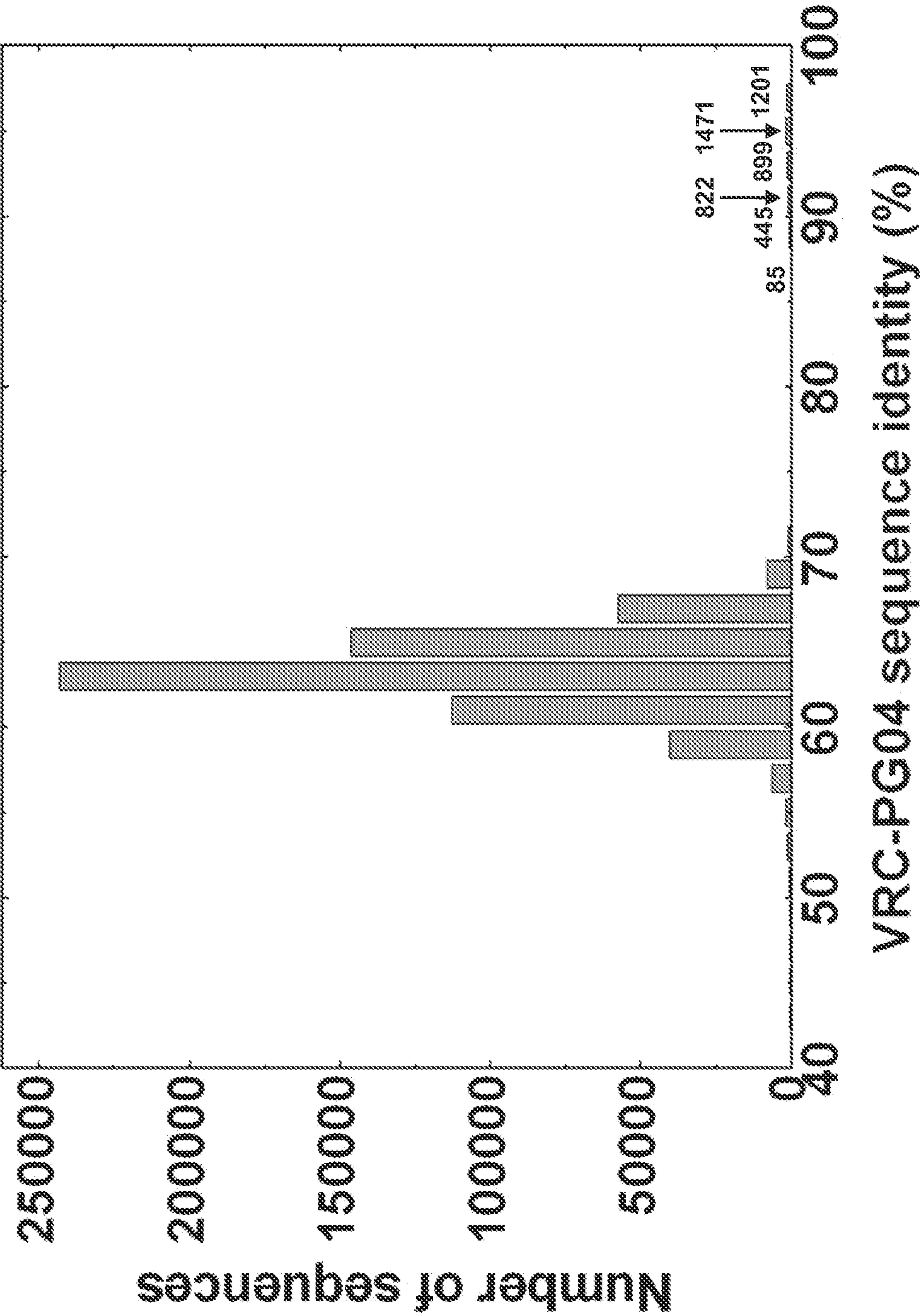
58/95



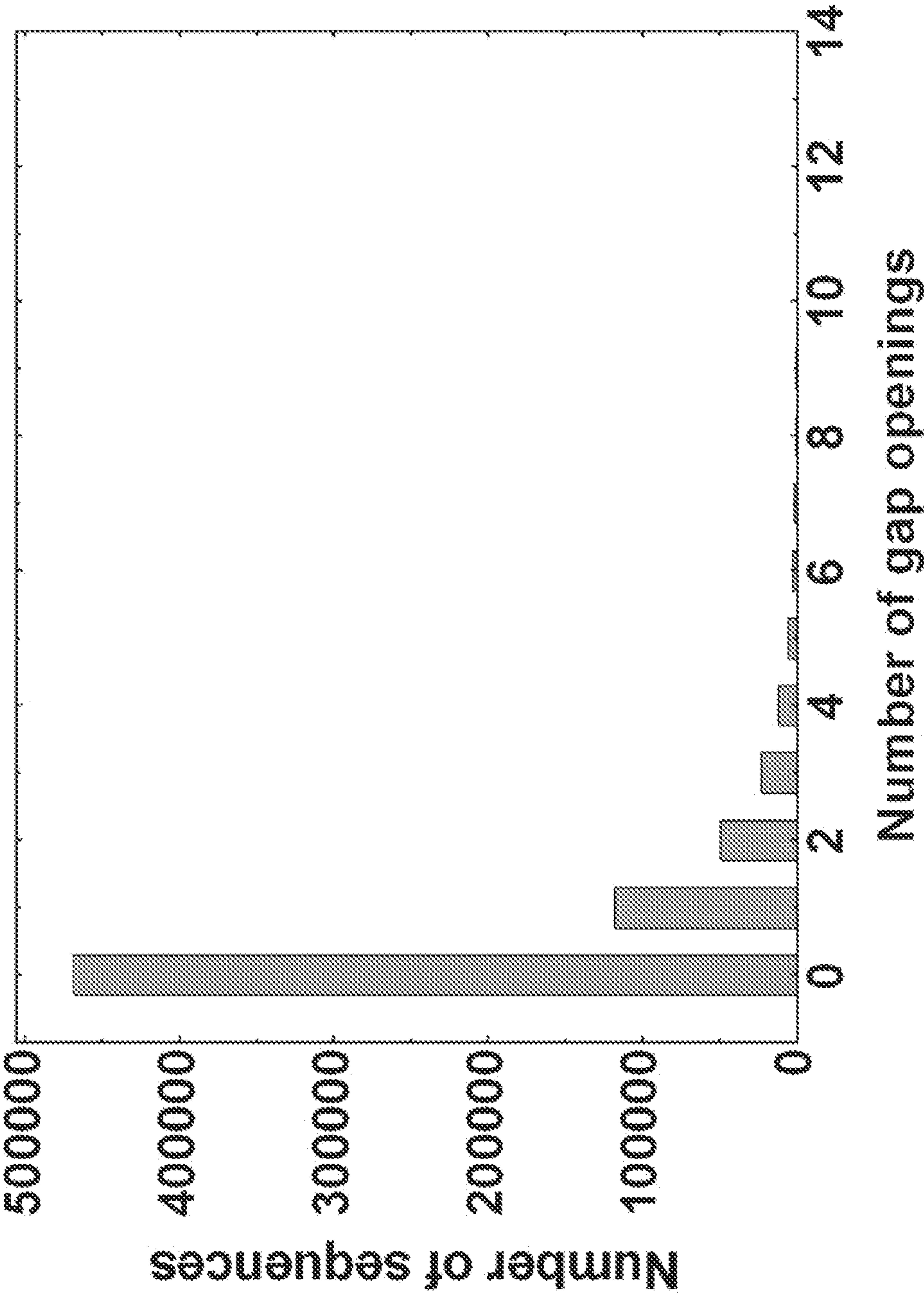
1. The divergence of full-length sequences that were assigned to non-IGHV1-2*02 germlines was calculated and plotted as a histogram.
2. A small group of highly divergent sequences were found to be centered at a divergence value of 29%, with 48 in the 26-28% bin, 150 in 28-30%, and rest beyond 30%.
3. 18 sequences were found to be more divergent than 30%, with 12 in the 30-32% bin, 4 in the 32-34% bin, and 2 in the 34-36%.

Figure A-19

Figure A-20

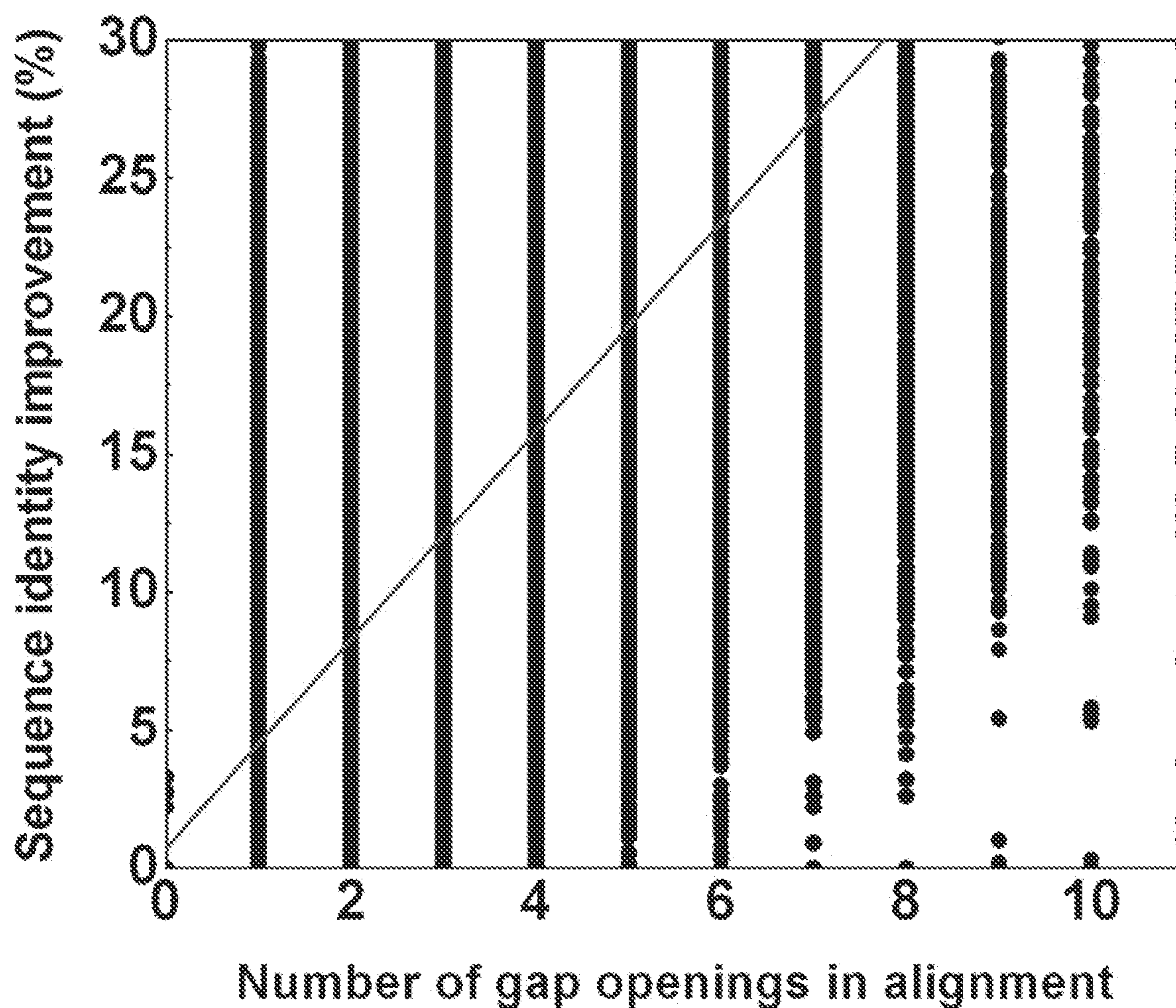


1. The sequence identity to VRC-PG04 H (at the nucleotide level) was calculated for all full-length sequences and plotted as a histogram.
2. A population of sequences were found to have a identity of 85 to 97% to VRC-PG04 H. The number of sequences in each bin is labeled on the histogram.



1. After aligned to the respective germlines, the number of gap openings in the variable region was calculated and plotted as a histogram for the whole data set.
2. 31.2% of the sequences were found to have at least one gap opening in the variable region alignment, which might be caused by 454 sequencing error or naturally occurring insertion /deletion.

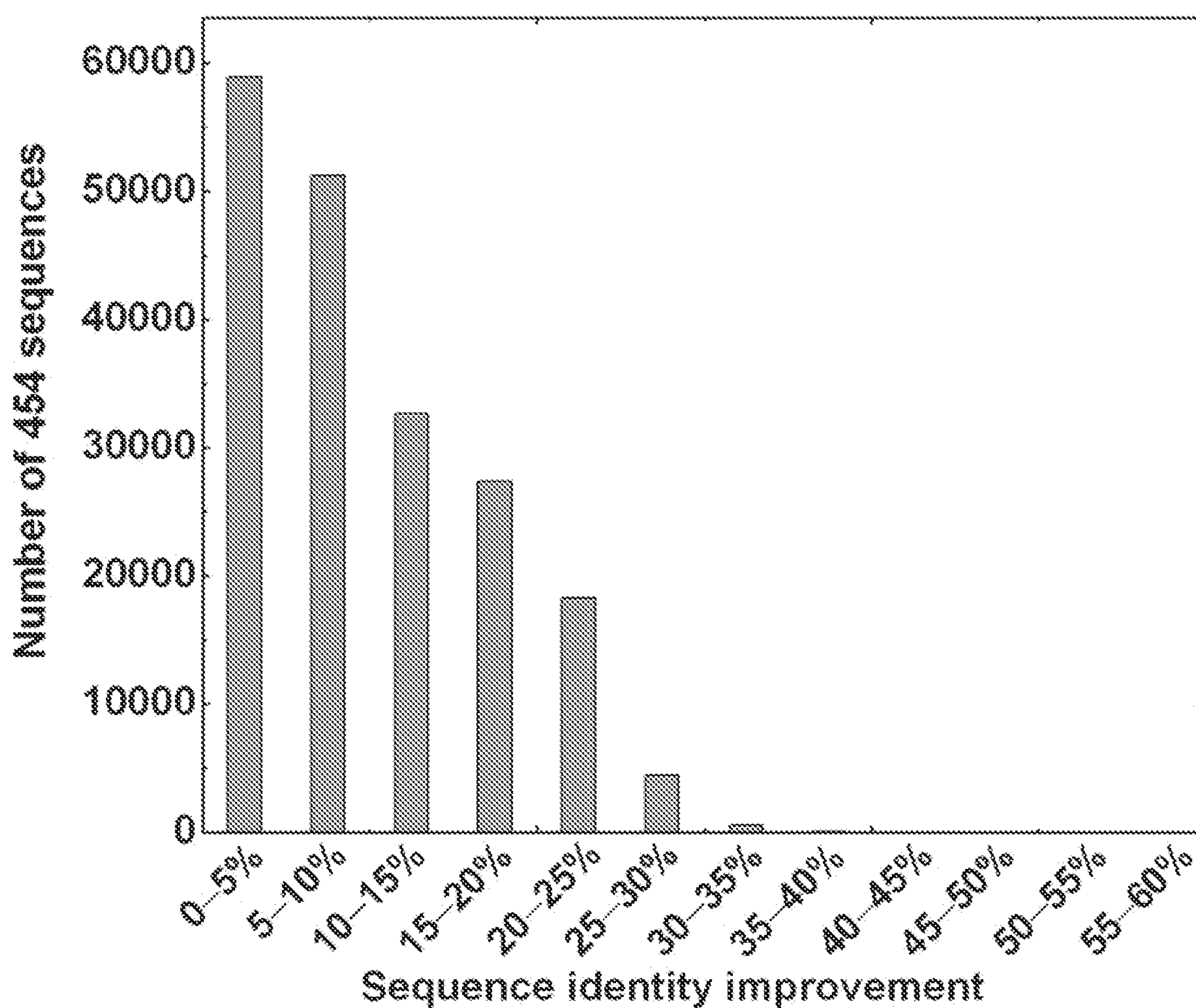
61/95



1. The sequencing errors in the variable region were corrected based on the respective germline and the improvement of sequence identity to the germline sequence was plotted as a function of number gap openings in the variable region.
2. The linear regression R value is 0.717 and the P-value is lower than 0.0001, suggesting that the correlation is significant.

Figure A-22

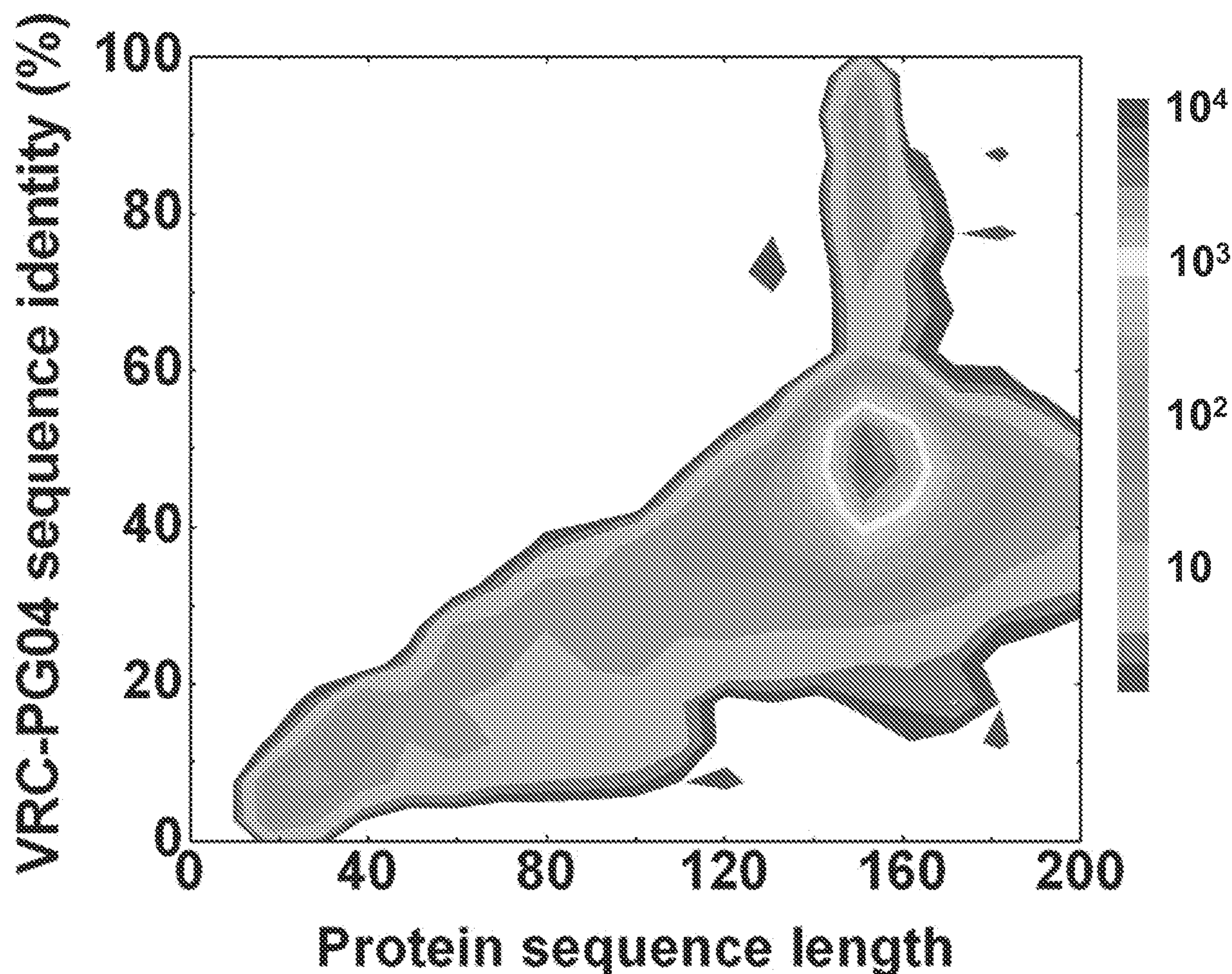
62/95



1. The improvement of sequence identity to the respective germline was calculated for the whole data set and plotted as a histogram.
2. The average improvement is 10.2%.

