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(71) Demandeur/Applicant:
CENTOCOR INC., US

(72) Inventeur/Inventor:
LU, JIN, US

(74) Agent: OGILVY RENAULT LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : PROCEDE ET APPAREIL PERMETTANT D'ANALYSER ET DE PRODUIRE DES SEQUENCES D'ACIDES AMINES ET D'ACIDES NUCLEIQUES D'ANTICORPS

(54) Title: METHOD AND APPARATUS FOR ANALYZING AND GENERATING HUMAN ANTIBODY AMINO ACID AND NUCLEIC ACID SEQUENCES

(57) Abrégé/Abstract:
The invention provides methods, computer programs, data and databases, computer readable media, computer systems, and/or apparatus that use, compare or generate data corresponding to at least one partial antibody or antibody fusion protein nucleic acid or amino acid sequence, on recordable media or in computer memory, such as engineered antibody or antibody fusion protein sequences that include any combination of partial antibody sequences, as well as comparisons between different human antibody partial or full sequences, wherein the present invention can be used, inter alia, for research, diagnostic and/or therapeutic products, methods and devices.

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(71) Applicant *(for all designated States except US)*: **CENTOCOR, INC.** [US/US]; 200 Great Valley Parkway, Malvern, Pennsylvania 19355 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **LU, Jin** [US/US]; 3756 Old Post Circle, Boothwyn, Pennsylvania 19061 (US).

(74) Agents: **JOHNSON, Philip, S.** et al.; One Johnson & Johnson Plaza, New Brunswick, NJ 08933 (US).

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(57) Abstract: The invention provides methods, computer programs, data and databases, computer readable media, computer systems, and/or apparatus that use, compare or generate data corresponding to at least one partial antibody or antibody fusion protein nucleic acid or amino acid sequence, on recordable media or in computer memory, such as engineered antibody or antibody fusion protein sequences that include any combination of partial antibody sequences, as well as comparisons between different human antibody partial or full sequences, wherein the present invention can be used, inter alia, for research, diagnostic and/or therapeutic products, methods and devices.

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JUMBO APPLICATIONS / PATENTS

THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE THAN ONE VOLUME.

THIS IS VOLUME __1___ OF __2___

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METHOD AND APPARATUS FOR ANALYZING AND GENERATING HUMAN ANTIBODY AMINO ACID AND NUCLEIC ACID SEQUENCES

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention provides methods, computer programs, data and databases, computer readable media, computer systems, and/or apparatus that use, compare or generate data corresponding to at least one partial or complete antibody or antibody fusion protein nucleic acid or amino acid sequence, on recordable media or in computer memory, such as an antibody or antibody fusion protein sequences that include any combination of partial antibody sequences, as well as comparisons between different human antibody partial or full sequences, wherein the present invention can be used, inter alia, for research, diagnostic and/or therapeutic products, methods and devices.

Related Art

Since the initiation of genome sequencing projects, such as the Human Genome Project, there has been an explosion of amino acid and nucleic acid sequence information. Advancements in the areas of nucleic acid sequencing and protein sequencing have also played an important role in this information explosion. However, the development and refinement of tools to analyze these sequences has barely kept pace with the information explosion. At the same time, development of sophisticated techniques for producing monoclonal antibodies (MABs) with unique specificity have evolved.

MABs can function as research reagents, diagnostics or therapeutics. Antibody based therapeutics can potentially treat a broad spectrum of health threats such as autoimmune disorders, cancers, infections, or poisonings. However, non-human antibodies contain amino acid sequences that are immunogenic in humans. Consequently, it is desirable to employ fully human or humanized antibodies to limit the immunogenicity problems caused by immunogenic sequences in human patients.

Human antibody sequences can be analyzed to attempt to determine potential structural and functional information. Such information can provide insights into antibody structure, posttranslational modification, and expression. This information in turn can be used to rationally alter antibody half-life, affinity, expression, and even

function. Such rational alterations can be accomplished by the deletion or substitution of single amino acid residues, or discrete regions, of an antibody.

To facilitate such rational approaches to antibody design it is necessary to have tools which enable the identification of conserved residues and regions of human antibodies. To meet this need there is a need for suitable methods, computer systems and networks, computer accessible databases, and/or algorithms.

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SUMMARY OF THE INVENTION

The present invention is directed to methods, computer programs, data, databases, computer readable media, computer systems, and/or apparatus for analyzing and generating human antibody sequences using novel approaches to analyze human antibody sequences and categorize classes, subclasses and components thereof, in order to provide searchable, analyzable and exportable databases and fields of amino acid and nucleic acid sequence data, as well as generating amino acid and nucleic acid sequence suitable to use in therapeutic and/or diagnostic antibodies, antibody fusion proteins or other protein sequences.

The present invention provides methods, computer programs, data and databases, computer readable media, computer systems, and/or apparatus that use, compare or generate data corresponding to at least one partial antibody or antibody fusion protein nucleic acid or amino acid sequence, on recordable media or in computer memory, such as engineered antibody or antibody fusion protein sequences that include any combination of partial antibody sequences, as well as comparisons between different human antibody partial or full sequences.

In one aspect of the present invention, a computer accessible database containing amino acid and/or nucleic acid sequences for consensus or engineered human antibodies or portions thereof is provided. The data in the database can optionally be processed and/or generated to filter out short and redundant sequences. In one embodiment of the database or data, there are provided at least one set of amino

acid or nucleic acid sequences corresponding to and comprising at least one set of human or human derived complementarity determing regions (CDRs), human heavy or light chain variable and/or constant region sequences, and/or human or human derived constant region sequences in the database. The data, in this non-limiting example of a database of the invention, can optionally be organized by grouping, superfamily, family and/or subfamily. Multiple data displays can optionally be available for analyzing, generating or viewing data in the database.

In a further aspect of the present invention, a BLAST or similar search engine is optionally further provided for searching, analyzing or generating at least one part of the database (see, e.g., as known in the art, e.g., but not limited to as disclosed in , Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997), entirely incorporated herein by reference).

In another aspect, the present invention provides at least one algorithm for generating at least one set of clustered alignments of human antibody amino acid or nucleic sequences. In one embodiment, an algorithm classifies the collected constant and/or variable region sequence data into superfamilies, families, and/or subfamilies. The classifications can optionally be based on annotations and sequence similarity.

In yet another aspect of the present invention, an additional algorithm displaying the frequency of substitutions at each position in the clustered alignment is provided. In one embodiment, an algorithm determines the prototypical sequence for a given subfamily and the frequency of each substitution (amino acid residue or gap) occurring at the prototype position.

In one aspect of the invention, a method for comparing, analyzing and/or generating human antibody amino acid and/or nucleic acid sequences is provided. The method comprises at least one of the following steps, such as, but not limited to, at least one of:

101. accessing suitable antibody sequence databases and collecting constant, complimentarity determing regions (CDRs), and/or variable region sequences;

102. subjecting the data collected in step **101** to Algorithm 1, wherein the sequences are classified into groups, superfamilies, and/or subfamilies;

103. performing sequence alignment on all sequences assigned to a given subfamily in step **102**;

104. displaying subfamily multiple sequence alignment result of step **103**;

105. accessing antibody sequence databases and collecting variable region sequences;

106. subjecting the data collected in step **105** to Algorithm 2, wherein the variable region sequences are classified into superfamilies and subfamilies;

107. performing multiple sequence alignment on all sequences assigned to a given subfamily in step **106**;

108. displaying subfamily multiple sequence alignment result of step **107**;

109. subjecting the multiple sequence alignment data generated in step **103** or **107** to Algorithm 3, wherein each amino acid substitution is examined and the substitution's frequency of occurrence at a given position is calculated;

110. determining the constant region subfamily prototype sequence and substitutions;

111. displaying the the constant region subfamily prototype sequence and substitutions generated by step **110**;

112. determining the variable region subfamily prototype sequence and substitutions;

113. displaying the variable region subfamily prototype sequence and substitutions generated by step **112**;

114. exporting the displayed results from step 104, 108, 111 or 113 to a web interface, wherein the displays can be viewed and BLAST searching can be performed.