Figure A-23

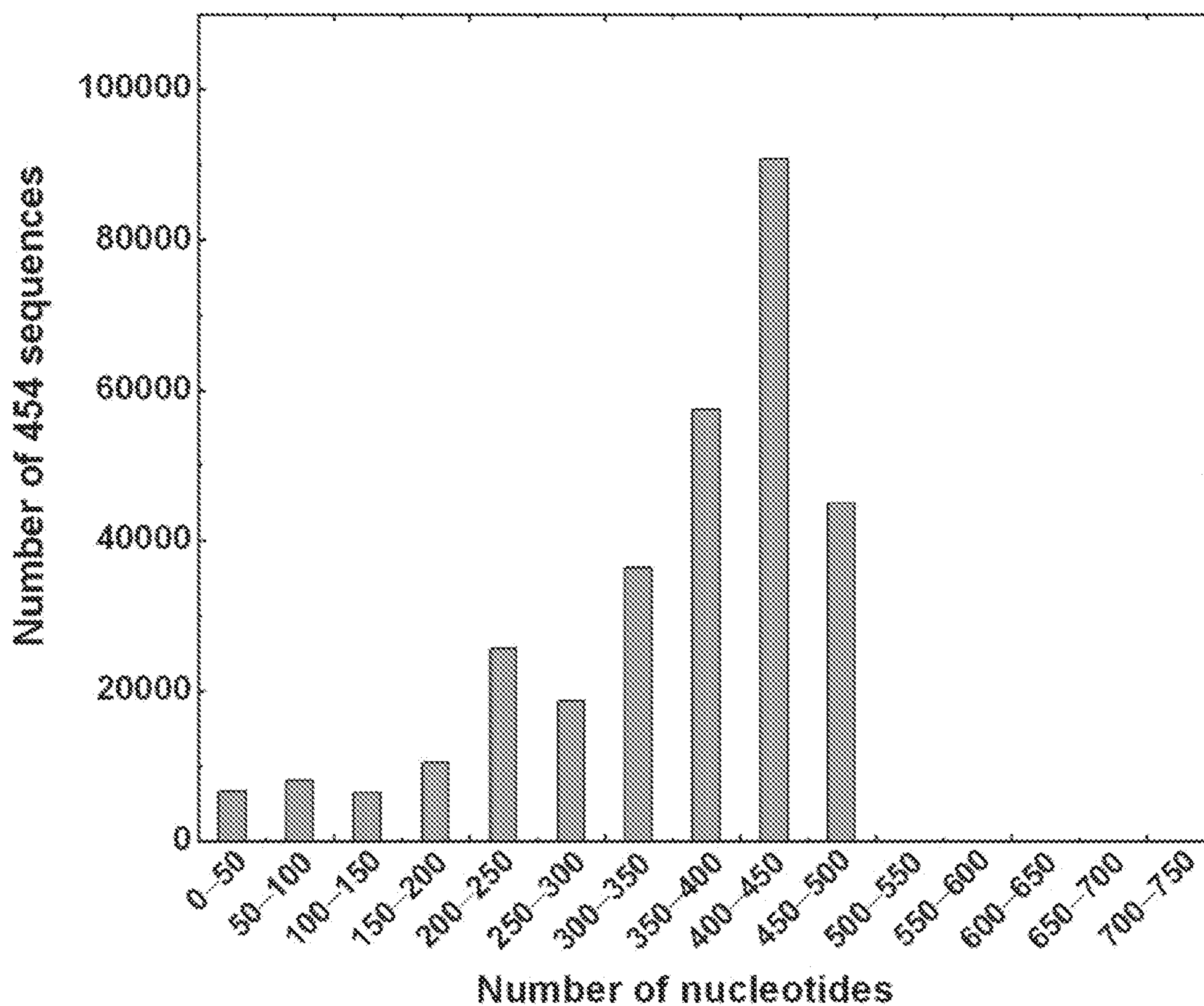
63/95



1. The protein sequences translated from corrected nucleotide sequences were plotted as a function of sequence length and sequence identity to VRC03 H.
2. A set of sequences that extend from the main population to the 100% identity to VRC-PG04 H was identified from the analysis. A subset of these sequences correspond to those identified by nucleotide-level divergence and sequence identity analyses.

Figure A-24

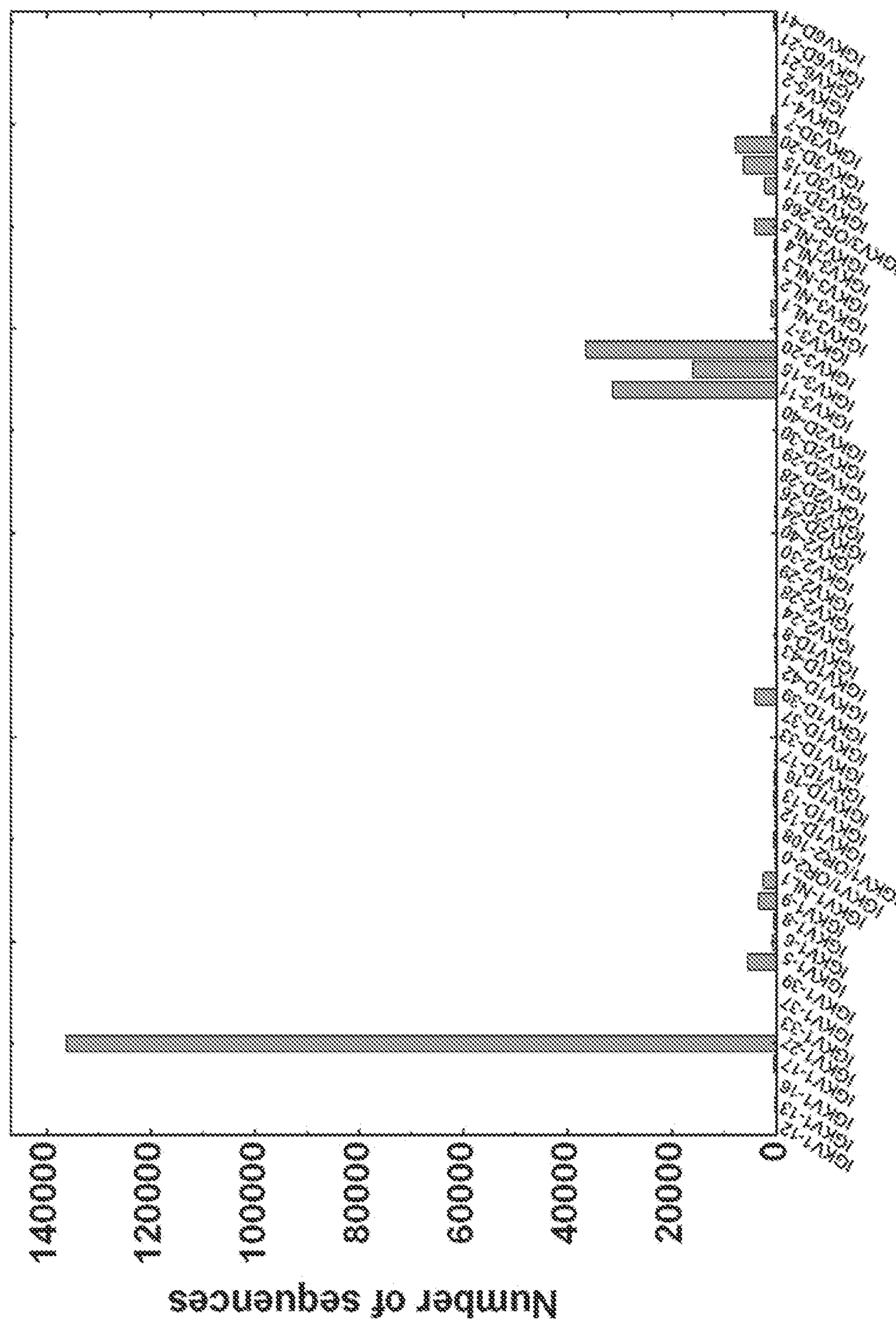
64/95



1. There are 305475 reads in the data set.
2. 230126 (or 75.3%) reads are longer than 300 nucleotides.
3. The average read length is 352.0.

Figure A-25

Figure A-26



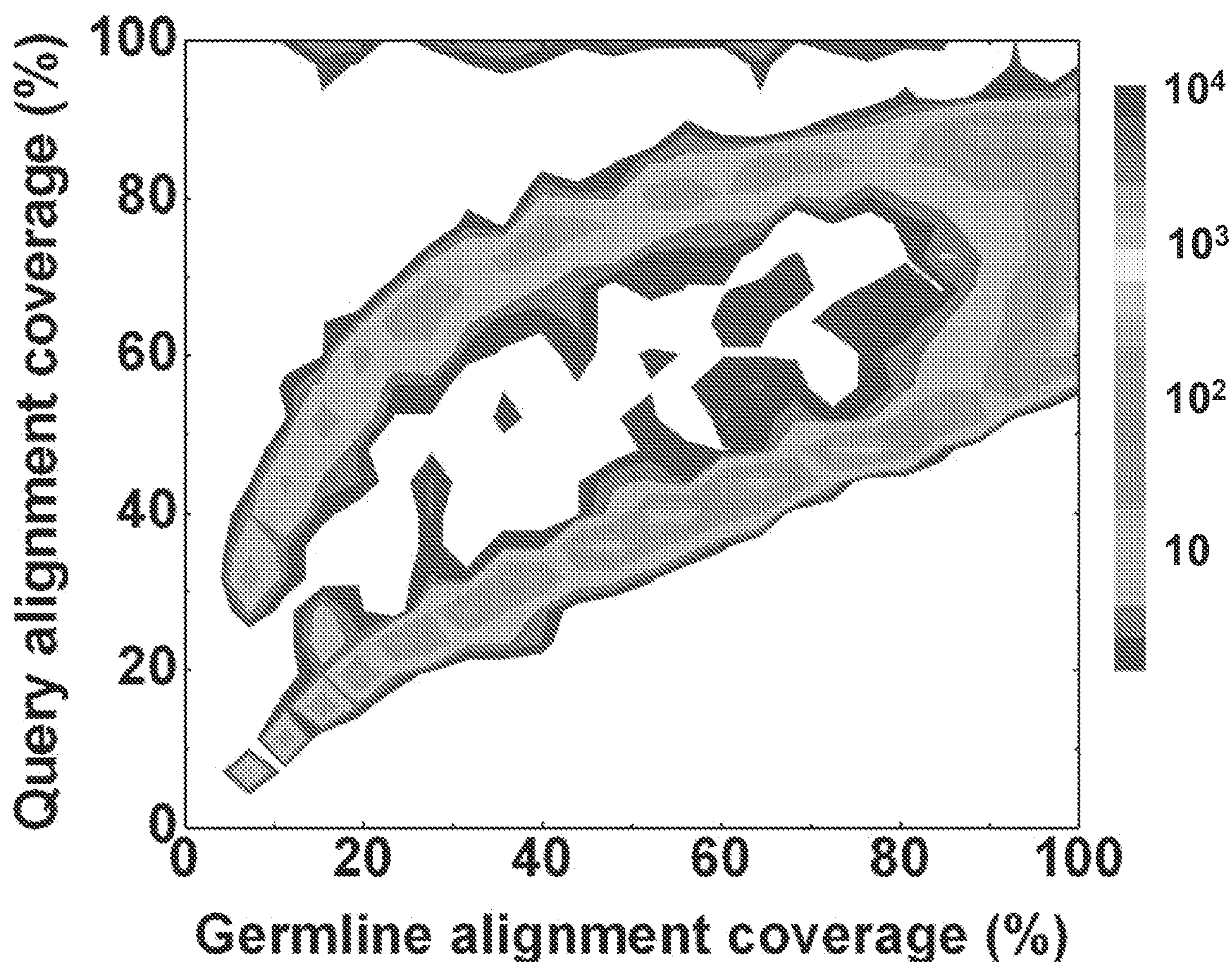
1. E-value from IGBlast was used to determine whether the assignment is reliable. A cutoff of 1.0E-3 was used in current analysis.
2. 260615 reads remained after removing the sequences with an E-value lower than 1.0E-3
3. Since VRC01 L and VRC03 L will be assigned to IGKV3-NL1*01 and IGKV3-NL5*01 by IGBlast, we included these two as possible germlines of VRC01 L and VRC03 L.
4. 70994 sequences were assigned to IGKV3-11*01, IGKV3-20*01, IGKV3-NL1*01, and IGKV3-NL5*01.

2. 260615 reads remained after removing the sequences with an E-value lower than 1.0×10^{-3}

3. Since VRC01 L and VRC03 L will be assigned to IGKV3-NL1*01 and IGKV3-NL5*01 by IGBlast, we included these two as possible germlines of VRC01 L and VRC03 L.

4. 70994 sequences were assigned to IGKV3-11*01, IGKV3-20*01, IGKV3-NL1*01, and IGKV3-NL5*01.

66/95



1. A metric – alignment coverage – was defined to characterize the effect of sequence length variation on the alignment of a 454 sequence to a germline gene.

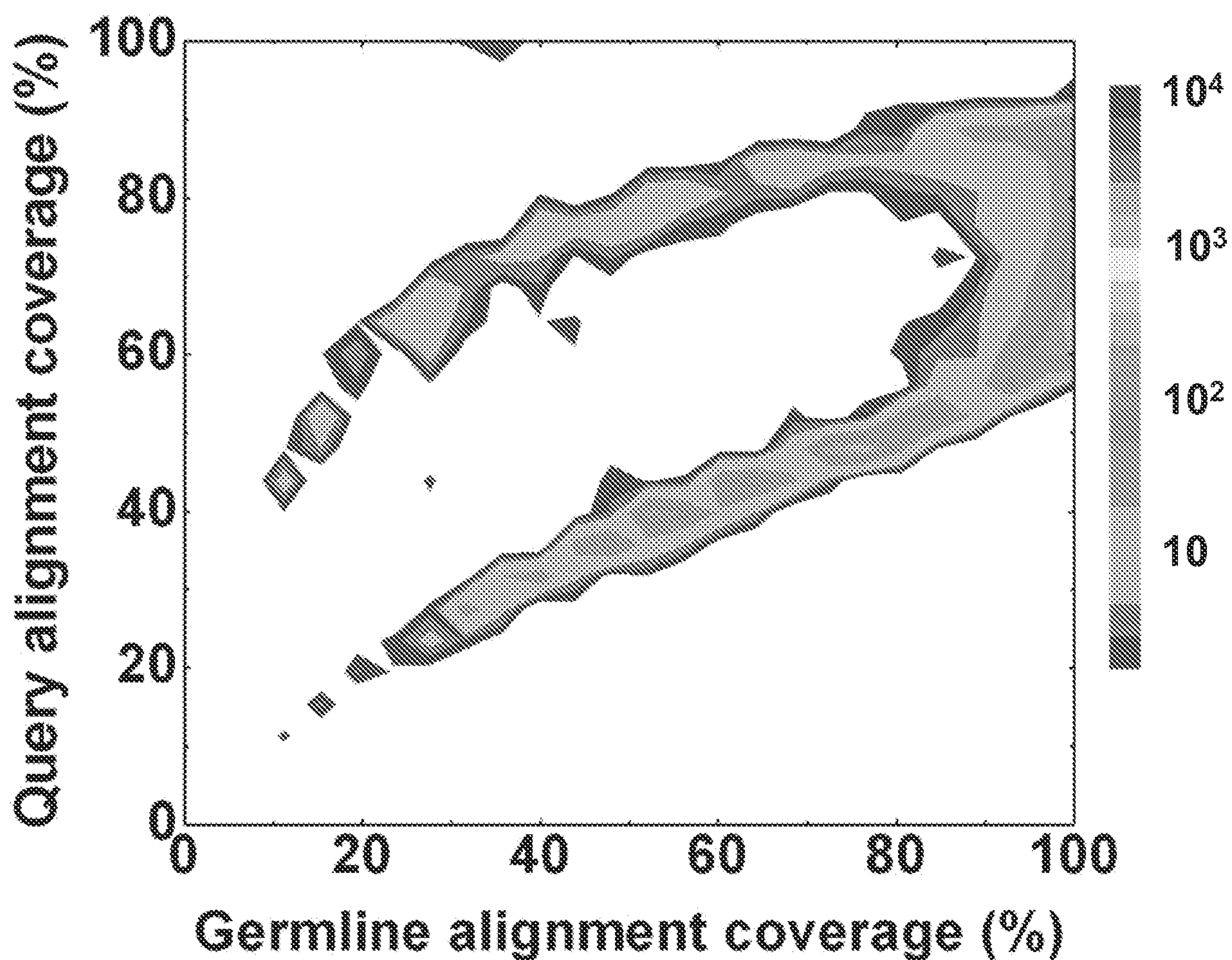
$$Coverage_{Germline} = \frac{Length(aligned\ region)}{Length(Germline\ gene)}$$

$$Coverage_{Query\ sequence\ from\ 454} = \frac{Length(aligned\ region)}{Length(Query\ sequence)}$$

2. When aligned to their respective germline genes, 103066 sequences (or 39.5%) could cover 95% of the germline sequence and thus were considered to contain the “complete” variable (V) gene.