In another aspect, the present invention provides a computer accessible database of clustered alignments of all human antibody amino acid sequences. In one non-limiting embodiment, the heavy chain variable region antibody superfamily consists of a total of 6628 unique sequences belonging to 9 subfamilies. In another embodiment, the light chain variable region kappa superfamily consists of 1730 unique sequences belonging to 6 subfamilies. In yet another embodiment, the light chain variable region lambda superfamily consists of 1209 unique sequences belonging to 15 subfamilies. In still another embodiment, there are 92 unique human constant region sequences belonging to 7 superfamilies and among them, IgA heavy chain constant region superfamily contains 2 subfamilies and the IgG heavy chain constant region superfamily contains 4 subfamilies.

In still a further aspect of the present invention, a computer program product is provided that has computer program logic recorded thereon for enabling a processor in

a computer system to analyze and generate human antibody nucleic acid or amino acid sequences. Such computer program logic includes at least one of the following:

at least one algorithm, sub-routine, routine or means for enabling the processor to access antibody sequence databases and collect human antibody constant region sequences, wherein the sequences are classified into superfamilies and subfamilies;

at least one algorithm, sub-routine, routine or means for enabling the processor to access public available databases and collect human antibody variable region sequences, wherein the sequences are classified into superfamilies and subfamilies; and

at least one algorithm, sub-routine, routine or means for enabling the processor to determine the prototypical sequence for a subfamily and the frequency of each amino acid substitution occurring at the prototype position.

In still a further aspect of the present invention, the present invention provides a computer network, wherein the computer accessible databases, algorithms and computerized search system of the invention are assembled and operated on. The computer network comprises a browser or workstation connected via a first network to a server. This first network can be connected via a second network to additional browsers or workstations.

Features and Advantages

The present invention provides methods, computer programs, data and databases, computer readable media, computer systems, and/or apparatus that use, compare or generate data corresponding to at least one partial antibody or antibody fusion protein nucleic acid or amino acid sequence, on recordable media or in computer memory, such as engineered antibody or antibody fusion protein sequences that include any combination of partial antibody sequences, as well as comparisons between different human antibody partial or full sequences, wherein the present invention can be used, inter alia, for research, diagnostic and/or therapeutic products, methods and devices.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is described with reference to the accompanying drawings. In the drawings, like reference numbers indicate identical or functionally similar elements.

FIG. 1 is a block diagram illustrating the overview of inputs, database assembly and analysis outputs in accordance with one embodiment of the present invention;

FIG. 2 is a block diagram illustrating Algorithm 1 analysis of constant region sequences in accordance with one embodiment for carrying out steps **101-104** shown in FIG. 1;

FIG. 3 is a block diagram illustrating Algorithm 2 analysis of variable region sequences in accordance with one embodiment for carrying out steps **105-108** shown in FIG. 1;

FIG. 4 is a block diagram illustrating Algorithm 3 for statistics distribution in accordance with one embodiment for carrying out steps **109-111** shown in FIG. 1;

FIG. 5 is a block diagram illustrating an exemplary computer system suitable for use with the present invention;

FIG. 6 is a screen shot (HTML page) depicting the content page with data display section hyperlinks exemplified using the heavy chain variable region Vh9 subfamily;

FIG. 7 is a screen shot depicting the first data display section wherein the raw optimized multiple sequence alignment with annotations of functional units generated by Algorithm 1 or 2 was exemplified using 25 Vh9 subfamily members;

FIG. 8 is a screen shot depicting the second data display section wherein the graphic alignment generated by Algorithm 1 or 2 was exemplified using 25 Vh9 subfamily members;

FIG. 9 is a screen shot depicting the third data display section generated by Algorithm 3 wherein the calculated amino acid distribution statistics for each prototype position distribution was exemplified using Vh9 subfamily sequence alignment, wherein the display is formatted for data export via a cut and paste function to programs such as Excel or Vector NTI; and

FIG. 10 is a screen shot depicting the fourth data display section presenting the same contents as the third data display in **Fig. 9**, wherein the display is designed for easy web page display on a computer monitor and for printing.

DETAILED DESCRIPTION OF THE EMBODIMENTS

The present invention provides methods, computer programs, data and databases, computer readable media, computer systems, and/or apparatus that use,

compare or generate data corresponding to at least one partial antibody or antibody fusion protein nucleic acid or amino acid sequence, on recordable media or in computer memory, such as engineered antibody or antibody fusion protein sequences that include any combination of partial antibody sequences, as well as comparisons between different human antibody partial or full sequences, wherein the present invention can be used, inter alia, for research, diagnostic and/or therapeutic products, methods and devices.

Definitions

"Bad clusterings" are clusters which are inconsistent with the subfamily clusters of a "reference database" or a "classification database."

A "browser" is a computer running a computer program for collecting and displaying accessible data.

"Calculated frequency" means the number of times a prototype residue occurs per the total number of sequences in the alignment inputted into Algorithm 3.

A "classification database" contains "reference sequences" which have been classified based on their germline subfamily. An example of a "classification database" is V Base (www.mrc-cpe.cam.ac.uk/vbase-ok.php?menu=901) which provides, when available, the germline subfamily of each variable heavy, variable light chain kappa, or variable light chain lambda included in the database.

A "cluster" is an organizational unit of sequences, or other string of characters, related by a given stringency. Clusters will vary depending on the chosen stringency.

A "duplicate sequence" in a collection of sequences is a sequence which is identical to at least one other sequence in the population.

"Gap frequency" is the percentage of all substitutions in a position which are gaps.

"Good clusterings" are consistent with the subfamily clusters of a "classification database."

"Known subfamily annotations" are annotations indicating the subfamily of a sequence.

"Known superfamily annotations" are annotations which indicate the superfamily of a sequence.

A "network" is an interconnected or interrelated chain, group, or system such as for example a system of computers connected by communications lines.

A "prototype residue" is the amino acid residue which occurs most frequently in a single position of a multiple sequence alignment.

A "prototype sequence" corresponds to a sequence of "prototype residues."

A "reference database" may be used to obtain heavy or light chain constant region "reference sequences." Swissprot is one example of such a database, but other databases in which the subfamily of the sequences deposited in the database is indicated can function as a "reference database."

"Reference sequences" are sequences which are known to belong to a given subfamily.

A "server" is a computer in a network which provides services to other computers in a network. Services provided by a server may include, for example, access to a database, files, and shared peripherals or the routing of files. A server may provide access to a database or files via a web server which provides such access to a "browser."

"Short sequences" are 70 amino acid residues or less.

"Substitutions" may be amino acid residues or gaps (no residue) occurring at a position in an aligned set of sequences.

A "workstation" is a computer for running the algorithms of the invention, assembling the database of the invention or for software development. A "workstation" is also capable of displaying data or operating as a "browser."

Network Structure

The computer accessible database, algorithms and computerized search system of the invention are assembled and operated on a computer network (Fig. 5). The computer network comprises a browser or workstation connected via a first network or a direct connection to a server. This first network can be connected via a second network to additional browsers or workstations.