Figure A-27

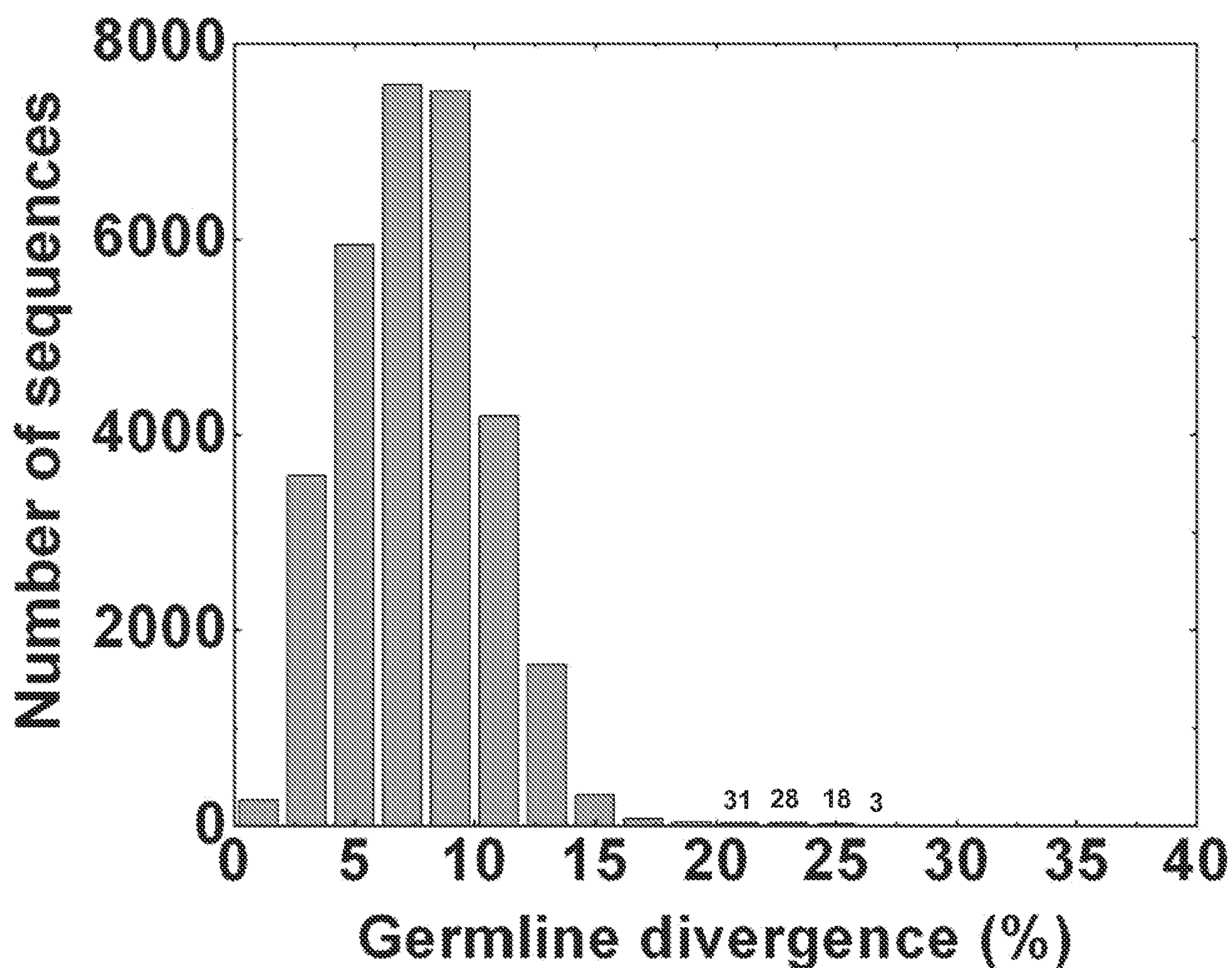
67/95



1. Alignment coverage was also used to characterize the sequences that were assigned to the 4 possible germline families, IGKV3-11*01, IGKV3-20*01, IGKV3-NL1*01, and IGKV3-NL5*01.
2. When aligned to the germline genes, 32878 sequences (or 46.3%) of these 4 families could cover 95% of the germline sequence and thus were considered to contain the "complete" V gene.

Figure A-28

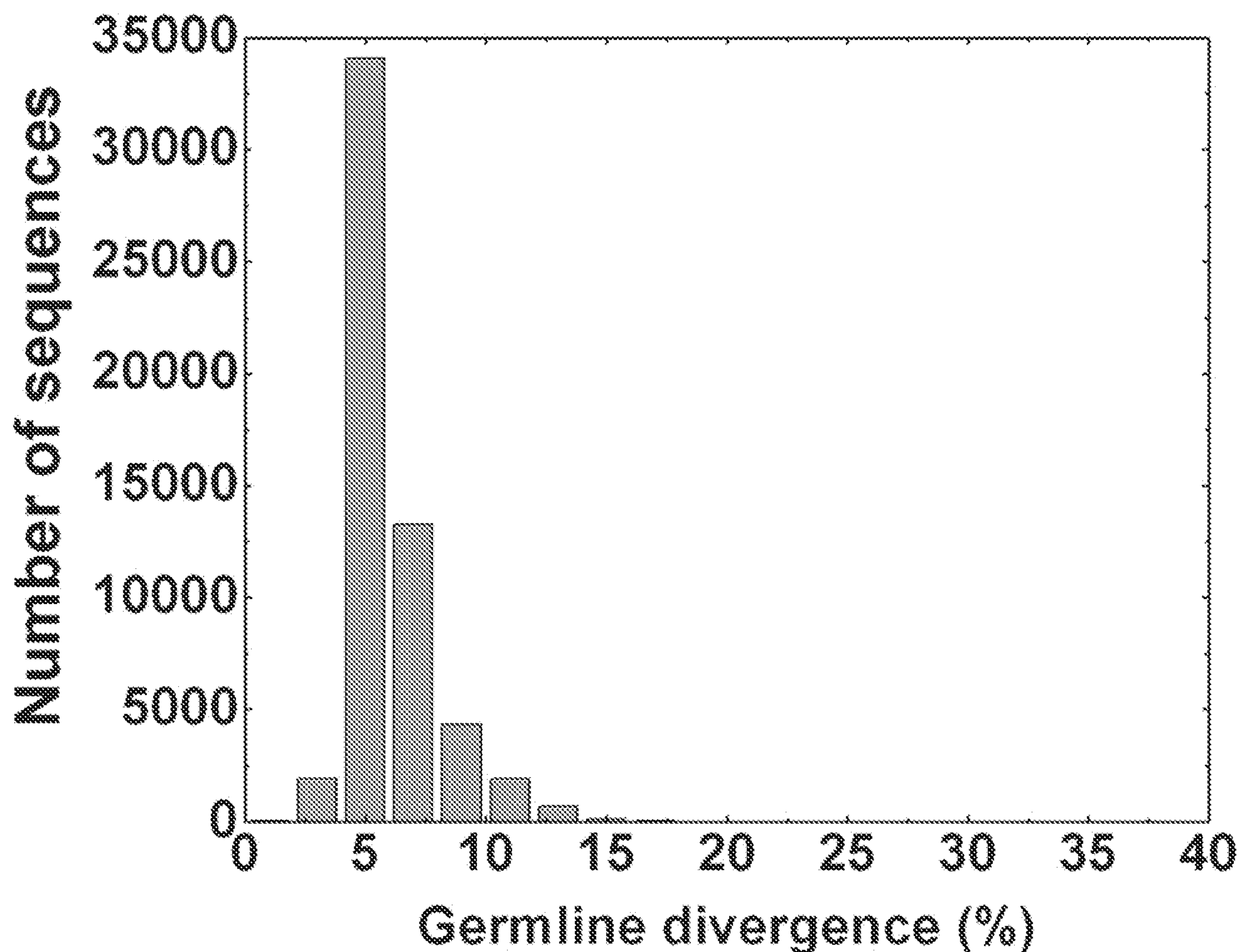
68/95



1. The full-length sequences were extracted from the data set using VRC01 H sequence as a template, resulting in a total of 87658 sequences with 31194 sequences assigned to the 4 possible germline families, IGKV3-11*01, IGKV3-20*01, IGKV3-NL1*01 and IGKV3-NL5*01.
2. The divergence of full-length sequences from these 4 germline families was calculated and plotted as a histogram.
3. A total of 80 sequences were found to have a divergence of 20% or higher, with 31 in the 20-22% bin, 28 in the 22-24% bin, 18 in the 24-26% bin, and 3 in the 26-28% bin, as labeled on the histogram.

Figure A-29

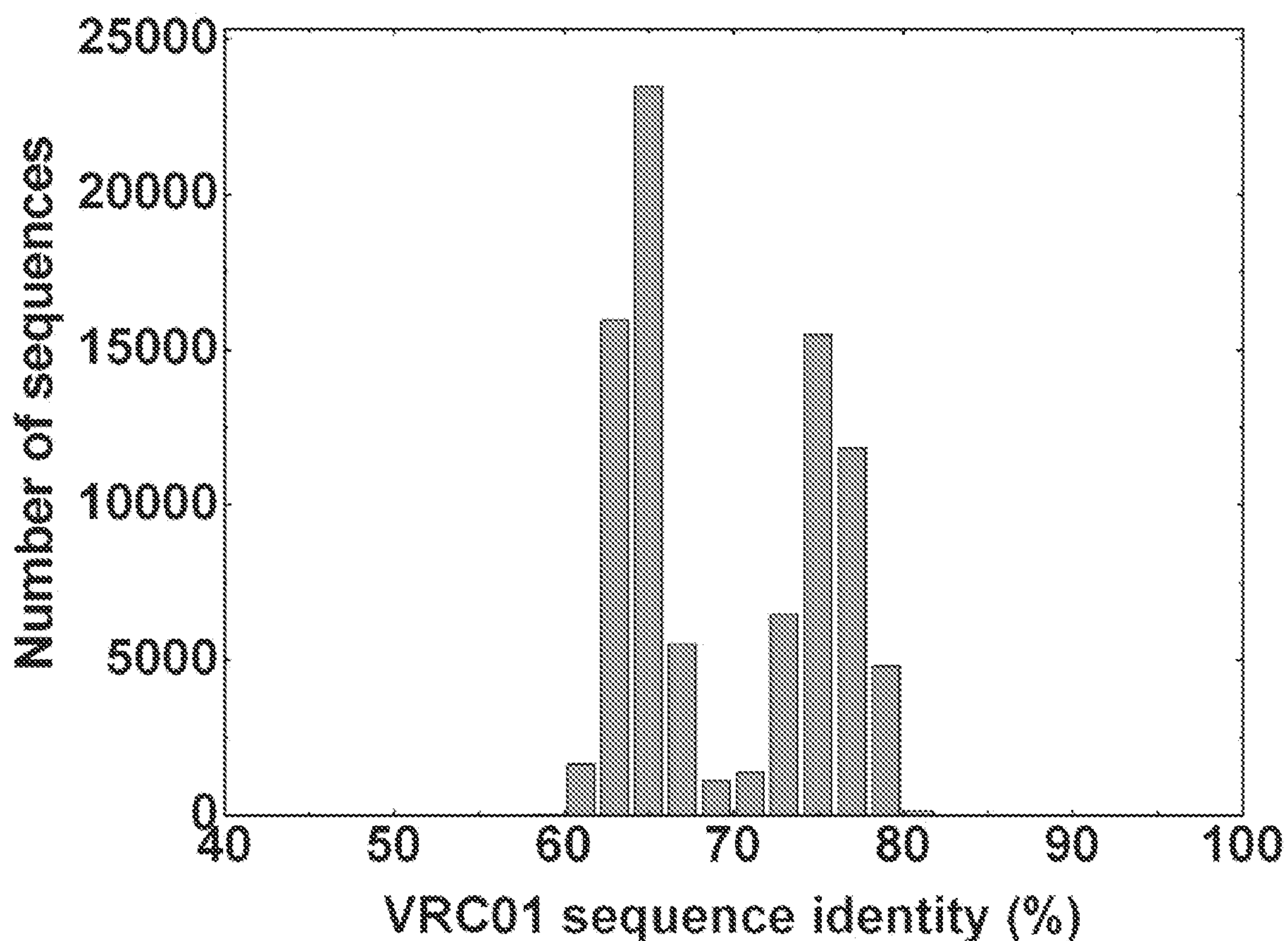
69/95



1. The divergence of full-length sequences that were assigned to other germline families was calculated and plotted as a histogram.
2. A total of 12 sequences were found to have a divergence of 20% or higher, with 7 in the 20-22% bin, 1 in the 22-24% bin, 2 in the 24-26% bin, and 2 in the 26-28% bin.

Figure A-30

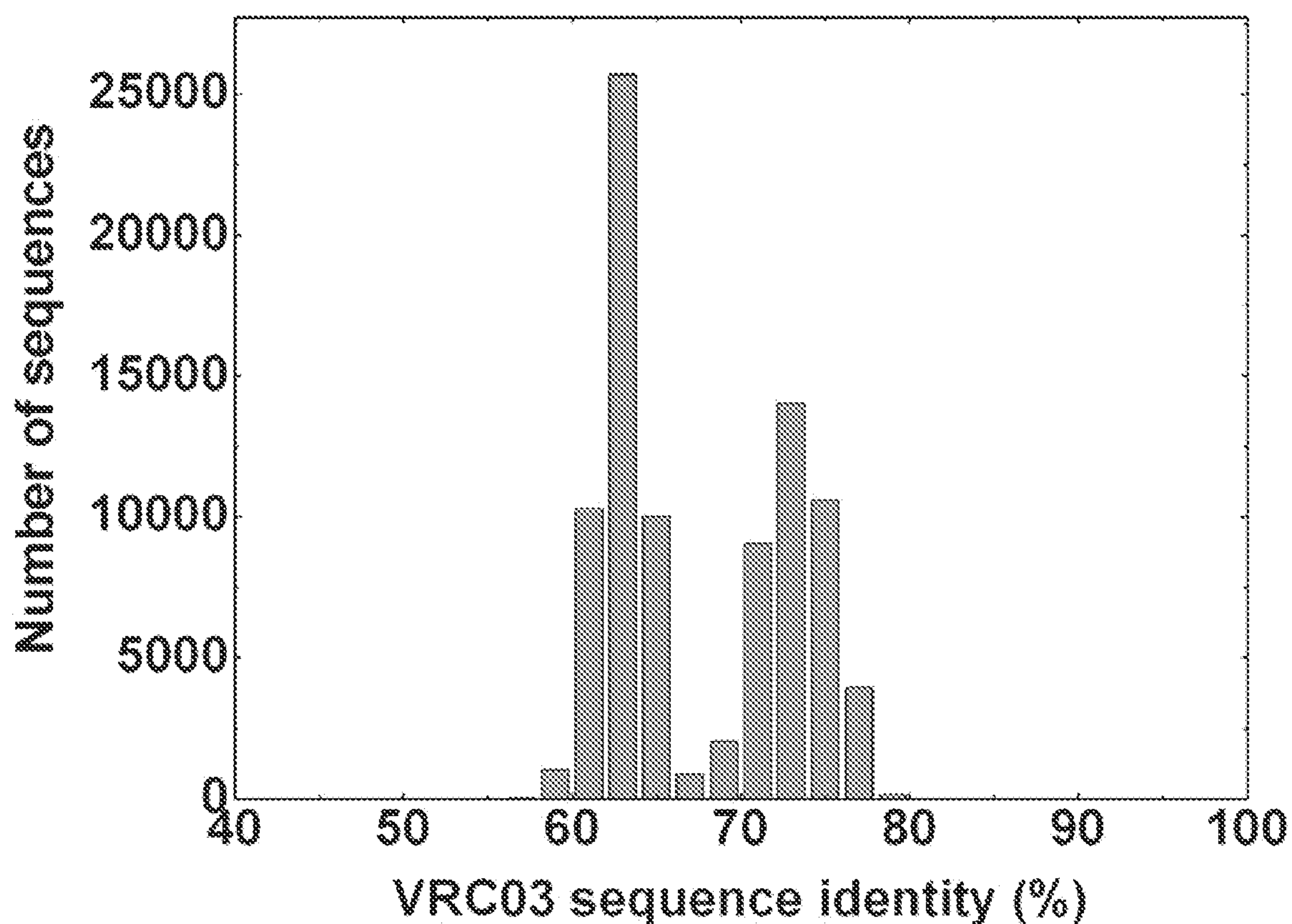
70/95



1. The sequence identity to VRC01 L (at the nucleotide level) was calculated for all full-length sequences and plotted as a histogram.
2. One sequence, #181371, was found to be in the 90-92% bin with a sequence identity of 92.0% to VRC01 light chain.
3. 43 sequences were identified to have the VRC01-like deletion, including #181371.

Figure A-31

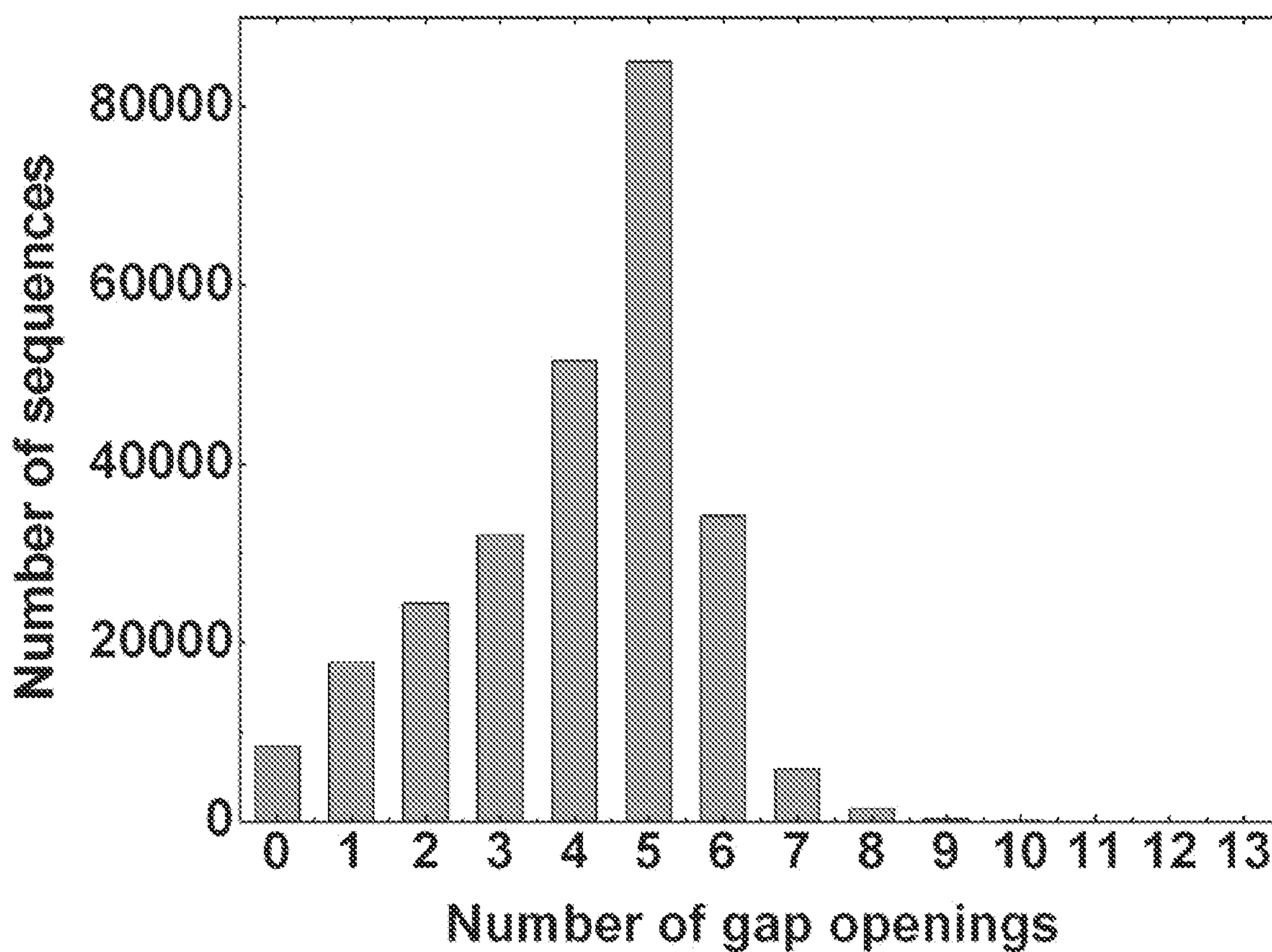
71/95



1. The sequence identity to VRC03 L (at the nucleotide level) was calculated for all full-length sequences and plotted as a histogram.
2. One sequence, #223454, was found to be in the 90-92% bin with a sequence identity of 90.3% to VRC03 light chain.
3. 55 sequences were identified to have the VRC03-like deletion

Figure A-32

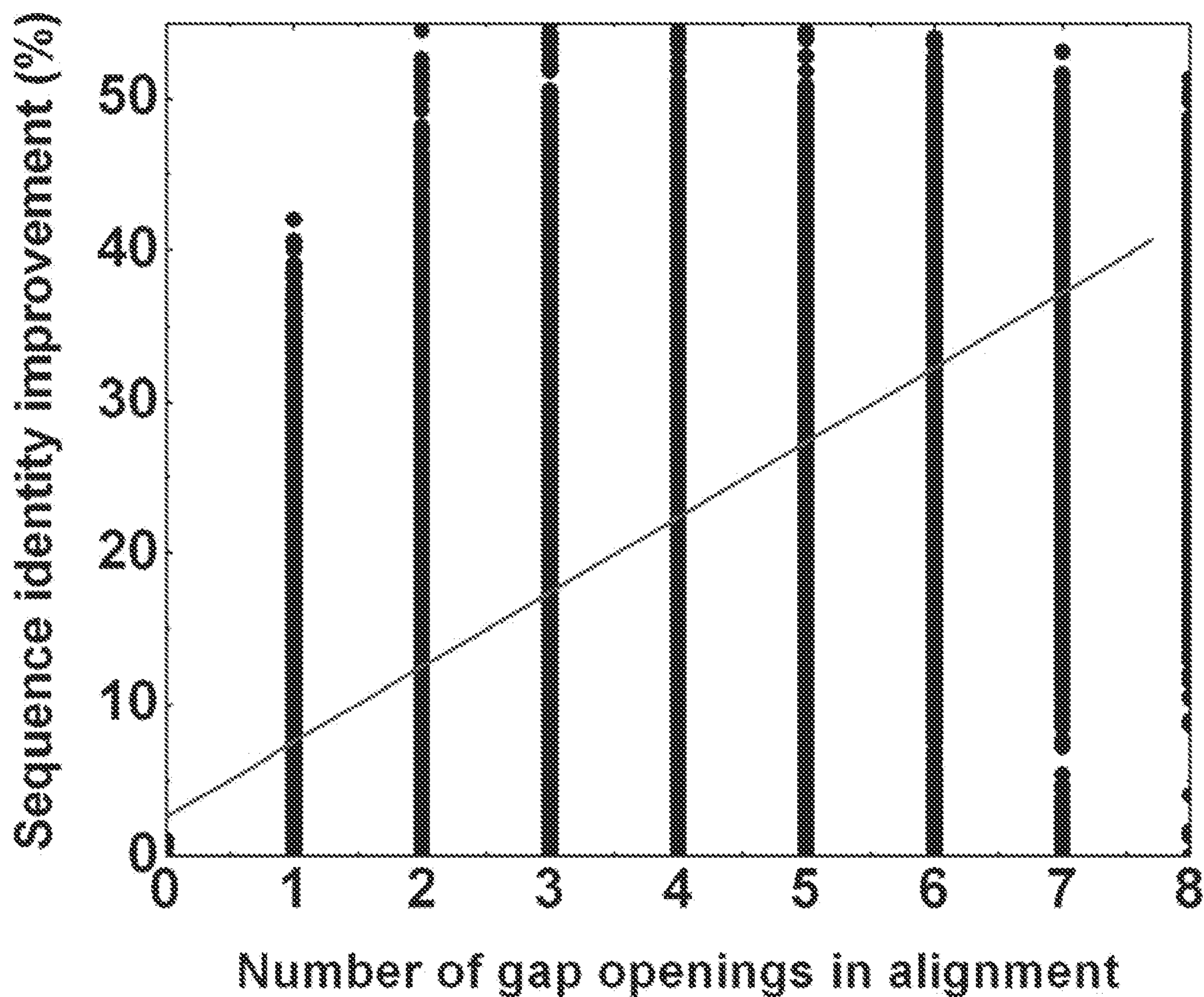
72/95



1. After aligned to the respective germlines, the number of gap openings in the variable region was calculated and plotted as a histogram for the whole data set.
2. 96.8% of the sequences were found to have at least one gap opening in the variable region alignment, which might be caused by 454 sequencing error or naturally occurring insertion/deletion.

Figure A-33

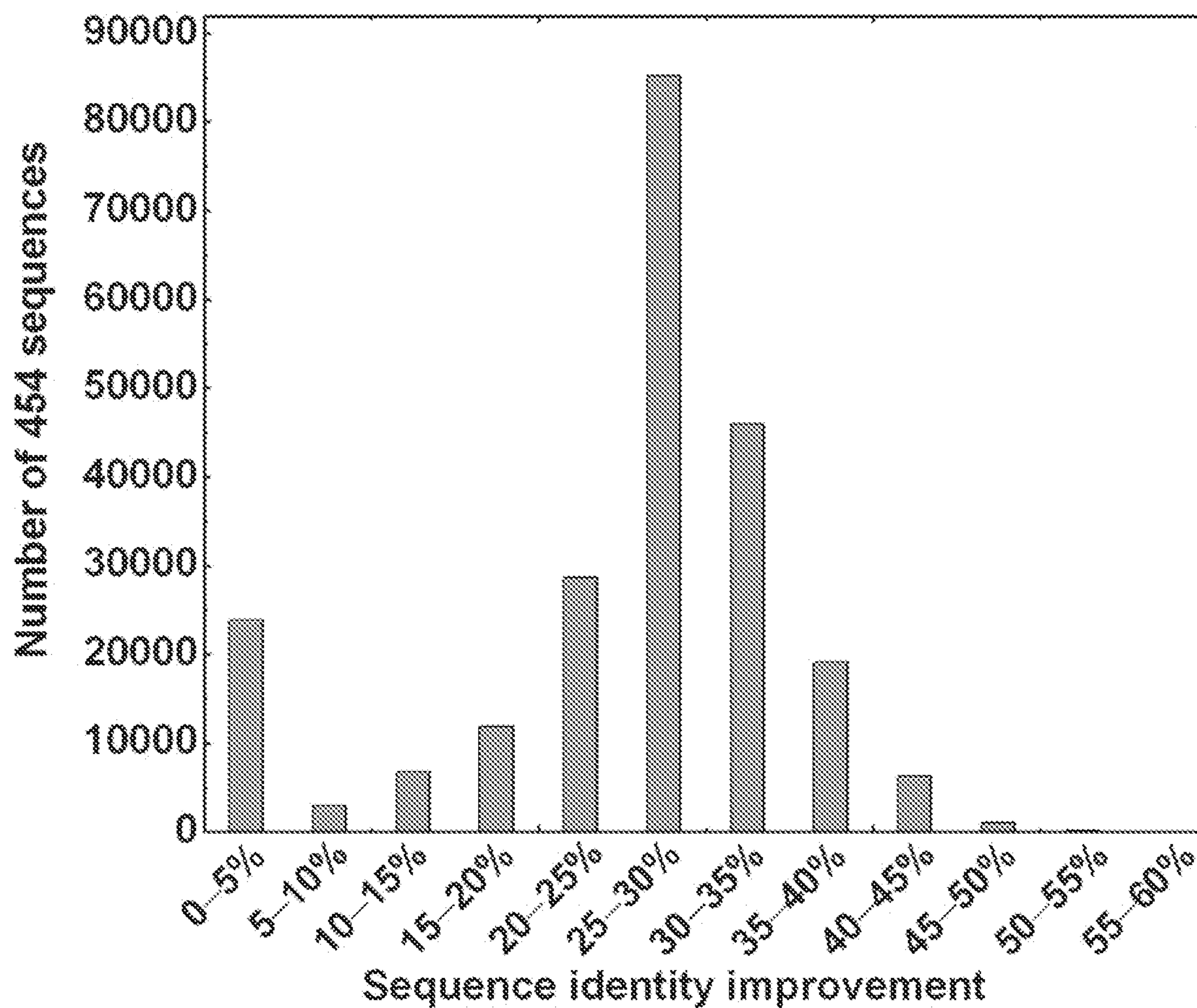
73/95



1. The sequencing errors in the variable region were corrected based on the respective germline and the improvement of sequence identity to the germline sequence was plotted as a function of number gap openings in the variable region.
2. The linear regression R value is 0.662 and the P-value is lower than 0.0001, suggesting that the correlation is significant.

Figure A-34

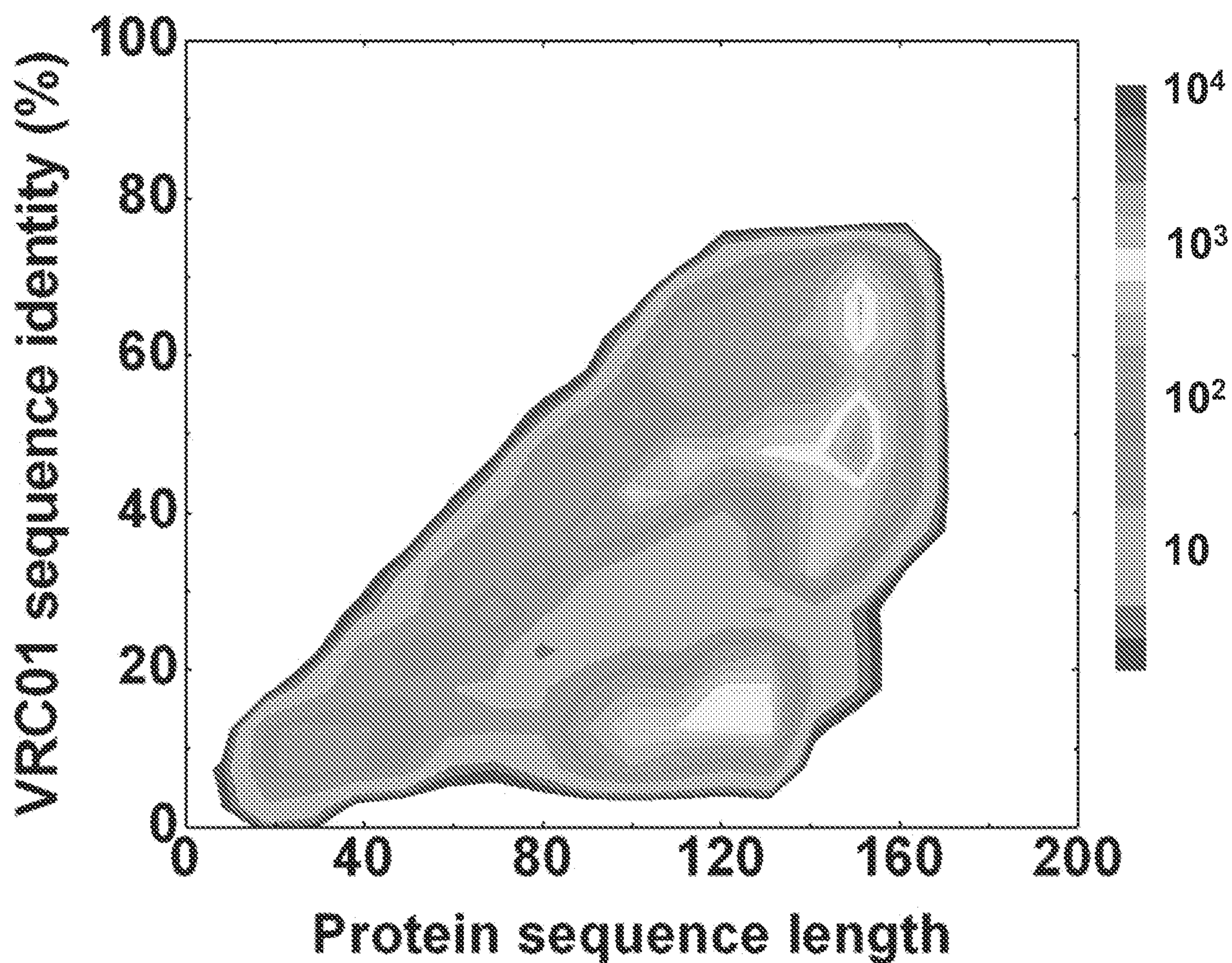
74/95



1. The improvement of sequence identity to the respective germline was calculated for the whole data set and plotted as a histogram.
2. The average improvement is 25.4%.

Figure A-35

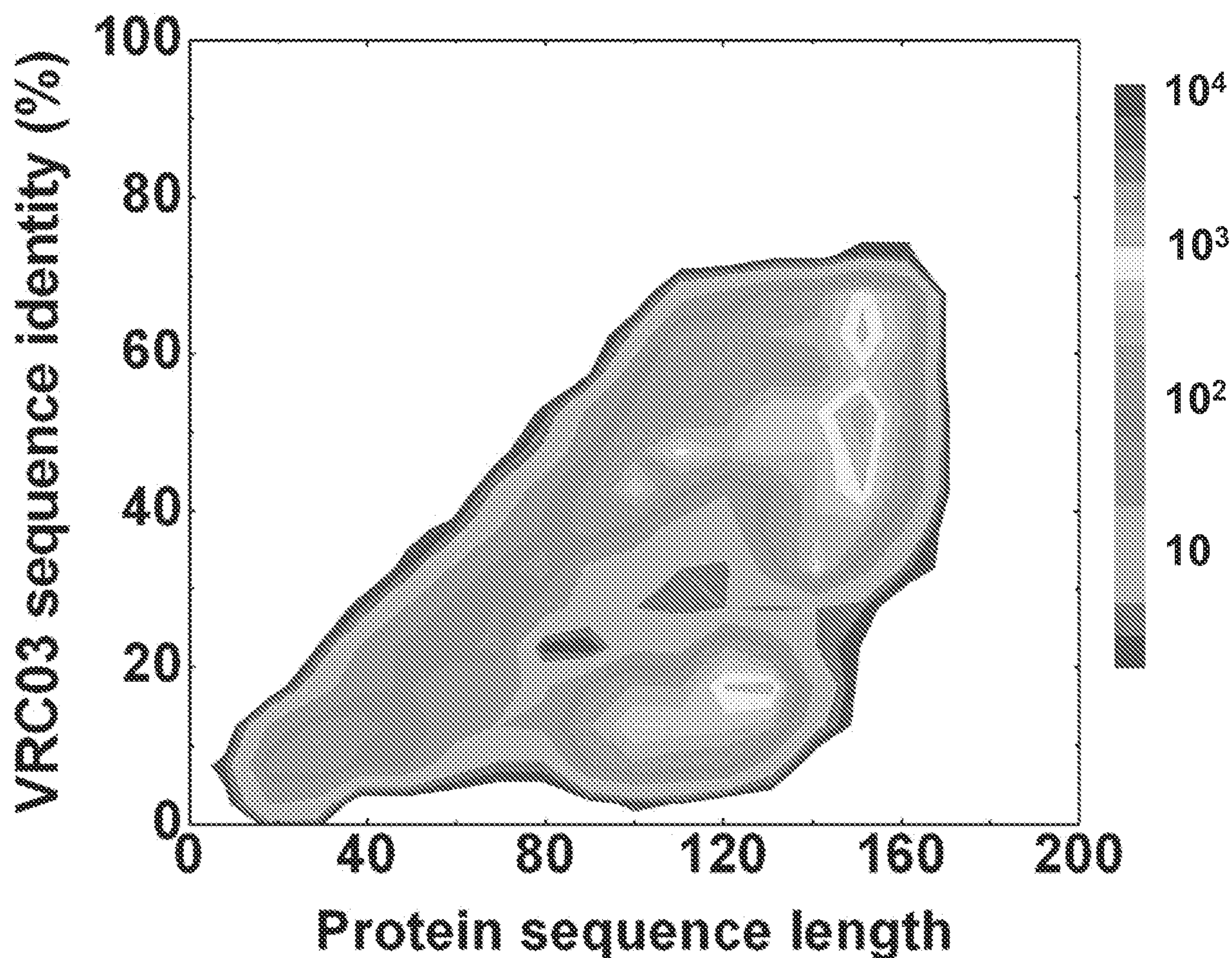
75/95



1. The protein sequences translated from corrected nucleotide sequences were plotted as a function of sequence length and sequence identity to VRC01 L.
2. No VRC01-like sequences were identified from this analysis except #181371.

Figure A-36

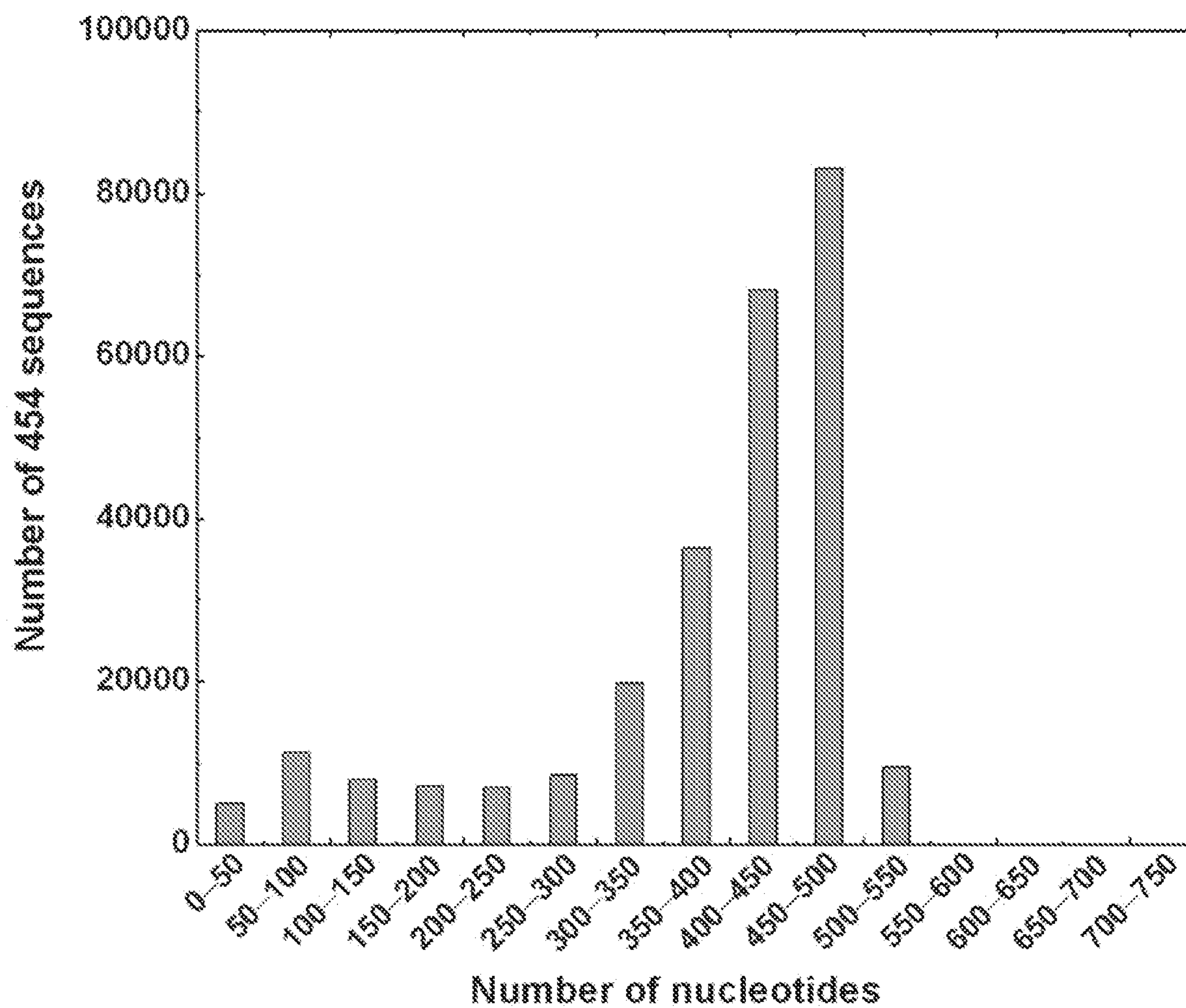
76/95



1. The protein sequences translated from corrected nucleotide sequences were plotted as a function of sequence length and sequence identity to VRC03 L.
2. No VRC03-like sequences were identified from this analysis except #223454.