Database contents and Features

The computer accessible database of the invention contains publicly available amino acid sequences for human antibodies. The data in the database is processed to filter out short and redundant sequences.

In one embodiment, there are 6628 unique human heavy chain variable region sequences, 1730 unique human light chain kappa variable region sequences, 1209 unique human light chain lambda variable region sequences, and 92 unique human constant region sequences in the database. The data in the database is organized by superfamily, and subfamily. Multiple data displays are available for viewing data in the database.

A BLAST or similar search engine is also provided for searching the database (e.g., as described in Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997), and/or as known in the art).

Superfamilies

The human antibody database, as a non-limiting exemplary embodiment,n contains non-redundant data, classified into 10 superfamilies (3 variable region sequence families and 7 constant region sequence families). The 3 variable region superfamilies are the heavy chain Vh, light chain V kappa and light chain V lambda superfamilies. The 7 constant region superfamilies are the heavy chain IgA, IgG, IgD, IgE, IgM, light chain constant kappa and light chain constant lambda superfamilies. Each superfamily, is further classified into at least one subfamilies (Table 1).

TABLE 1: Non-Limiting example of consensus antibody amino sequences according to the present invention

SEQ ID NO		Ab region	AA NO	regions FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
1	heavy chain variable region	Vh1	125	1-31	32	33-46	47	48-79	80	81-125
2		Vh2	97	1-30	31	32-45	46	47-78	79	80-97
3		Vh3a	102	1-30	31	32-45	46	47-78	79	80-102
4		Vh3b	102	1-30	31	32-45	46	47-78	79	80-102
5		Vh3c	94	1-30	31	32-45	46	47-78	79	80-94
6		Vh4	106	1-30	31	32-45	46	47-78	79	80-106
7		Vh5	97	1-30	31	32-45	46	47-78	79	80-97
8		Vh6	91	1-30	31	32-45	46	47-78	79	80-91
9		Vh7	91	1-30	31	32-45	46	47-78	79	80-91
10	light chain variable region	κ1_4	73	1-23	24	25-39	40	41-72	73	
11		κ2	73	1-23	24	25-39	40	41-72	73	
12		κ3	73	1-23	24	25-39	40	41-72	73	
13		κ5	73	1-23	24	25-39	40	41-72	73	
14		κ νεω1	67	1-17	18	19-33	34	35-66	67	
15		κ νεω2	65	1-15	16	17-31	32	33-64	65	
16		λ1α	72	1-22	23	24-38	39	40-71	72	
17		λ1β	73	1-23	24	25-39	40	41-72	73	
18		λ1χ	72	1-22	23	24-38	39	40-71	72	
19		λ3α	72	1-22	23	24-38	39	40-71	72	
20		λ3β	72	1-22	23	24-38	39	40-71	72	
21		λ3χ	72	1-22	23	24-38	39	40-71	72	
22		λ3ε	72	1-22	23	24-38	39	40-71	72	
23		λ4α	72	1-22	23	24-38	39	40-71	72	

24		λ4β	72	1-22	23	24-38	39	40-71	72	
25		λ5	75	1-22	23	24-39	40	41-74	75	
26		λ6	74	1-22	23	24-38	39	40-73	74	
27		λ7	72	1-22	23	24-38	39	40-71	72	
28		λ8	72	1-22	23	24-38	39	40-71	72	
29		λ9	72	1-22	23	24-38	39	40-71	72	
30		λ10	72	1-22	23	24-38	39	40-71	72	

SEQ ID NO			TOTAL AA NO	regions						
				CH1	hinge1	hinge2	hinge3	hinge4	CH2	CH3
31	heavy chain constant region	IgA1	354	1-102	103-122				123-222	223-354
32		IgA2	340	1-102	103-108				109-209	210-340
33		IgD	384	1-101	102-135	136-159			160-267	268-384
34		IgE	497	1-103					104-210	211-318
35		IgG1	339	1-98	99-113				114-223	224-339
36		IgG2	326	1-98	99-110				111-219	220-326
37		IgG3	377	1-98	99-115	116-130	131-145	146-160	161-270	271-377
38		IgG4	327	1-98	99-110				111-220	221-327
39		IgM	476	1-104					105-217	218-323
40	light chain constant region	Igkc	107							
41		Igl	107							

Subfamilies

Subfamilies are sorted and displayed from largest to smallest based on the number of sequences in each subfamily. The heavy chain variable region antibody

superfamily consists of a total of 6628 unique sequences belonging to 9 subfamilies. The light chain variable region kappa and light chain variable region lambda superfamilies contain 6 and 15 subfamilies respectively. The light chain variable region kappa superfamily has 1730 sequences; the light chain variable region lambda superfamily contains 1209 sequences. The heavy chain constant region superfamilies consist of IgA, IgG, IgD, IgE, and IgM. The IgA heavy chain constant region superfamily contains two subfamilies and the IgG heavy chain constant region superfamily has 4 subfamilies. The other heavy chain constant region superfamilies each contain a single subfamily. The total number of unique heavy chain constant regions sequences in the database is 92. The light chain kappa and lambda superfamilies each contain a single subfamily.

Algorithms

Two algorithms (Figs. 2 and 3) classify the collected data in the database into 10 different superfamilies (3 for variable region sequences, 7 for constant region sequences) and into corresponding subfamilies based on annotations and sequence similarity. A third algorithm (Fig. 4) determines the prototypical sequence for a subfamily and the frequency of each substitution (amino acid residue or gap) occurring at the prototype position. All three algorithms can be used to construct the database of the invention or a portion thereof (overviewed in Fig. 1).

Algorithm 1: Generation of constant region multiple sequence alignments and subfamily assignments

The first algorithm (Fig. 2) collects, classifies, and analyzes the heavy and light chain constant regions of human antibodies.

First, a preparation step is performed in which human antibody databases such as Kabat (immuno.bme.nwu.edu; available via NCBI), NCBI (www.ncbi.nlm.nih.gov), SwissProt (www.ebi.ac.uk/swissprot/; available via NCBI), PIR (pir.georgetown.edu), published patent sequence databases (e.g. PAT database; available via NCBI) or other publicly available data sources are made accessible to a workstation for further analysis. See Fig. 2, Step **201**.

Second, a process step is performed in which data for human light and heavy chain constant region amino acid sequences are collected. In this step, human light and heavy chain constant region sequences are collected based on annotations indicating the

sequence is an antibody or immunoglobulin, that the sequences is a *Homo sapiens* (human) sequence, and that the sequences is a heavy or light chain constant region sequence. Data collected in this step includes annotations, sequences, sequence names, accession numbers and the like. See Fig. 2, Step **202**.

Third, a process step is performed in which duplicate and short sequences are removed from the collected data. See Fig. 2, Step **203**.

Fourth, a process step is performed in which data for light and heavy chain constant region sequences with known superfamily annotations is collected and grouped by superfamily. In the context of this step known superfamily annotations indicate that a sequence is a heavy chain constant IgA, IgD, IgE, IgG or IgM superfamily member or alternatively that the sequence is a light chain constant lambda or kappa superfamily member. Sequences lacking known superfamily annotations are not collected at this step. See Fig. 2, Step **204**.

Fifth, a decision step is performed in which it is determined if a sequence has known subfamily annotations. See Fig. 2, Step **205**.

The fifth step is a branch point. After this step sequences with known subfamily annotations are assigned to subfamilies through different steps than those without known subfamily annotations. Each branch (Branch A and Branch B) is described below. After the sequences have been assigned to subfamilies they are further processes in a sixth step.

The sixth step, is process step in which a multiple sequence alignment is performed on all sequences assigned to a given subfamily. A program such as, for example, CLUSTALW (Higgins et al., Nucleic Acids Res. 22:4673-4680 (1994)) may be used to perform multiple sequence alignments. See Fig. 2, Step **206**.

The seventh step is a terminal step in which the result of the preceding steps, a multiple sequence alignment of all the sequences in a given constant region subfamily, may either be displayed or input into Algorithm 3. Data may be displayed in the first section data display described below. See Fig. 2, Step **207**.

<u>Branch A: Processing of sequences with known subfamily annotations</u>

First, a process step is performed in which sequences with known subfamily annotations are collected. See Fig. 2, Step **205A1**.

Second, a process step is performed in which these sequences are assigned to subfamilies. See Fig. 2, Step **205A2**.

After these steps the sequences with known subfamily annotations are processed identically to sequences without known subfamily annotations. Identical processing resumes at step 6. See Fig. 2, Step **206**.

Branch B: Processing of sequences without known subfamily annotations

First, a process step is performed in which sequences without known subfamily annotations are collected. See Fig. 2, Step **205B1**.

Second, a process step is performed in which multiple sequence alignment and phylogeny tree analysis is performed to generate clusters of sequences. A program such as, for example, CLUSTALW may be used to perform multiple sequence alignments. The sequences processed in this step include both collected sequences without known subfamily annotations and reference sequences from a reference database. The reference sequences have known subfamily annotations such as for example IgG1, IgG2, IgA1, IgA2 and the like. Including reference sequences in this step provides a tag which can be used to determine which subfamily each cluster generated by the multiple alignment and phylogeny tree analysis corresponds to. See Fig. 2, Step **205B2**.

Third, a process step is performed in which the sequences are assigned to subfamilies based on which reference sequences cluster with them in the phylogeny tree. See Fig. 2, Step **205B3**.

Fourth, a process step is performed in which the subfamily assignments are validated by user examination of the scientific literature. Subfamily assigments are validated if they are consistent with subfamily assignments described in the scientific literature. See Fig. 2, Step **205B4**.

After these steps the sequences without known subfamily annotations are processed identically to sequences with known subfamiliy annotations. Identical processing resumes at step **206**. See Fig. 2, Step **206**.

Algorithm 2: Generation of variable region multiple sequence alignments and subfamily assignments

The second algorithm (Fig. 3) collects, classifies, and analyzes the heavy and light chain variable regions of human antibodies.

First, a preparation step is performed in which human antibody databases such as Kabat (immuno.bme.nwu.edu; available via NCBI), NCBI (www.ncbi.nlm.nih.gov), SwissProt (www.ebi.ac.uk/swissprot/; available via NCBI),

PIR (pir.georgetown.edu), published patent sequence databases (e.g. PAT database; available via NCBI) or other publicly available data sources are made accessible to a workstation for further analysis. See Fig. 3, Step **301**.

Second, a process step is performed in which data for human light and heavy chain variable region amino acid sequences is collected. In this step, human light and heavy chain variable region sequences are collected based on annotations indicating the sequence is an antibody or immunoglobulin, that the sequences is a *Homo sapiens* (human) sequence, and that the sequences is a heavy or light chain variable region sequence. Data collected in this step includes annotations, sequences, sequence names, accession numbers and the like. See Fig. 3, Step **302**.

Third, a process step is performed in which duplicate and short sequences are removed from the collected data. See Fig. 3, Step **303**.

Fourth, a process step is performed in which data for light and heavy chain variable region sequences with known superfamily annotations is collected and grouped by superfamily. In the context of this step known superfamily annotations indicate that a sequence is a heavy chain variable region, light chain lambda variable region, or light chain kappa variable region superfamily member. Sequences lacking known superfamily annotations are not collected at this step. See Fig. 3, Step **304**.

Fifth, a process step is performed in which sequences within each superfamily are clustered to identify the corresponding subfamilies. This clustering is based on sequence similarity and is performed using a single linkage clustering algorithm (e.g. the BlastClust program; ftp://ftp.ncbi.nih.gov/blast/executables). See Fig. 3, Step **305**.

Sixth, a decision step is performed in which the subfamily clusters are compared to the germline subfamilies of a classification database (e.g. V Base antibody database) and it is decided if the clustering is a good clustering or bad clustering. This comparison is possible because variable region reference sequences from each germline subfamily found in the classification database are present among the variable region sequences which have been collected and clustered. See Fig. 3, Step **306**.

One example of good clustering, in the context of the sixth step, occurs when each cluster of collected sequences contains reference sequences belonging solely to a single germline subfamily of the classification reference. Those of ordinary skill in the art will also recognize other examples of good clustering.

One example of bad clustering, in the context of the sixth step, occurs when a single cluster of collected sequences contains reference sequences belonging to several different germline subfamilies. Those of ordinary skill in the art will also recognize other examples of bad clustering.

If bad clustering is detected a process step is performed in which the clustering parameters (e.g. overlap, percent sequence identity and the like) of the single linkage clustering algorithm are adjusted. The clustering and validation steps are then repeated until a good cluster is obtained. See Fig. 3, Step **306A**.

The seventh step is performed when good subfamily clustering is obtained. This step is a process step in which a multiple sequence alignment of the sequences in each subfamily cluster is performed. A program such as, for example, CLUSTALW may be used to perform multiple sequence alignments. See Fig. 3, Step **307**.

Eighth, a decision step is performed in which these alignments are determined to be good or bad. Bad or good alignments may be recognized by those skilled in the art by examination of a given alignment. See Fig. 3, Step **308**.

If the alignment is bad it is improved by removing sequences or adjusting the alignment. See Fig. 3, Step **308A**.

The ninth step is performed, when a good alignment is obtained. The ninth step is a terminal step in which the result of the preceding steps, a multiple sequence alignment of all the sequences in a given variable region subfamily, may either be displayed or input into Algorithm 3. Data may be displayed in the first section data display described below. See Fig. 3, Step **309**.

Algorithm 3: Generation of prototype amino acid sequences and calculation of substitution frequency

A third algorithm (Fig. 4) reports each amino acid substitution and the substitution's frequency of occurrence at a given position in a subfamily's prototype sequence.

First, a preparation step is performed in which multiple sequence alignment and data formatting instructions are inputted by a user into the data initiation module. See Fig. 4, Step **401**.

The inputted multiple sequence alignment data may be generated by Algorithm 1 or Algorithm 2. See Fig. 2 or 3. Such data includes multiple sequence alignments corresponding to a variable or constant region subfamily. The data

formatting instructions specify the number of amino acid prototype positions, substitutions and other information to display per row. This information is used by the data formulation module described below in step **410**. See Fig. 4, Step **410**.

Second, a process step is performed in which the multiple sequence alignment data is parsed to collect the substitutions occurring at all positions in a set of aligned sequences. See Fig. 4, Step **402**.

Third, a process step is performed in which a single position in the multiple sequence alignment is examined. See Fig. 4, Step **403**.

Fourth, a process step is performed in which each substitution occurring at a single examined position in a set of aligned sequences is collected. See Fig. 4, Step **404**.

Fifth, a process step is performed in which all the substitutions occurring at a single position in a set of aligned sequences are counted and collected. See Fig. 4, Step **405**.

Sixth, a process step is performed in which a calculation of the frequency of each substitution is made and the substitutions are sorted. Substitutions are sorted from most common to least common based on the number of times the substitution occurs in a single position. See Fig. 4, Step **406**.