Figure A-37

77/95



1. There are 263764 reads in the data set.
2. 197503 (or 74.9%) reads are longer than 350 nucleotides.
3. The average read length is 383.1.

Figure A-38

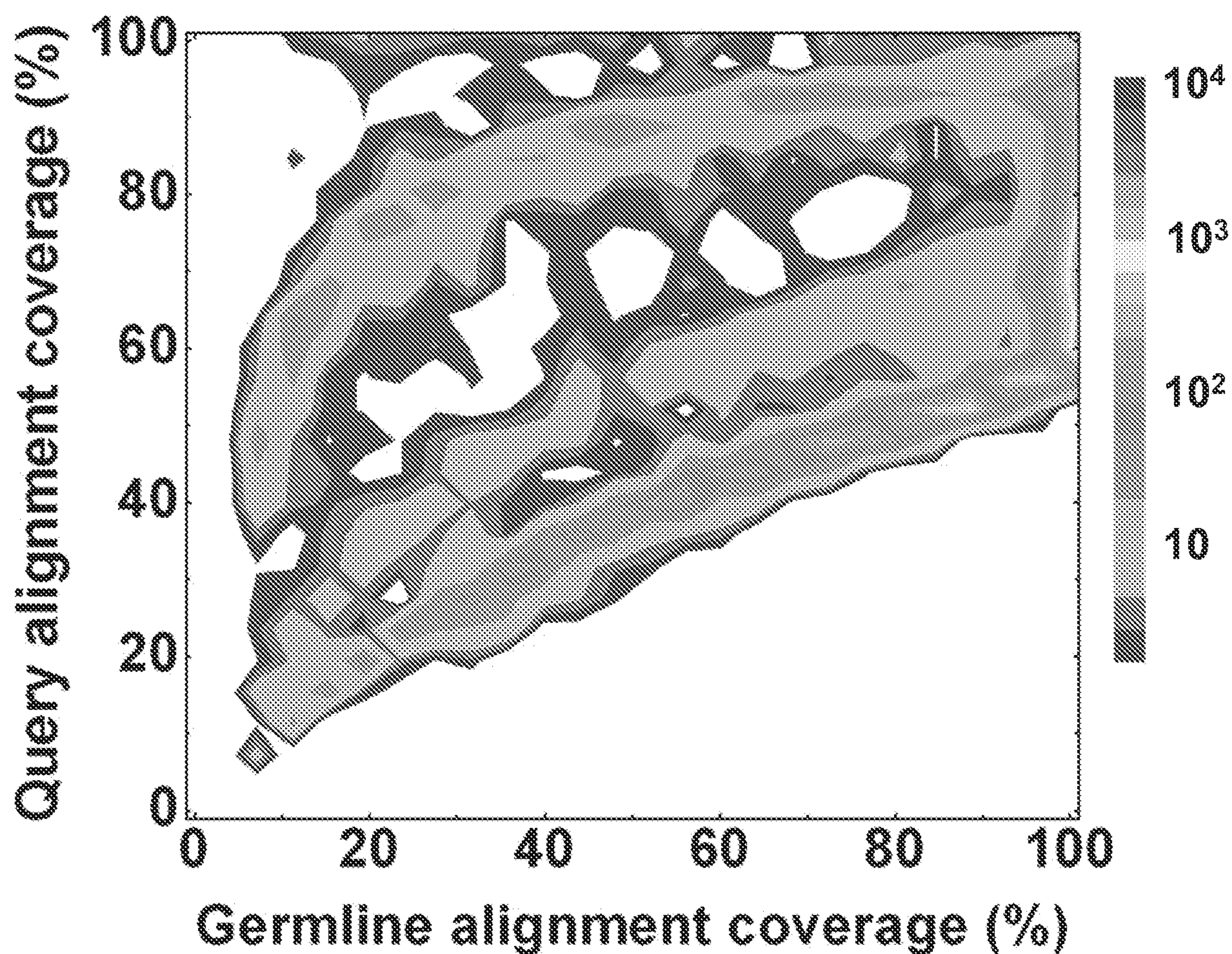
Figure A-39

78/95



1. E-value from IGBlast was used to determine whether the assignment is reliable. A cutoff of 1.0E-3 was used in current analysis.
2. 246261 reads remained after removing the sequences with an E-value lower than 1.0E-3
3. 48061 sequences were assigned to IGHV1-2 family with four possible alleles, IGHV1-2*01, IGHV1-2*02, IGHV1-2*03 and IGHV1-2*04.

79/95



1. A metric – alignment coverage – was defined to characterize the effect of sequence length variation on the alignment of a 454 sequence to a germline gene.

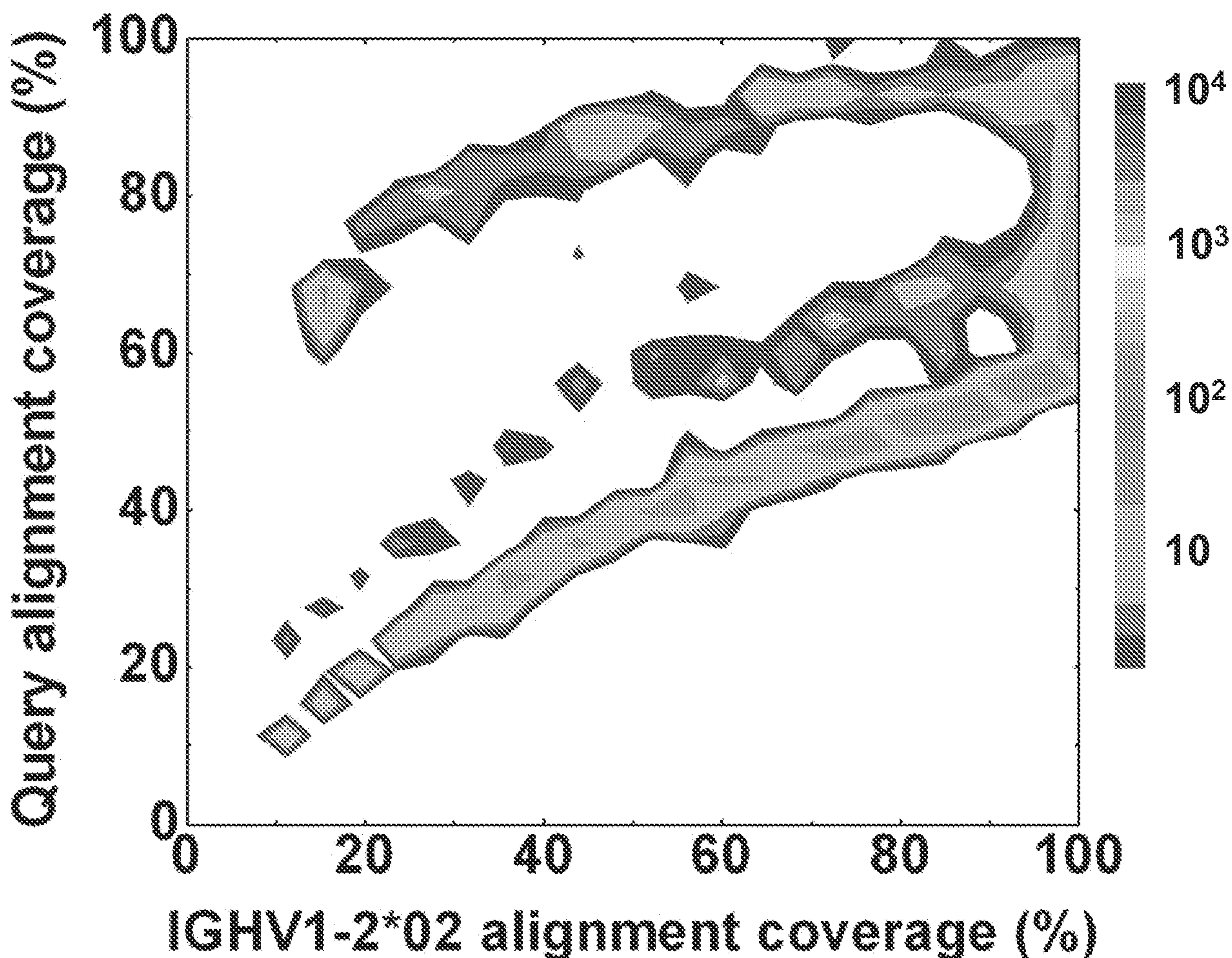
$$Coverage_{Germline} = \frac{Length(aligned\ region)}{Length(Germline\ gene)}$$

$$Coverage_{Query\ sequence\ from\ 454} = \frac{Length(aligned\ region)}{Length(Query\ sequence)}$$

2. When aligned to their respective germline genes, 131457 sequences (or 53.4%) could cover 95% of the germline sequence and thus were considered to contain the “complete” variable (V) gene.

Figure A-40

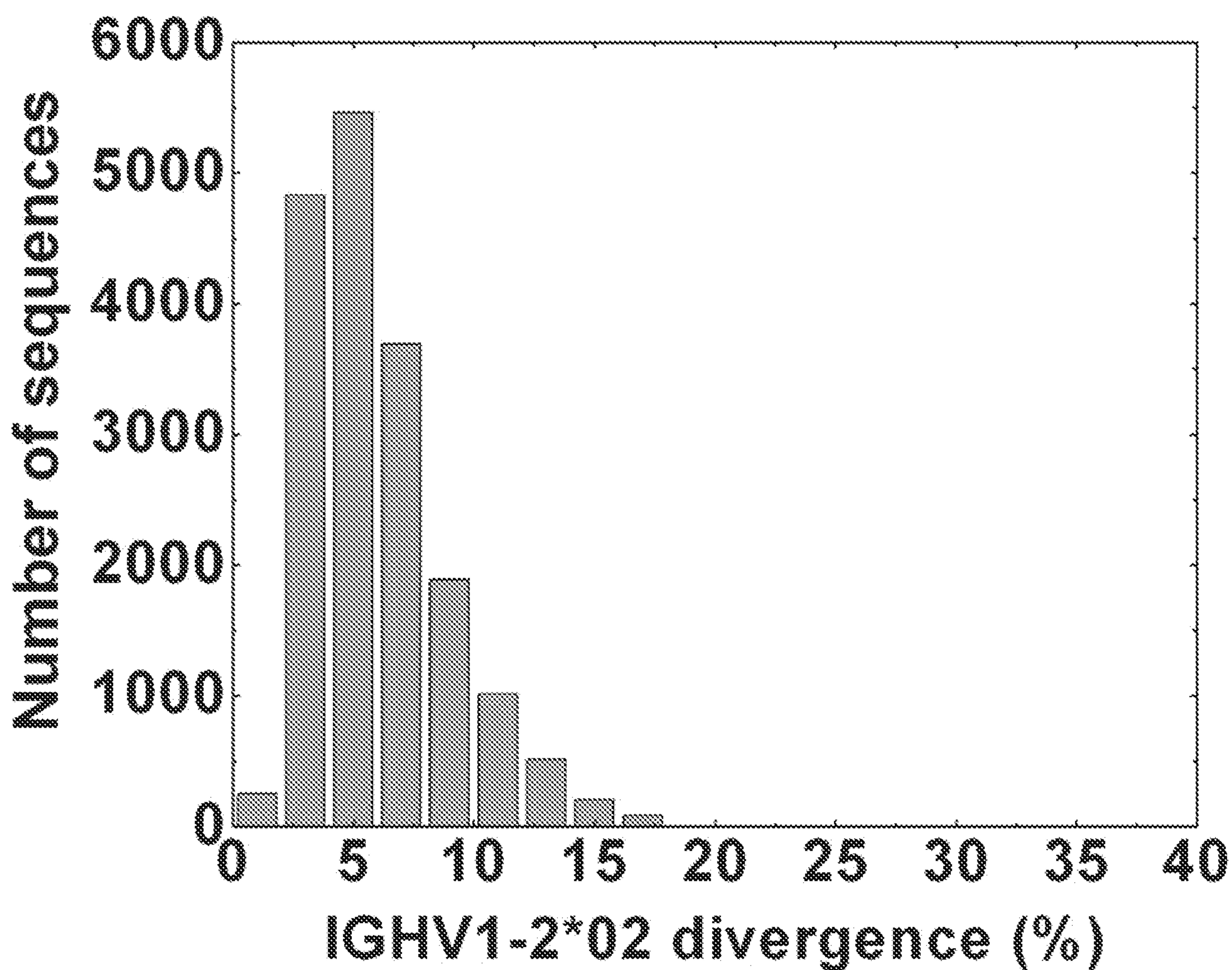
80/95



1. Alignment coverage was also used to characterize the sequences that were assigned to IGHV1-2*02 family. Note that alleles IGVH1-2*01, IGHV1-2*03 and IGHV1-2*04 were also considered in the calculation due to their high similarities to IGHV1-2*02.
2. When aligned to the germline genes, 27101 sequences (or 56.4%) of IGHV1-2*02 family could cover 95% of the germline sequence and thus were considered to contain the "complete" IGHV1-2*02 gene.

Figure A-41

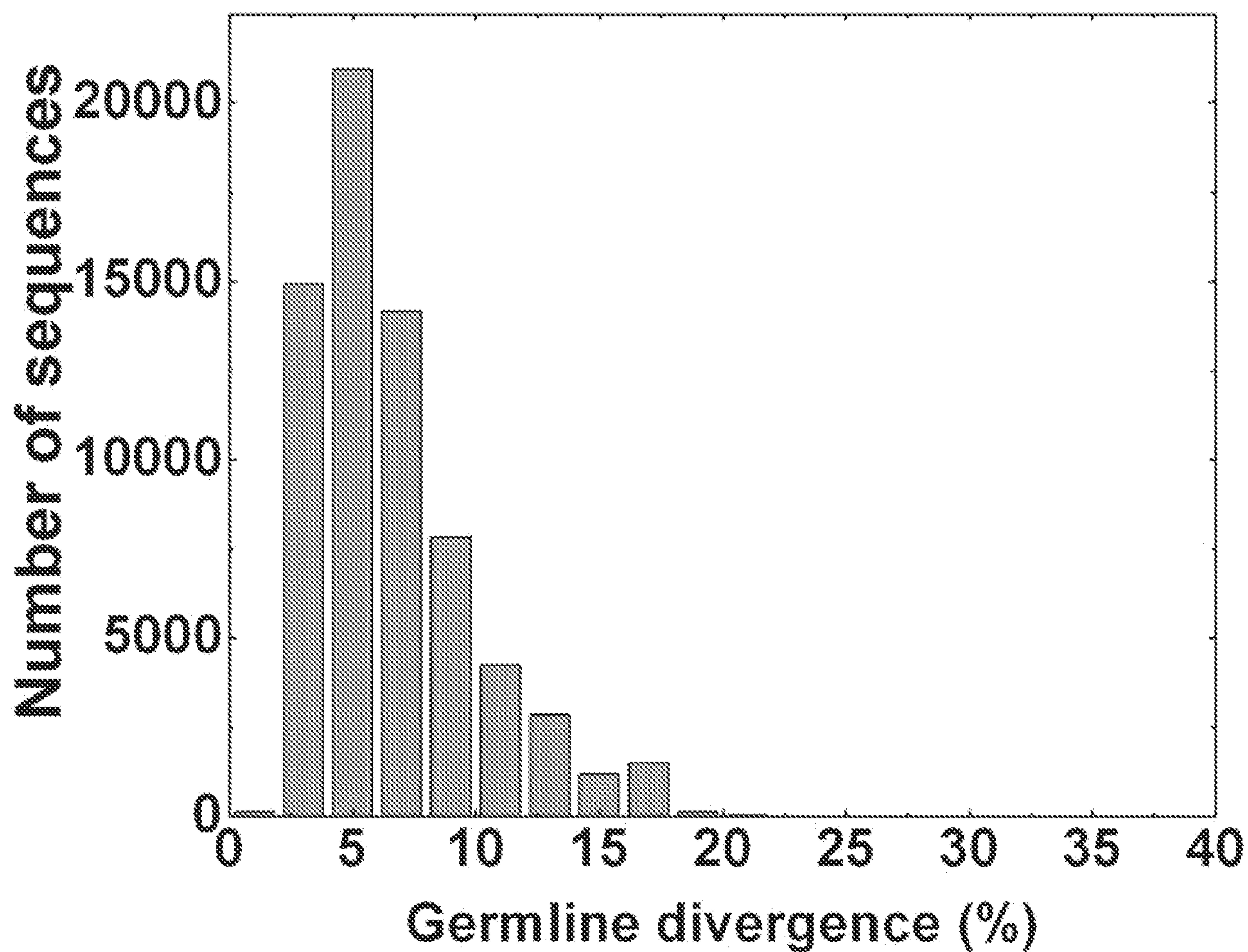
81/95



1. The full-length sequences were extracted from the data set using VRC01 H sequence as a template, resulting in a total of 85851 sequences with 17945 sequences assigned to IGHV1-2*02 family (IGHV1-2*01, IGHV1-2*03 and IGHV1-2*04 alleles included).
2. The divergence of full-length IGHV1-2*02 sequences was calculated and plotted as a histogram.
3. No sequences were found to be more divergent than 24%