Seventh, a decision step is performed in which it is determined if an amino acid residue is the most frequent substitution occurring in a position. If an amino acid residue is the most frequently occurring substitution the decision is to proceed to step 8. See Fig. 4, Step **407**.

If a gap is the most frequently occurring substitution the decision is to proceed to step 407A1 described below. Step **407A1** is a branch for the processing of positions in which the most frequent substitution is a gap. See Fig. 4, Step **407A1**.

Eighth, a process step is performed in which the most frequently occurring amino acid residue in a position is designated to be the prototype residue for the position. See Fig. 4, Step **408**.

Ninth, a process step is performed in which a count is made of the number of times each substitution occurs in a position and the calculated frequency for the position's prototype residue is generated. See Fig. 4, Step **409**.

Tenth, a process step is performed by the data formulation module in which the preceding steps are repeated via a do-loop for each position in the inputted multiple

sequence alignment. After these steps have been performed for each position in the alignment the module reads the formatting instructions inputted by the user into the data initiation module of step **401**. The data is then formatted for display. See Fig. 4, Step **410**.

The eleventh step is a terminal step in which the result of the preceding steps, a prototype sequence and substitutions for each position of this sequence, may be displayed. Data may be displayed in the third or fourth section data displays described below. See Fig. 4, Step **411**.

Branch A: Processing of positions in which the most frequent substitution is a gap

First, a process step is performed in which a calculation is made of the gap frequency. See Fig. 4, Step **407A1**.

Second, a decision step is performed in which it is determined if the gap frequency is more or less than 99 percent. See Fig. 4, Step **407A2**.

If the gap frequency is more than 99 percent the position is removed from the dataset and steps three through seven are repeated. See Fig. 4, Step **407B1**.

If the gap frequency is less than 99 percent the most frequent amino acid residue is identified and step eight is performed. See Fig. 4, Step **407B2**.

Database organization and data displays

For each subfamily in a given superfamily there are four data display sections (Fig. 6).

First section data display

In the first section data display there is an optimized multiple sequence alignment which consists of all sequences in a subfamily and includes annotations of functional units such as frameworks, CDRs, CH1-4, or hinge regions (Kabat et al., 1991) (Fig. 7). The source of each sequence can be discerned through their names, e.g. lcl|Kab_000794 which indicates the sequence is from the Kabat database (Johnson, G. et al., 2000) (Fig. 7). The data for the first section displays can be generated via algorithm 1 or 2 as appropriate.

Second section data display

In the second section data display, which is a graphic alignment, each amino acid is color coded according to its charge, hydrophobicity or other properties (Fig. 8). The conserved regions and broad pattern for alignments can easily be observed in the

second section display. The data for the second section data displays can be generated by algorithm 1 or 2 as appropriate. A program such as, for example, JALVIEW (http://www.ebi.ac.uk/~michele/jalview/dist/) may be used to generate a second section data display.

Third section data display

The third section displays calculated amino acid distribution statistics for each prototype positition identified by algorithm 3 in an alignment of subfamily sequences (Fig. 9). The first line indicates each numbered amino acid position of a prototype sequence. The second line shows the prototypical residue found in the numbered position. The third line indicates how many times the prototype amino acid occurs in a given position relative to the total number of sequences in the subfamily. The other lines display all other possible substitutions occurring at each prototype position and the number of times each substitution occurs in a given prototype position. These substitutions are sorted by their frequency of occurrence at each prototype position. The third section data display is also formatted for data import via a cut and paste function to programs such as Excel or Vector NTI. Data and formatting for the third section data display can be generated via algorithm 3.

Fourth section data display

The fourth section data display is a distribution list for the Vh9 subfamily which has the same contents as the section 3 display (Fig. 10). This section, however, is designed for easy web page display on a computer monitor and printing. To accomplish this, each line of the fourth section display shows substitution data for no more than 10 prototype amino acid positions. Annotations denoting the framework, CDR, CH1-4, and hinge regions may also added to the data displayed in this section or selected data from this section.

Example 1

Use of the database for human antibody immunogenicity prediction.

The database of the invention can be used to predict whether a given antibody could be tolerated in humans without an adverse immune response. For example, a scientist wishing to determine if an antibody might generate an adverse response will obtain the sequences of the antibody's heavy and light chain variable and constant regions. The scientist will then use these heavy chain and light chain sequences to query the database through its BLAST searching feature. By reviewing

the results of these BLAST searches the scientist can determine, for example, that the heavy and light chain variable regions of his antibody have a high level of similarity (e.g.>90% identical) to known human light and heavy chain variable region sequences. Most of the sequences differences occur in the CDRs; a minority occur in the frameworks. Similarly, the scientist can determine that the heavy and light chain constant regions are highly similar (e.g. >99% identical) to known human heavy and light chain constant region sequences. This information suggests to the scientist that the antibody is very human like and unlikely to generate an adverse immune response. This conclusion can be confirmed by performing a similar search in a database of mouse antibody sequences.

Example 2

Use of the database for human antibody scaffold alteration.

In some instances it may be desirable to substitute a portion of one antibody with a portion of a second antibody. It may also be desirable to make amino acid substitutions. For example, a scientist may wish to replace a murine heavy chain variable region framework 1 with a human framework, or eliminate a post-translational modification site.

By using the BLAST feature of the invention a scientist could identify the human heavy chain variable region framework 1 region which is most similar to the murine framework 1 region to be substituted. The human framework 1 identified could then be substituted into the variable region of the antibody of interest.

Similarly, a scientist could eliminate a post-translational modification site in the constant region by using the BLAST feature of the database to determine which heavy chain constant region subfamily the antibody of interest belongs to. The scientist could then examine the Section 3 or 4 data displays for this subfamily and find the region corresponding to the post-translational modification site of interest. The scientist can then use the substitution frequency information for this region to select those substitutions which occur in the subfamily, but eliminate the post-translational modification site.

References

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3. Knappik, A., Ge, L., Honegger, A., Pack, P., Fischer, M., Wellnhofer, G., Hoess, A., Wolle, J., Plückthun, A. and Virnekas, B. 2000. Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides. J.Mol.Biol. 296: 57-86.

4. Kabat, E. A., Wu, T. T., Perry, H. M., Gottesman, K.S. and Foeller, C. 1991. Sequences of Proteins of Immunological Interest. 5th edit, NIH publication no. 91-3242. US Department of Health and Human Services, Washington, DC.

5. Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.

6. Higgins D., Thompson J., Gibson T., Thompson J.D., Higgins D.G., and Gibson T.J.(1994), CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting,position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

DEMANDES OU BREVETS VOLUMINEUX

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

CECI EST LE TOME __1___ DE __2___

NOTE: Pour les tomes additionels, veillez contacter le Bureau Canadien des Brevets.

JUMBO APPLICATIONS / PATENTS

THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE THAN ONE VOLUME.

THIS IS VOLUME __1___ OF __2___

NOTE: For additional volumes please contact the Canadian Patent Office.