Figure A-42

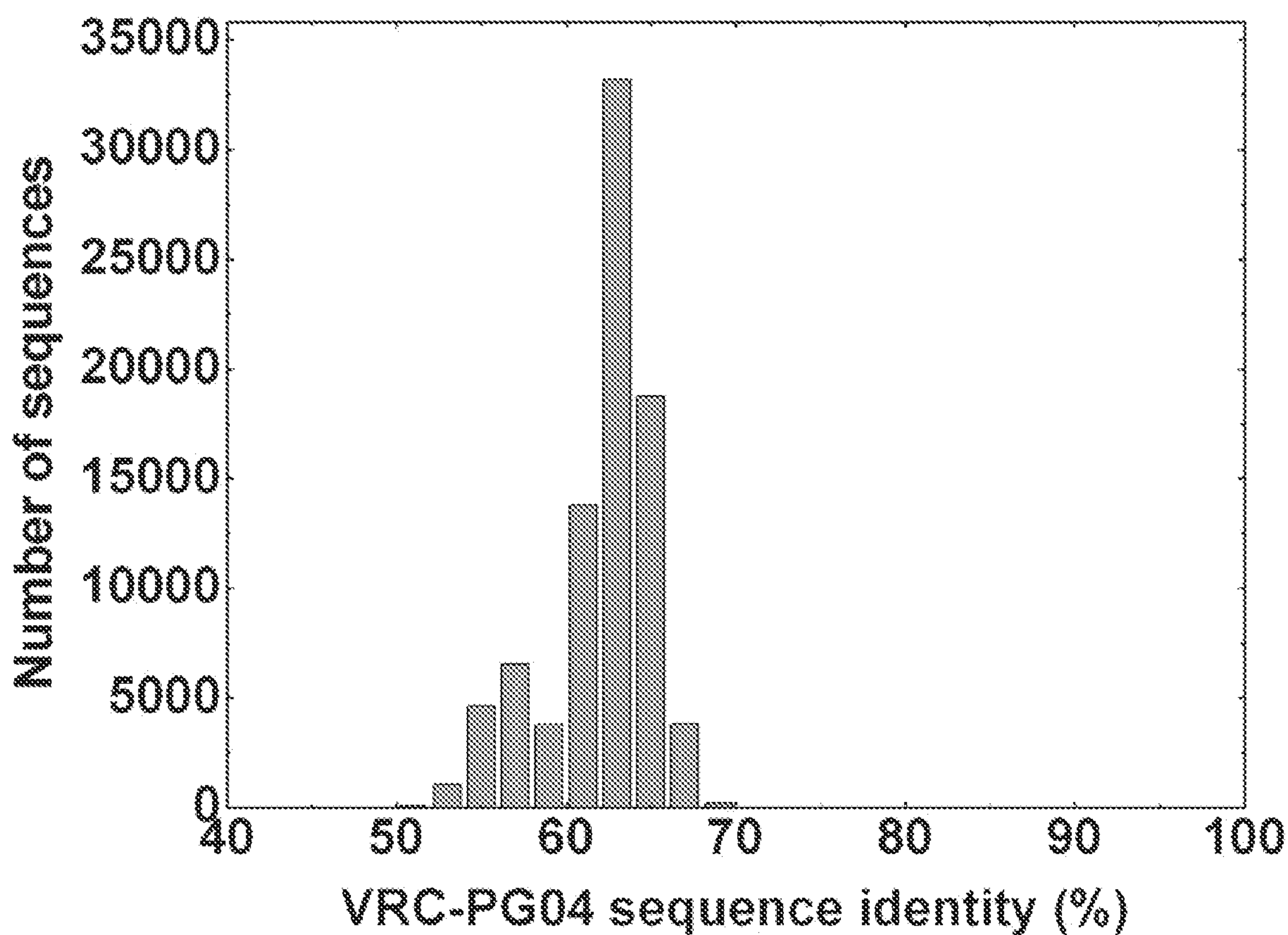
82/95



1. The divergence of full-length sequences that were assigned to non-IGHV1-2*02 germlines was calculated and plotted as a histogram.
2. Only three sequences were found to be more divergent than 24%.

Figure A-43

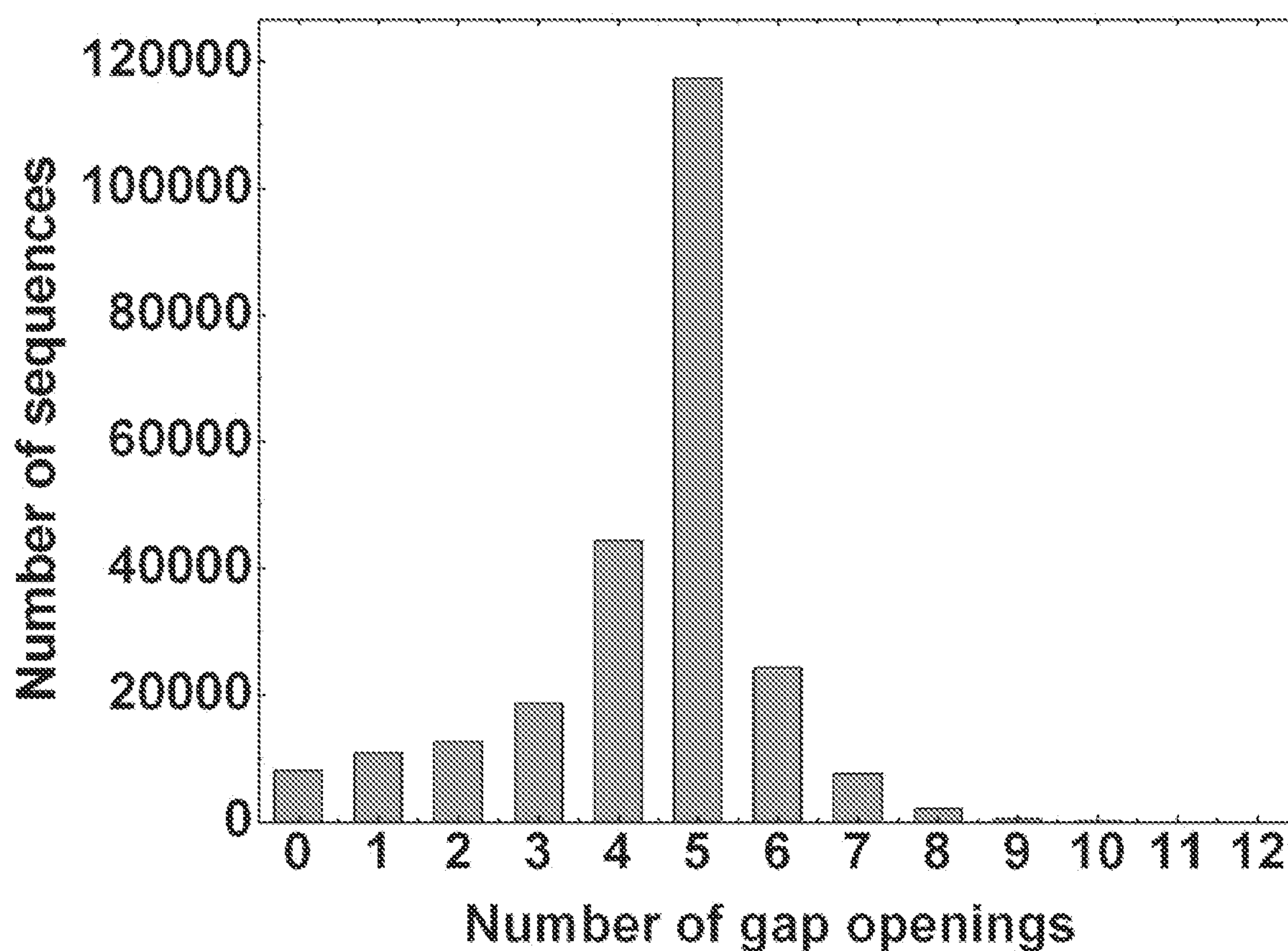
83/95



1. The sequence identity to VRC-PG04 H (at the nucleotide level) was calculated for all full-length sequences and plotted as a histogram.
2. No sequences in the data set were found to be over 72% identical to the VRC-PG04 H sequence.

Figure A-44

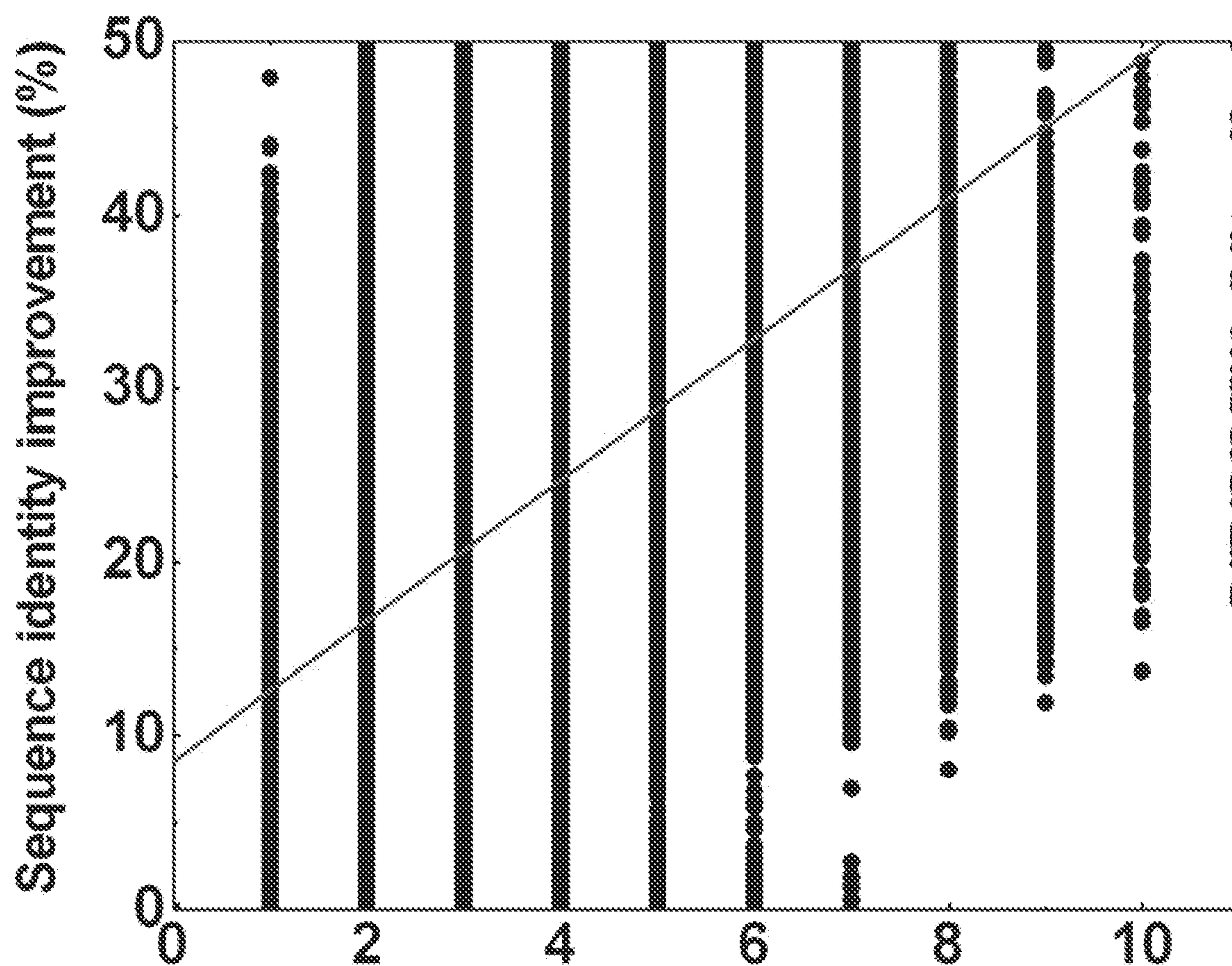
84/95



1. After aligned to the respective germlines, the number of gap openings in the variable region was calculated and plotted as a histogram for the whole data set.
2. 96.7% of the sequences were found to have at least one gap opening in the variable region alignment, which might be caused by 454 sequencing error or naturally occurring insertion/deletion.

Figure A-45

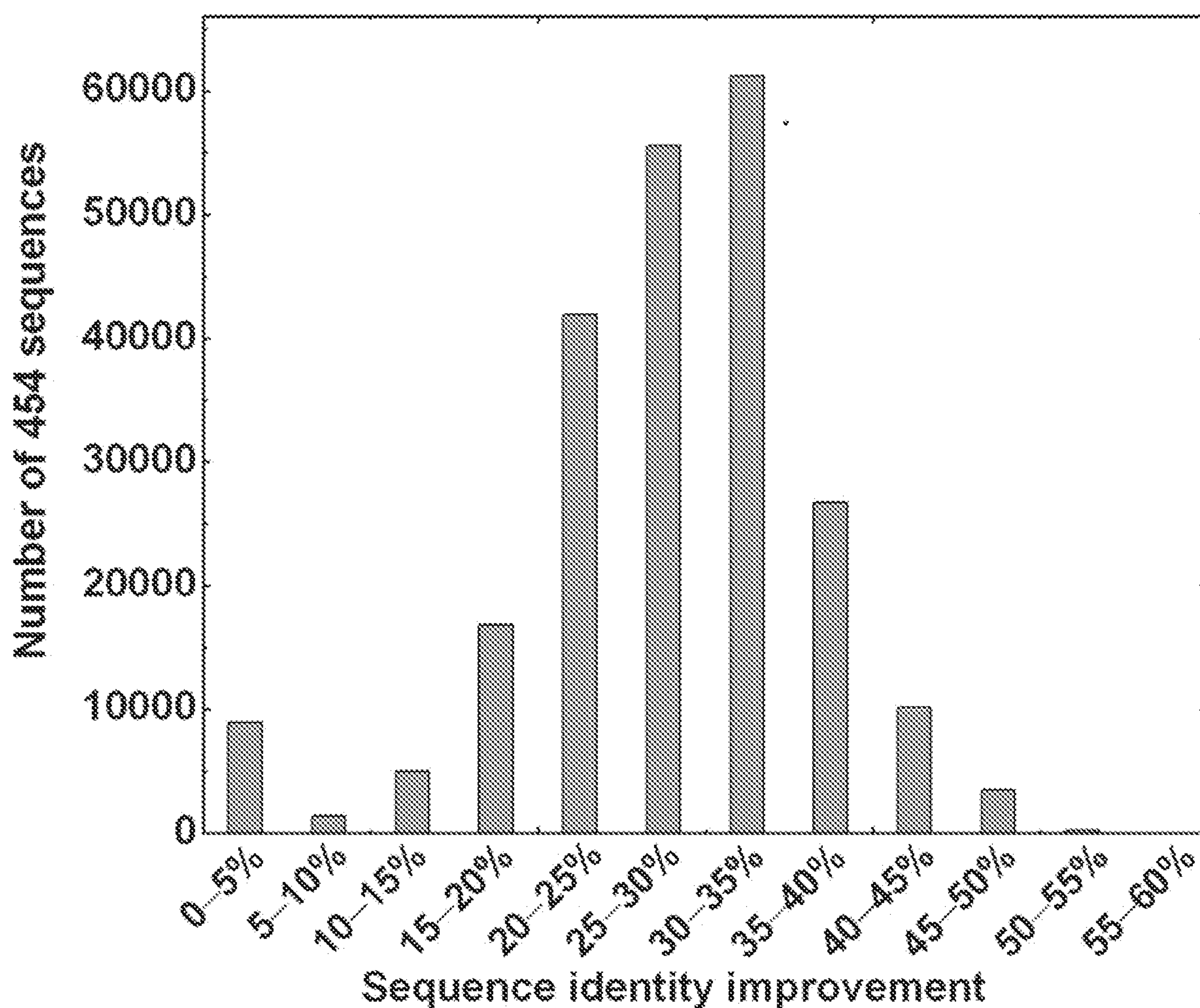
85/95



1. The sequencing errors in the variable region were corrected based on the respective germline and the improvement of sequence identity to the germline sequence was plotted as a function of number gap openings in the variable region.
2. The linear regression R value is 0.583 and the P-value is lower than 0.0001, suggesting that the correlation is significant.

Figure A-46

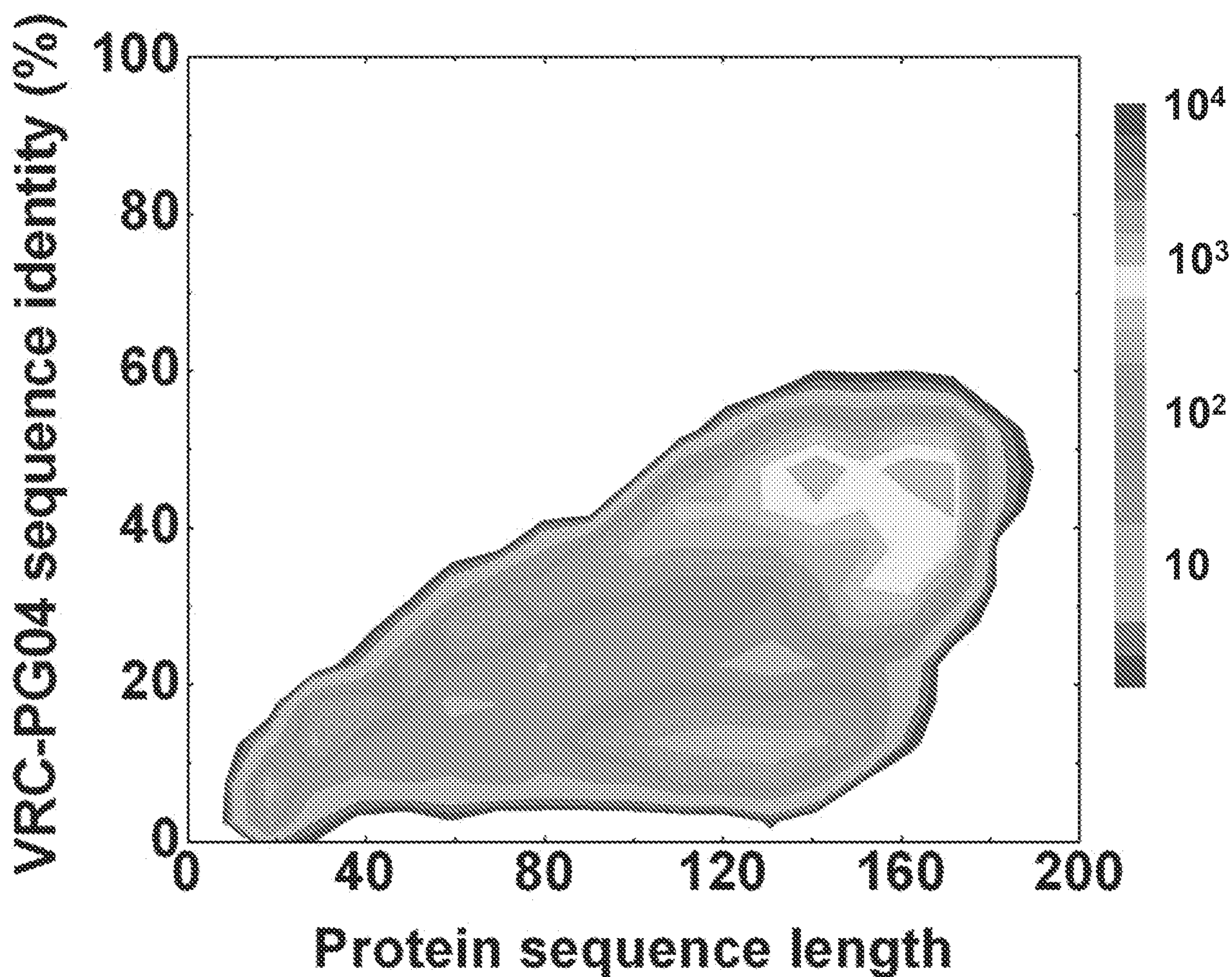
86/95



1. The improvement of sequence identity to the respective germline was calculated for the whole data set and plotted as a histogram.
2. The average improvement is 27.9%.

Figure A-47

87/95



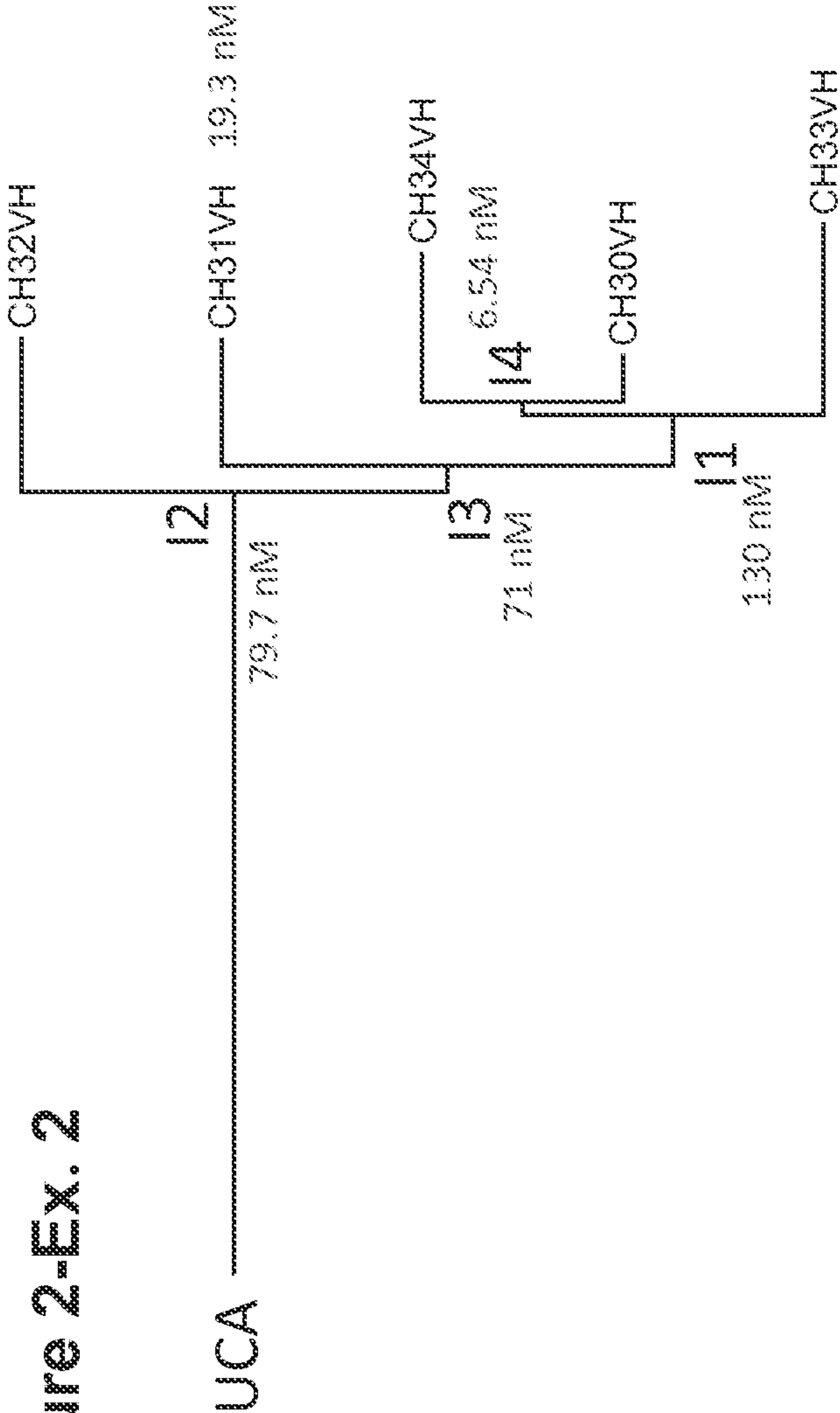
1. The protein sequences translated from corrected nucleotide sequences were plotted as a function of sequence length and sequence identity to VRC-PG04 H.
2. No VRC-PG04-like sequences were identified from this analysis.

Figure A-48

Figure 2-Ex. 2

CH3x SPR Binding Data
(a244 gd Δ11)

Kd



Tree from Kepler, SPR data from Alam

CH3x Light Chain

CDR1

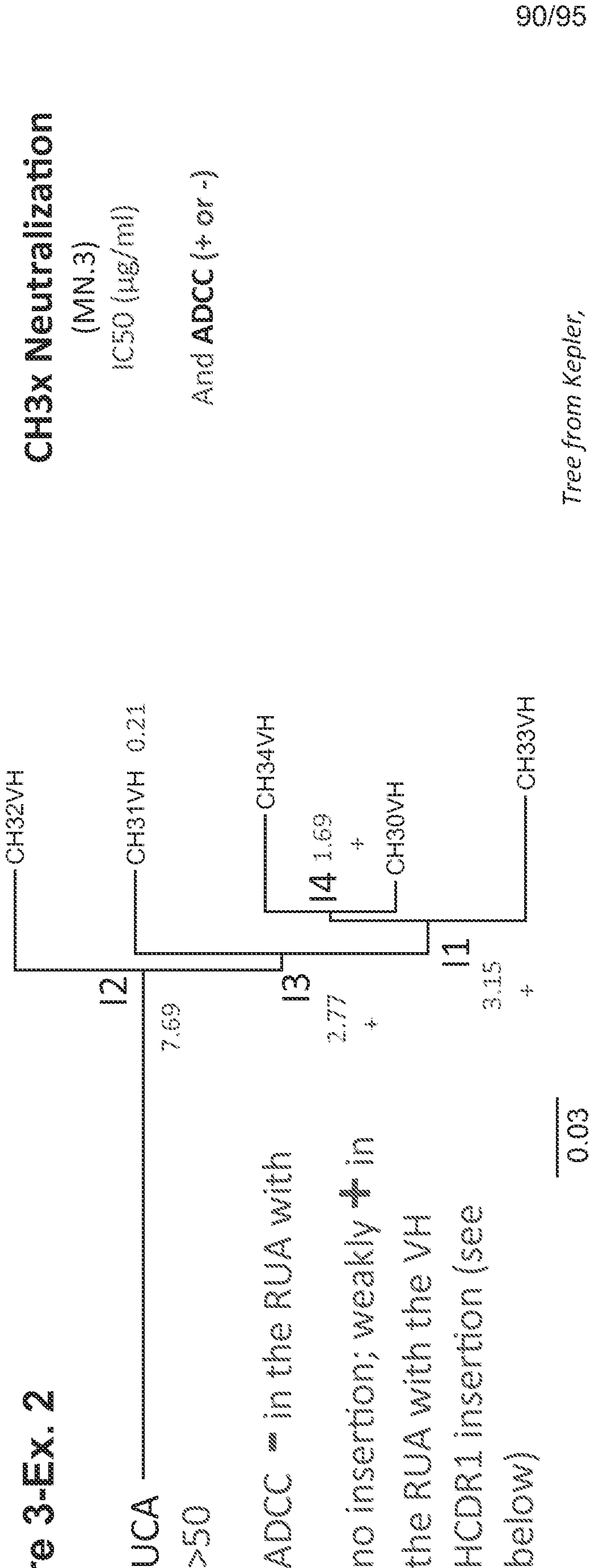
CDR2

I2 DIQMTQSPSSLSASLGDRVTITTCQASRGIGKDLNWYQQKPGKAPKLLVSDASTILEGGVPSRFSGSGFHQ
I3
I1
I4
31 A.....T.....

CDR3

I2 NESLTISSLQAEDVATYFCQQYEYTFGGQGTKVDIK
I3
I1 P.....
I4 P.....
31

Figure 3-Ex. 2



CH3x Heavy Chain		insertion	
		CDR1	CDR3
I2	QVQLVQSGAAVRKPGASVTVSCKFAEDID	DYSPHWVNEAPEHYIHFLRQAPGQQLLEWLAWM	NPTNGAVNYAWQLQGRVTATRDRSMTTAFLEVKSLRSDDTAVYYCARAQKRGSEWYAHWGQGPVVVSS
I3			
I1			
I4			
CH31		Y.....F.....	
		CDR2	CDR3
I2			
I3			
I1			
I4			
CH31		Y.N.....	

Figure 4-Ex. 2

ADCC mediated by CH31-lineage mAb
Target= BaL-infected CEM.NKR_{CCR5}
Effector= purified NK cell V/V phenotype
E:T ratio= 5:1
mAb tested from 40 to 0.0004µg/ml in 10-fold dilution
6 hour incubation using Luciferase read-out
Cut-off= 5% Specific Killing
Data points= avg±SD of 3 experiments in duplicate
All graphs are on the same scale

Control mAbs tested in each assay

- Synagis
- A32_AAA
- VRC01
- Cut-off

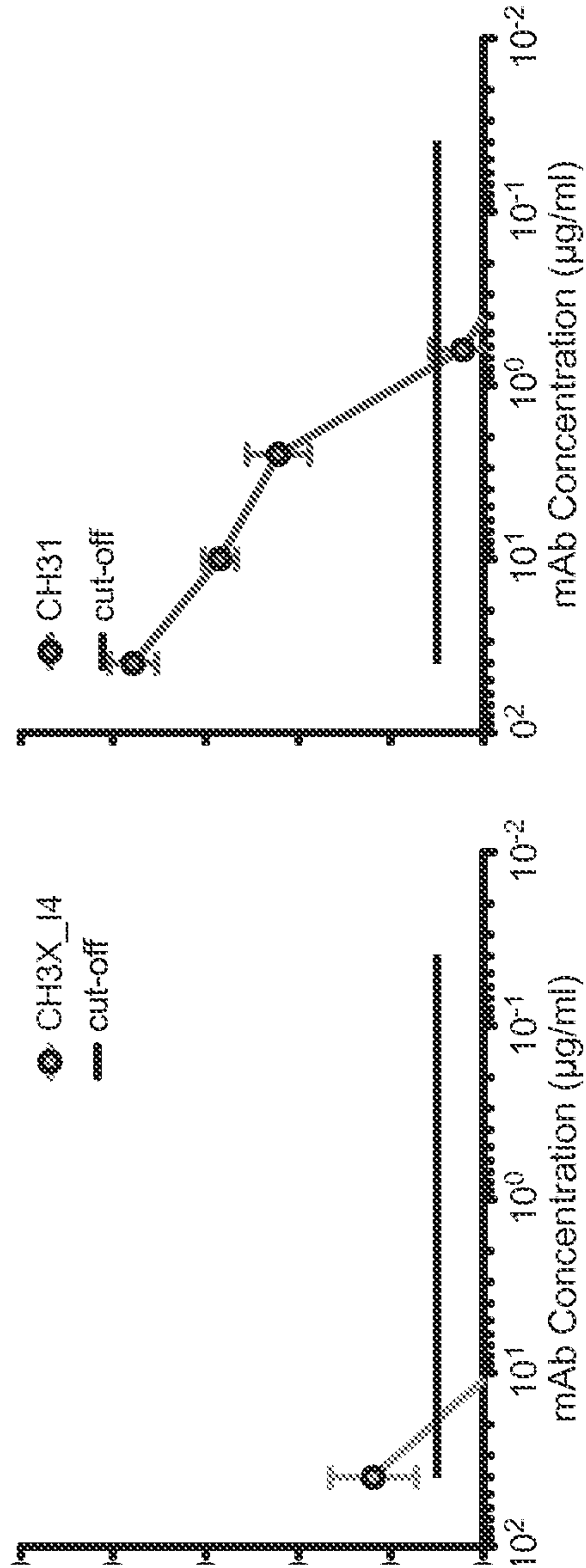
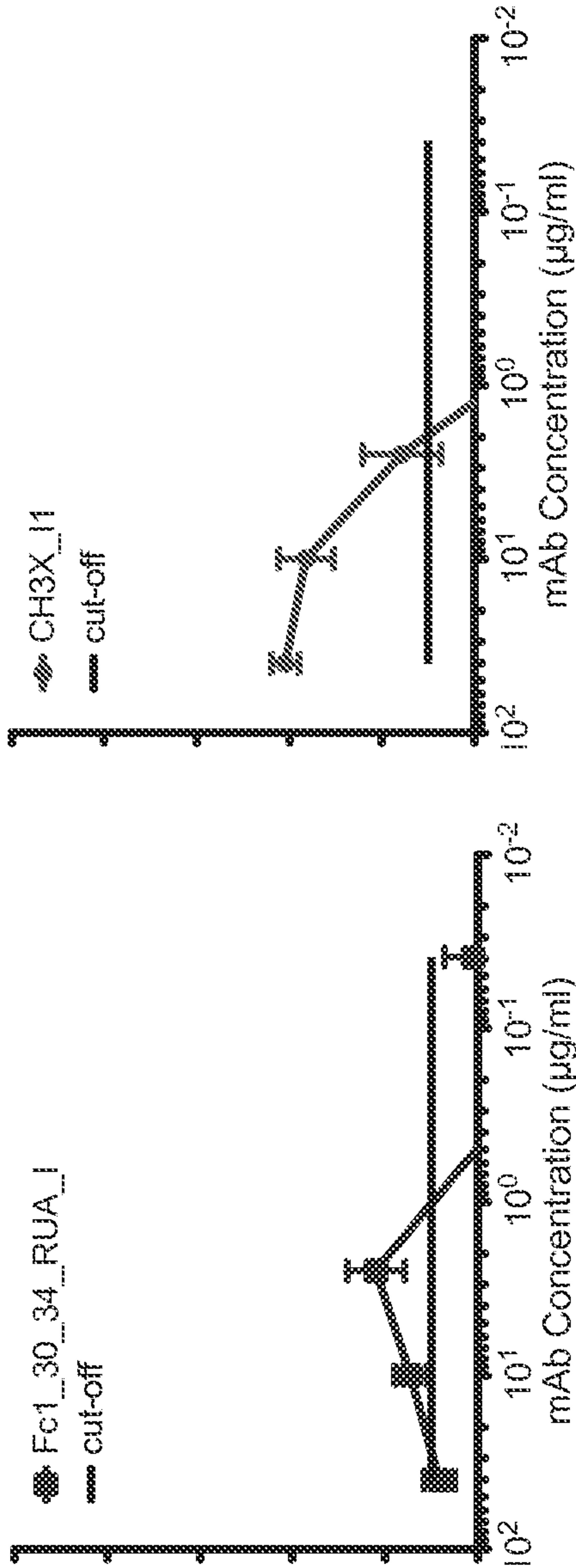
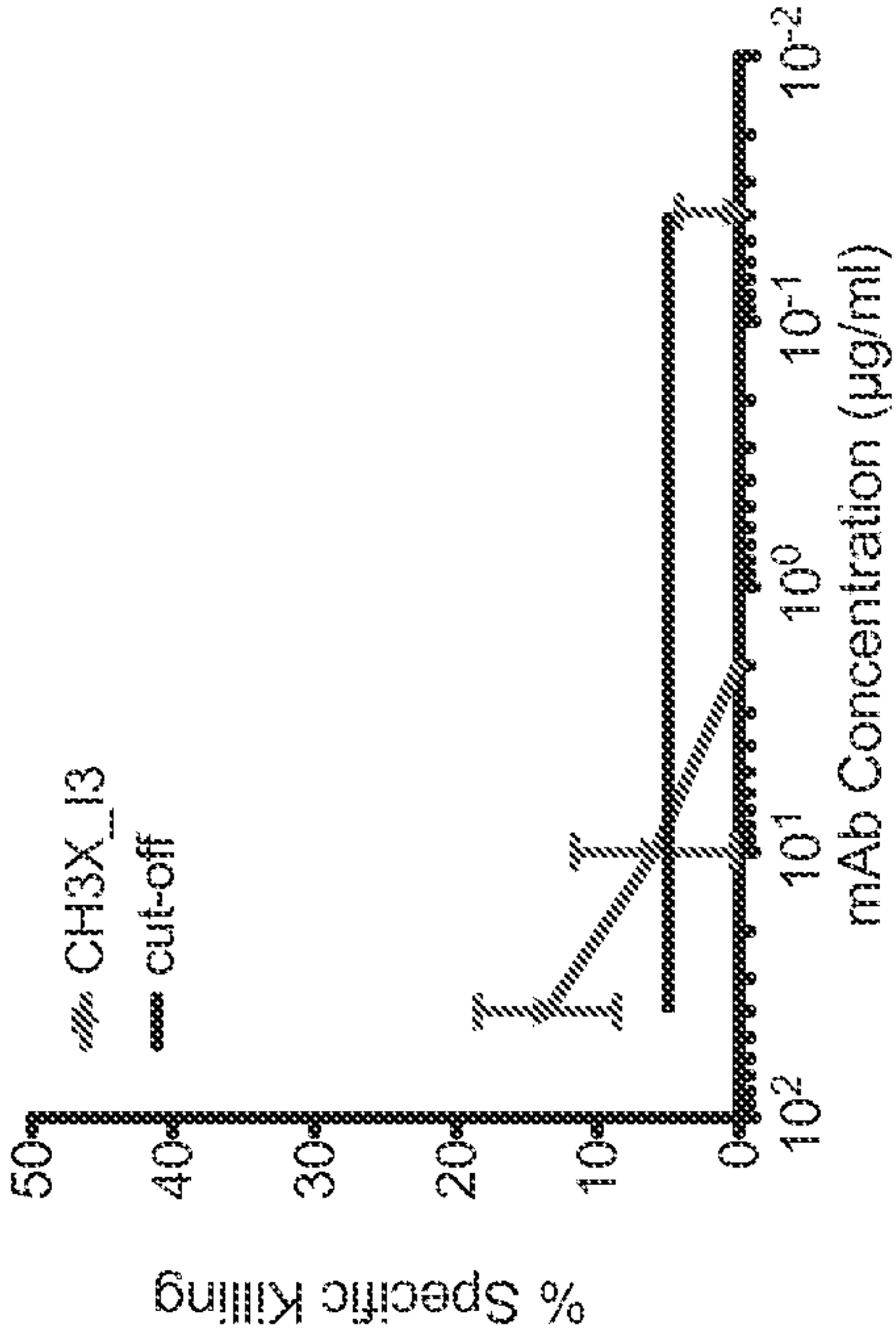
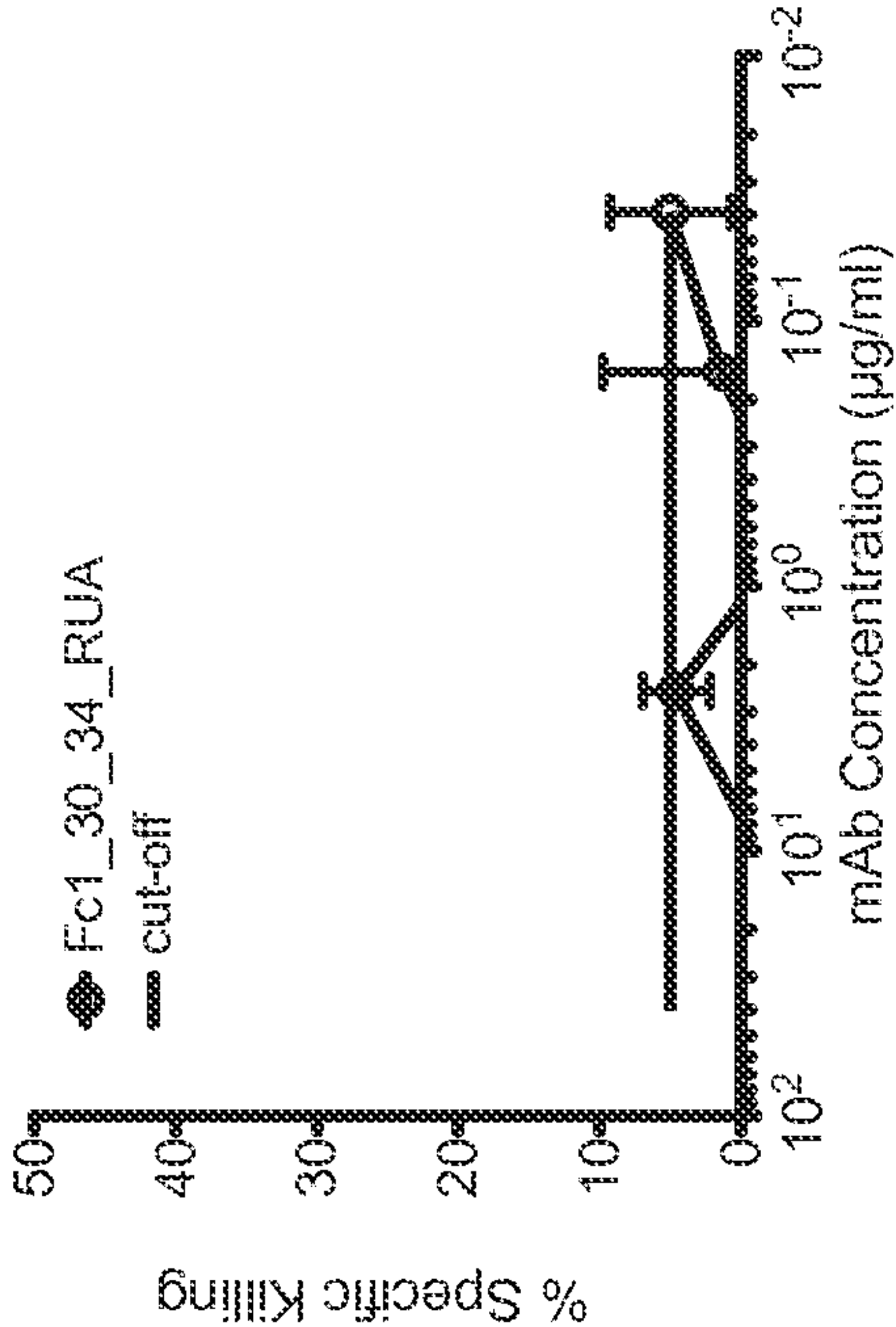
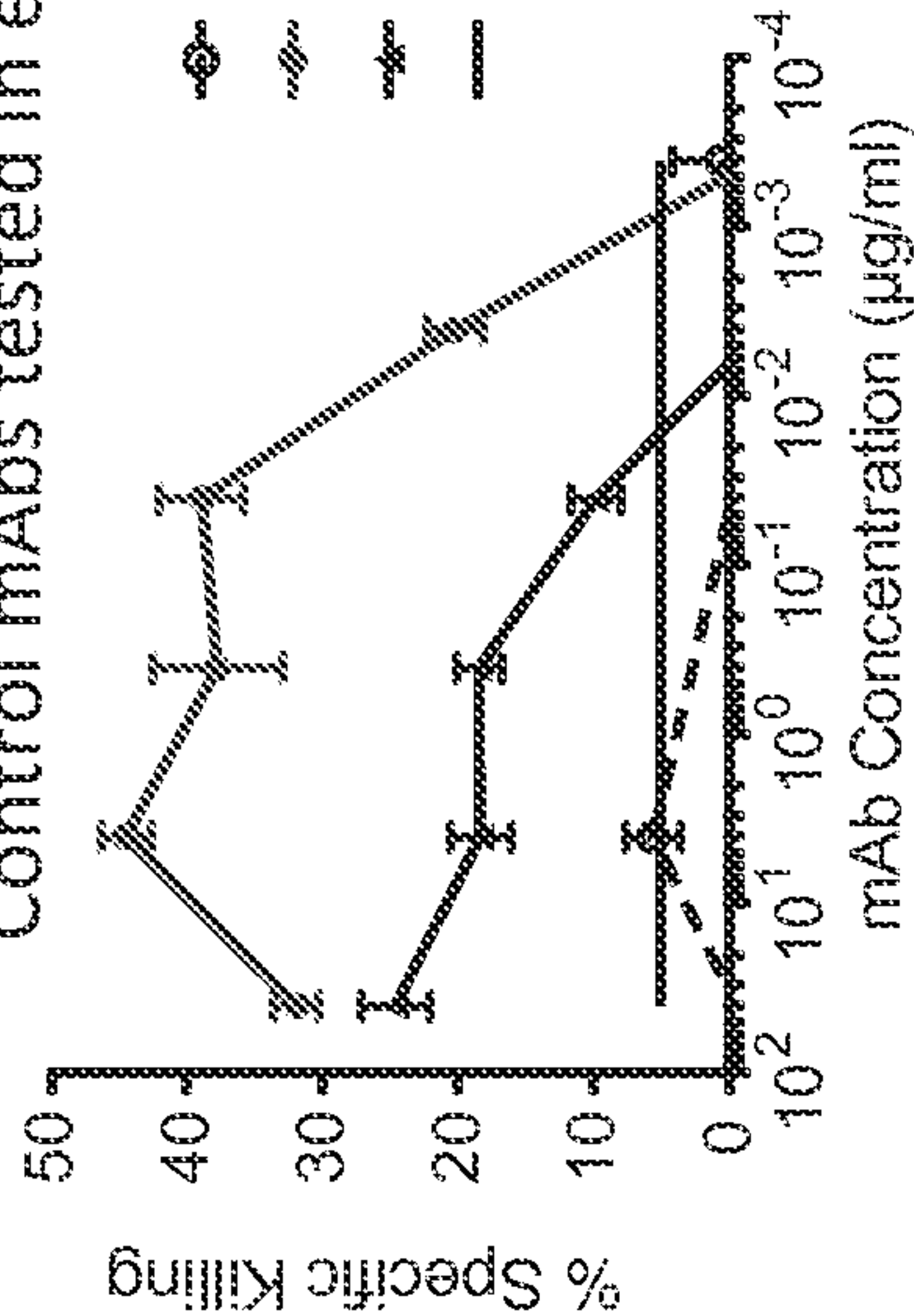