What Is Claimed Is:

1. A method for selecting, generating, comparing or analyzing human or human derived antibody or antibody fusion protein amino acid or nucleic acid sequences, comprising:

(a) accessing suitable antibody sequence databases and collecting constant, complimentarity determing regions (CDRs), and/or variable region sequences;

(b) subjecting the data collected in step (a) to Algorithm 1, wherein the sequences are classified into groups, superfamilies, and/or subfamilies;

(c) performing sequence alignment on all sequences assigned to a given subfamily in step (b);

(d) displaying subfamily multiple sequence alignment result of step (c);

(e) accessing antibody sequence databases and collecting variable region sequences;

(f) subjecting the data collected in step (e) to Algorithm 2, wherein the variable region sequences are classified into superfamilies and subfamilies;

(g) performing multiple sequence alignment on all sequences assigned to a given subfamily in step (f);

(h) displaying subfamily multiple sequence alignment results of step (g);

(i) subjecting the multiple sequence alignment data generated in step (c) or (g) to Algorithm 3, wherein each amino acid substitution is examined and the substitution's frequency of occurrence at a given position is calculated;

(j) determining the constant region subfamily prototype sequence and substitutions;

(k) displaying the the constant region subfamily prototype sequence and substitutions generated by step (j);

(l) determining the variable region subfamily prototype sequence and substitutions;

(m) displaying the variable region subfamily prototype sequence and substitutions generated by step (l);

(n) exporting the displayed results from step (d), (h), (k) or (m) to a web interface, wherein the displays can be viewed and BLAST searching can be performed.

2. Any invention described herein.

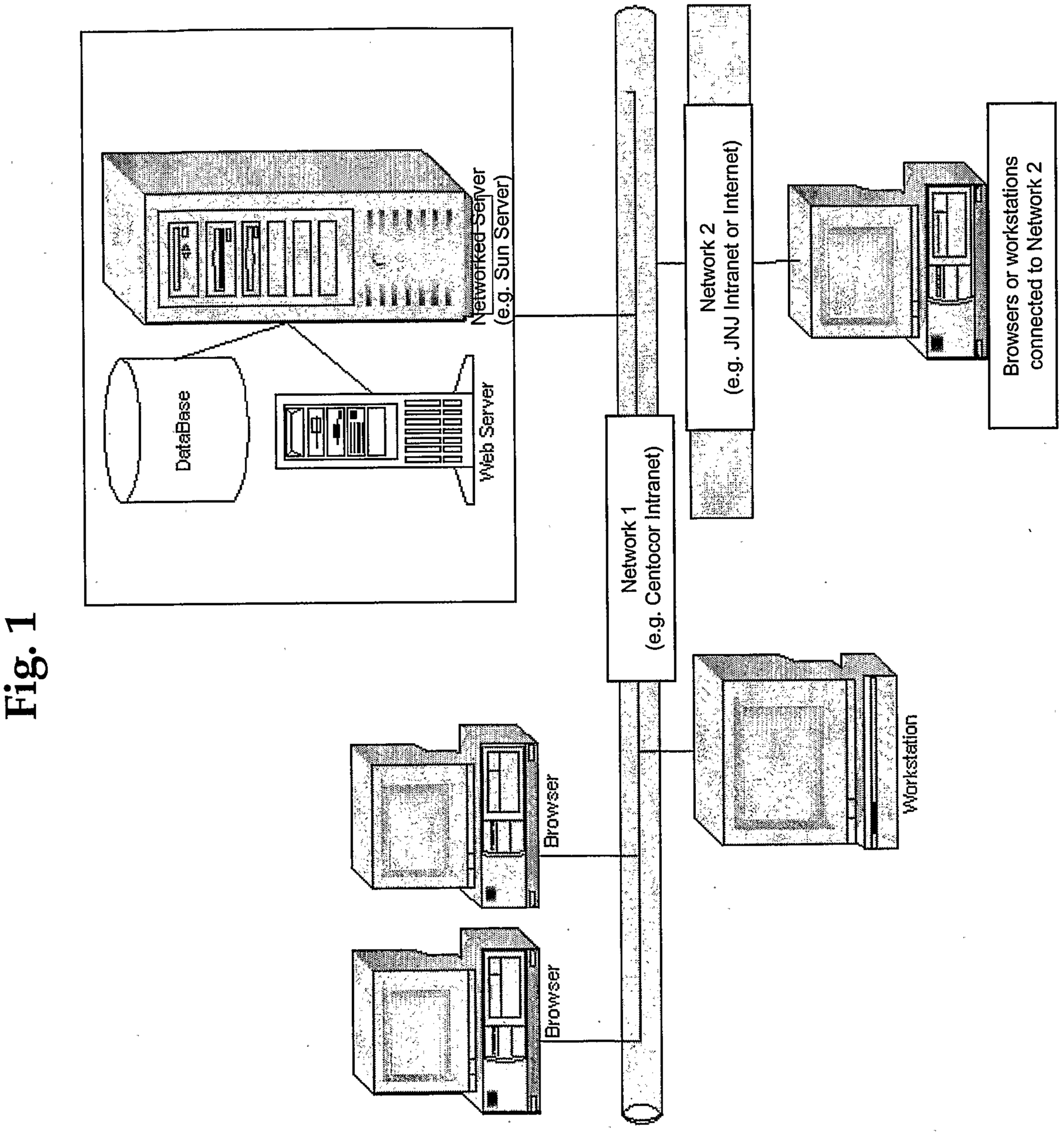

Networked Server
(e.g. Sun Server)
DataBase
Web Server
Network 1
(e.g. Centocor Intranet)
Network 2
(e.g. JNJ Intranet or Internet)
Browsers or workstations
connected to Network 2
Browser
Browser
Workstation

Fig. 1

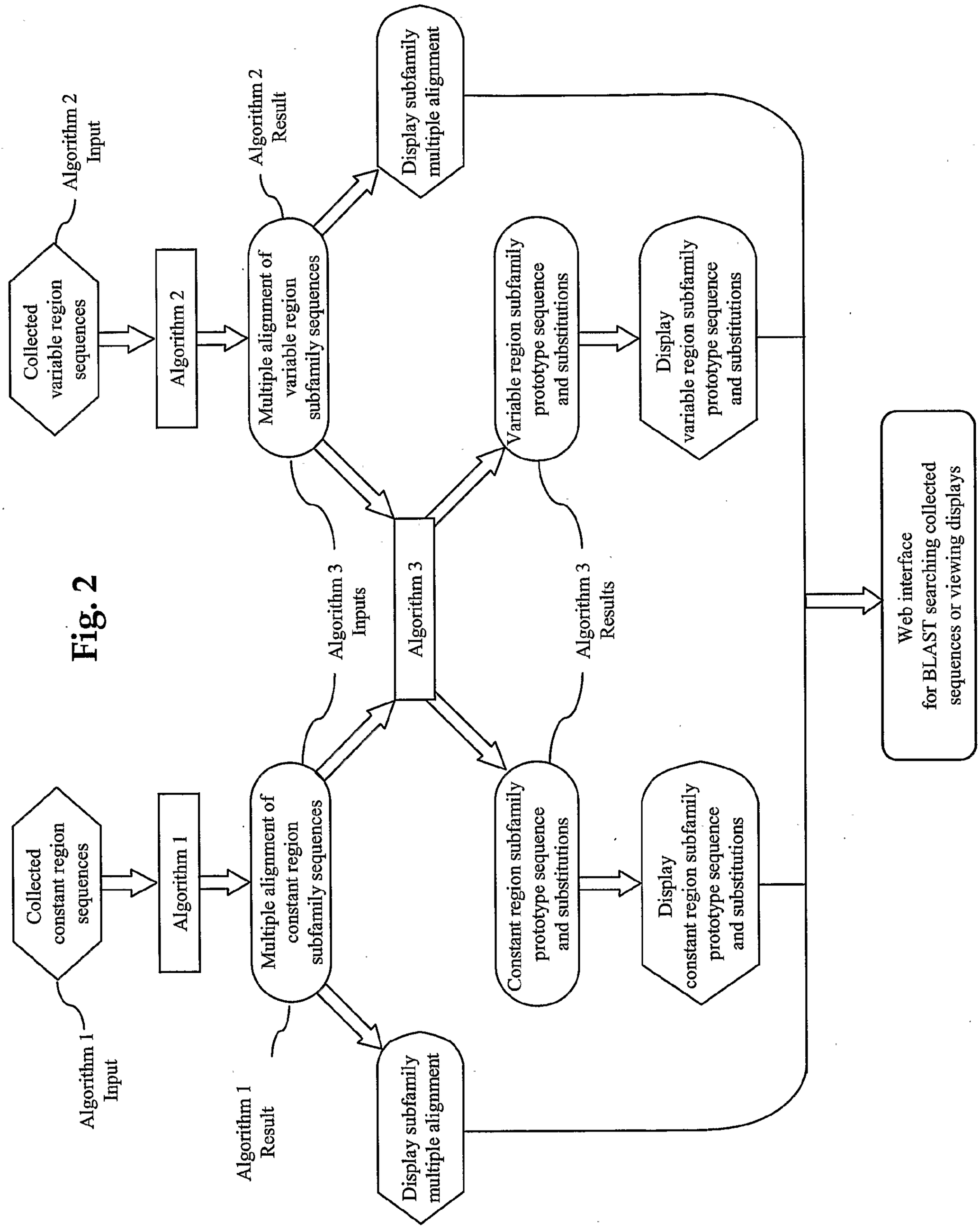
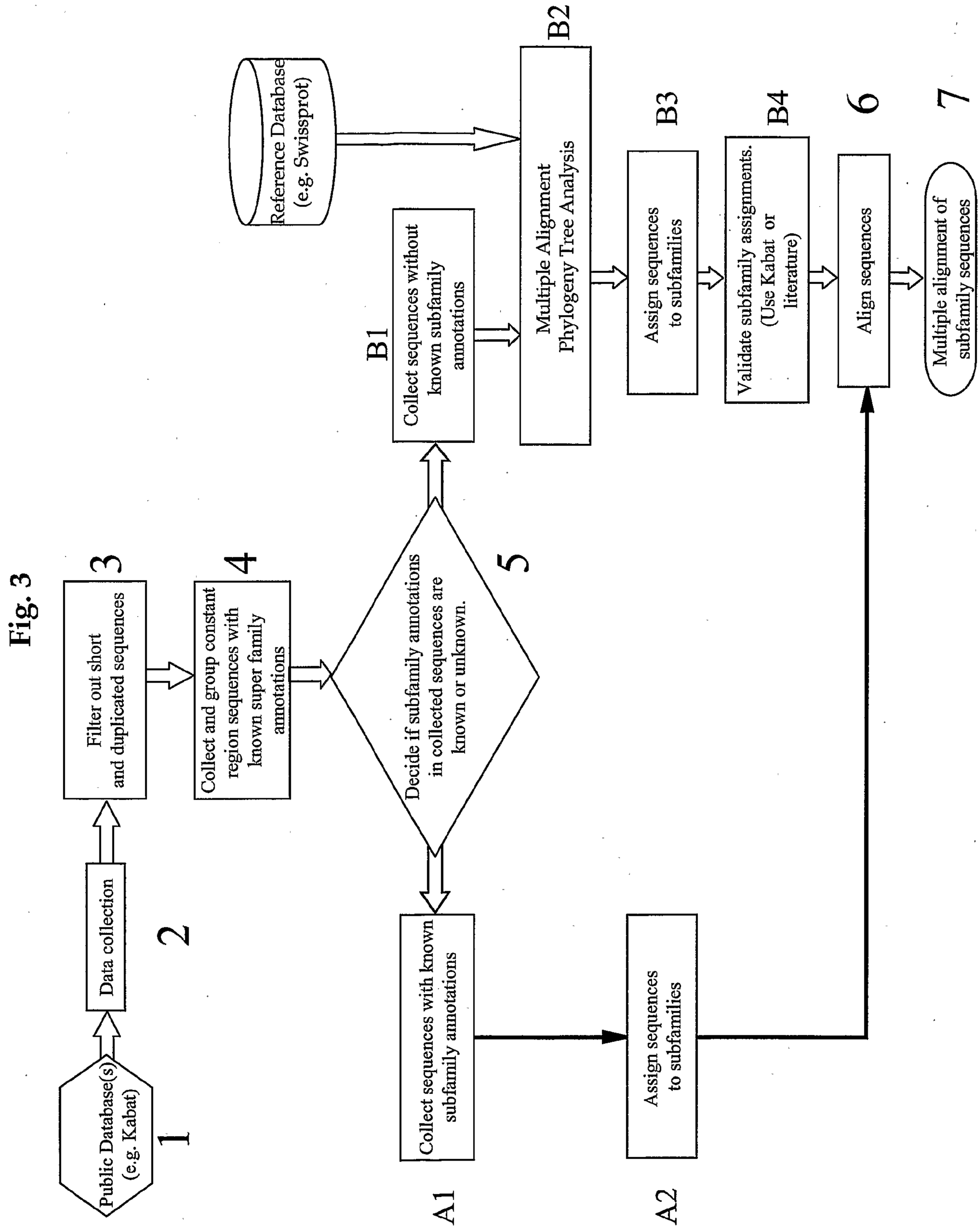

Collected variable region sequences
Algorithm 2 Input
Algorithm 2
Multiple alignment of variable region subfamily sequences
Algorithm 2 Result
Display subfamily multiple alignment
Variable region subfamily prototype sequence and substitutions
Display variable region subfamily prototype sequence and substitutions
Collected constant region sequences
Algorithm 1 Input
Algorithm 1
Multiple alignment of constant region subfamily sequences
Algorithm 1 Result
Display subfamily multiple alignment
Algorithm 3 Inputs
Algorithm 3
Constant region subfamily prototype sequence and substitutions
Algorithm 3 Results
Display constant region subfamily prototype sequence and substitutions
Web interface for BLAST searching collected sequences or viewing displays

Fig. 2

Fig. 3


Public Database(s)
(e.g. Kabat)
1
Data collection
2
Filter out short
and duplicated sequences
3
Collect and group constant
region sequences with
known super family
annotations
4
Decide if subfamily annotations
in collected sequences are
known or unknown.
5
Collect sequences with known
subfamily annotations
A1
Assign sequences
to subfamilies
Validate subfamily assignments.
(Use Kabat or
literature)
Align sequences
Multiple alignment of
subfamily sequences
A2
Reference Database
(e.g. Swissprot)
B1
Collect sequences without
known subfamily
annotations
B2
Multiple Alignment
Phylogeny Tree Analysis
B3
Assign sequences
to subfamilies
B4
Validate subfamily assignments.
(Use Kabat or
literature)
6
Align sequences
7
Multiple alignment of
subfamily sequences