Figure 5-Ex. 2

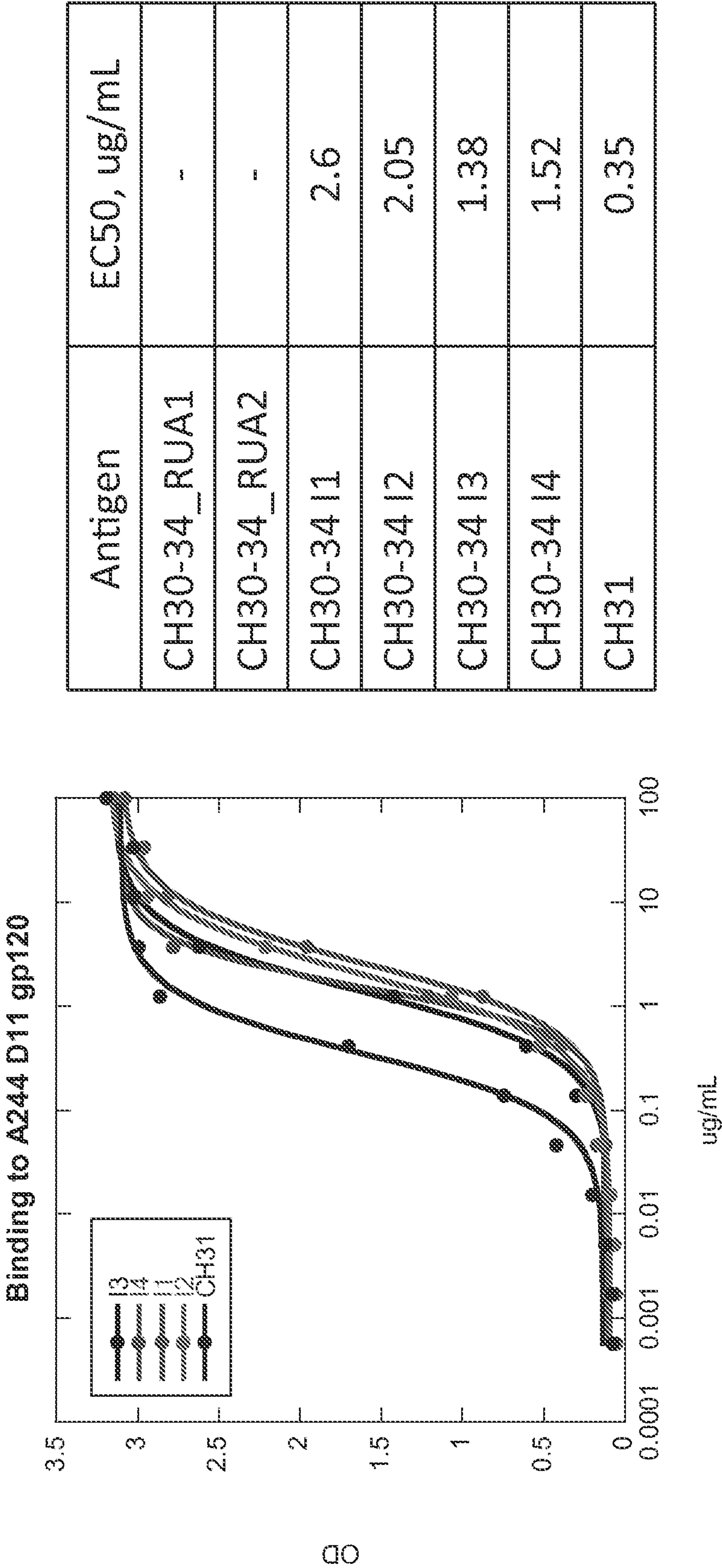
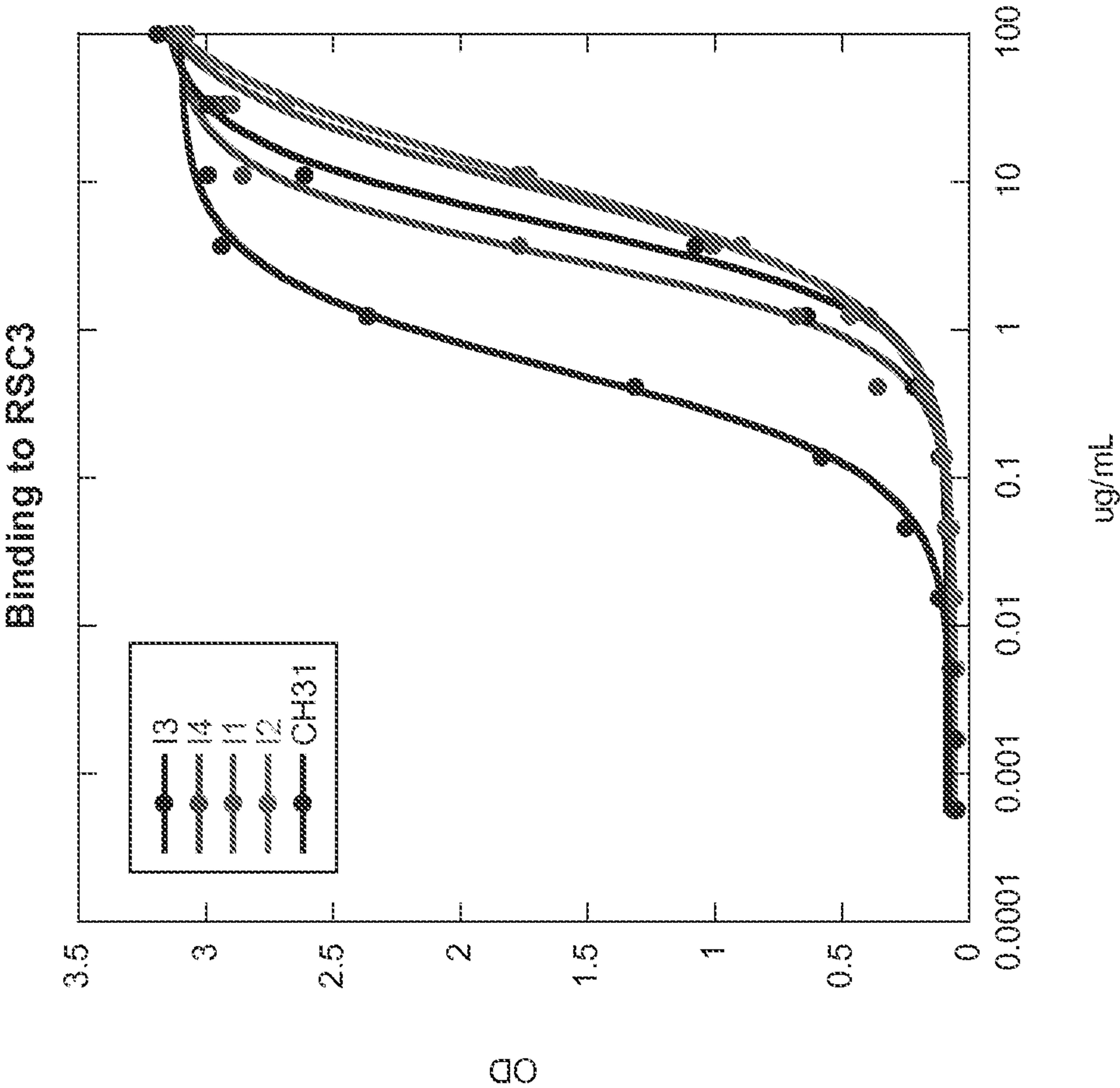
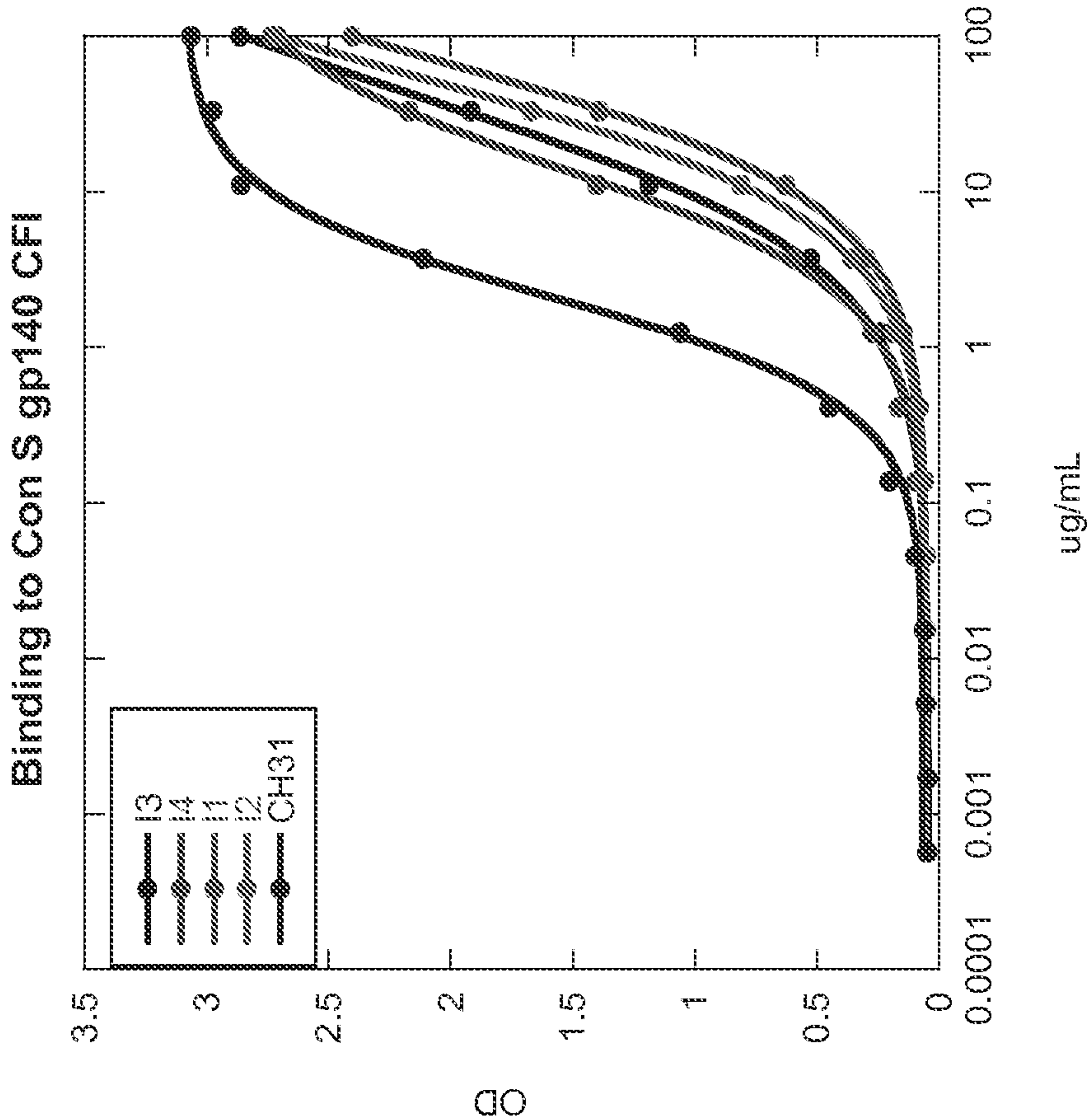


Figure 6-Ex. 2



Antigen	EC50, ug/mL
CH30-34_RUA1	-
CH30-34_RUA2	-
CH30-34 I1	9.1
CH30-34 I2	10.8
CH30-34 I3	5.1
CH30-34 I4	3.1
CH31	0.52

Figure 7-Ex. 2



Antigen	EC50, ug/mL
CH30-34_RUA1	-
CH30-34_RUA2	-
CH30-34 I1	71.6
CH30-34 I2	52.8
CH30-34 I3	38.0
CH30-34 I4	14.2
CH31	2.1

Figure 8-Ex. 2

B Cell Lineage-Based Approach to Vaccine Design