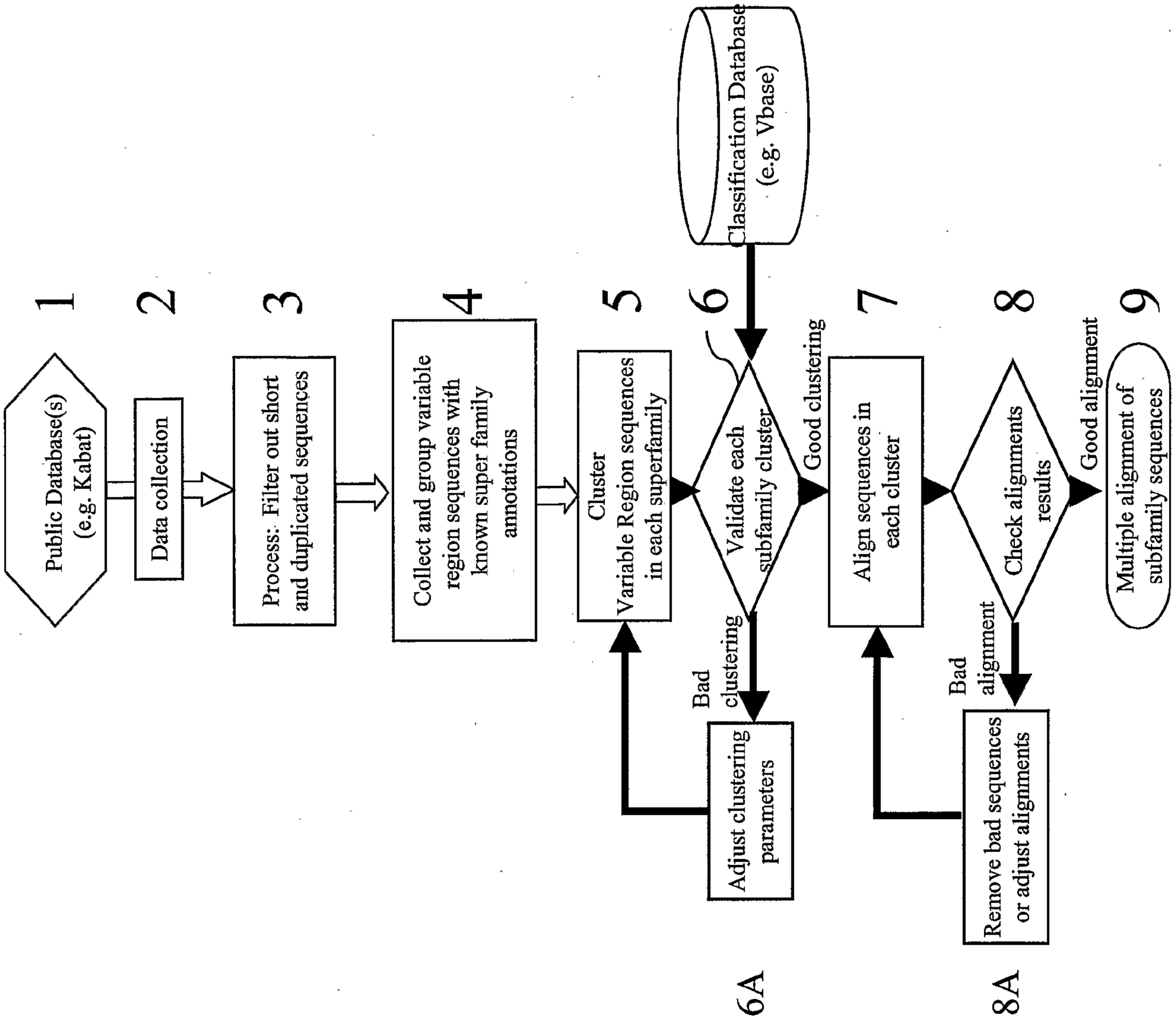

1
Public Database(s) (e.g. Kabat)
2
Data collection
3
Process: Filter out short and duplicated sequences
4
Collect and group variable region sequences with known super family annotations
5
Cluster Variable Region sequences in each superfamily
6
Validate each subfamily cluster
Bad clustering
Adjust clustering parameters
6A
Good clustering
7
Align sequences in each cluster
8
Check alignments results
Bad alignment
Remove bad sequences or adjust alignments
8A
Good alignment
9
Multiple alignment of subfamily sequences
Classification Database (e.g. Vbase)

Fig. 4

Fig. 5

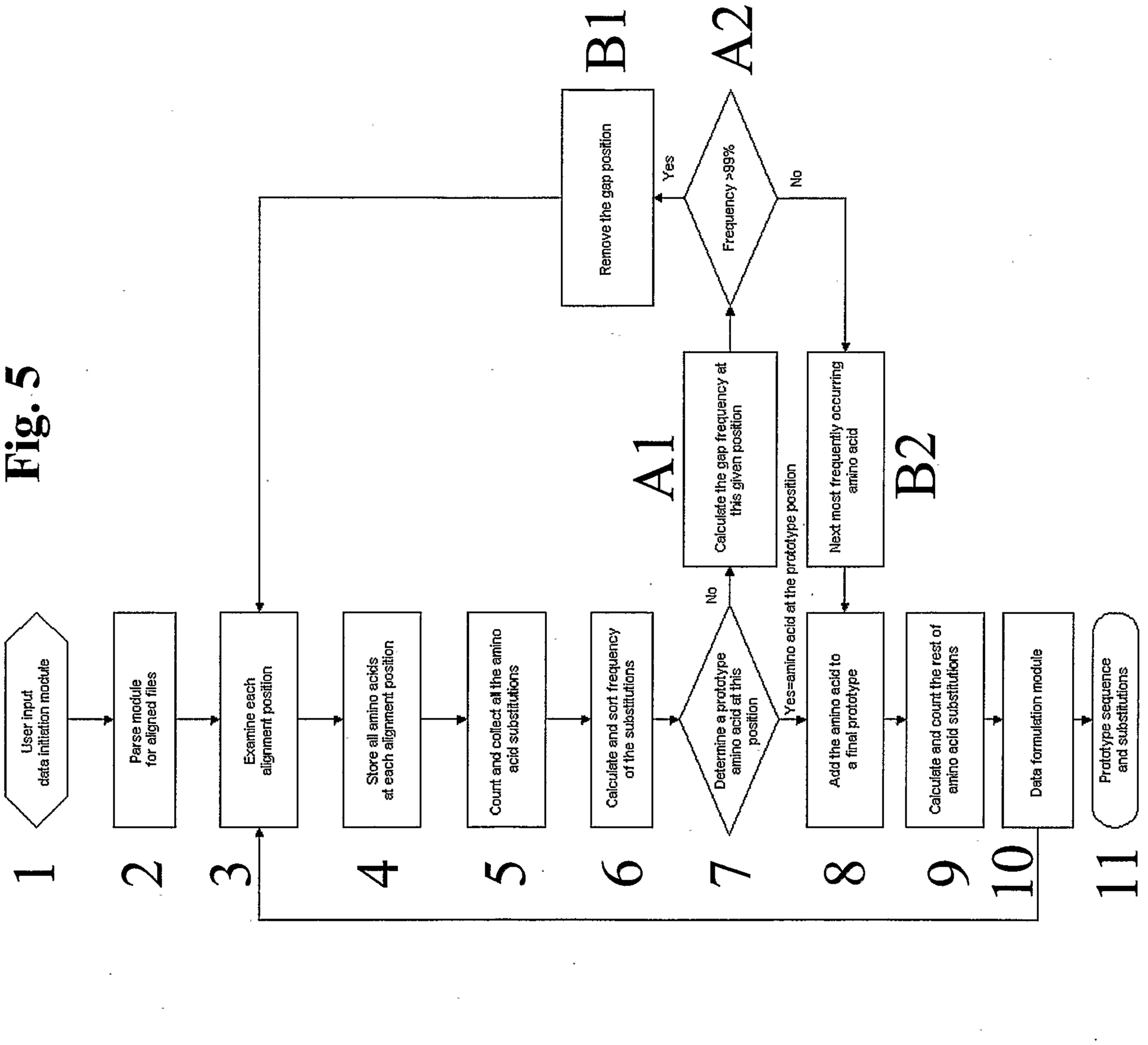
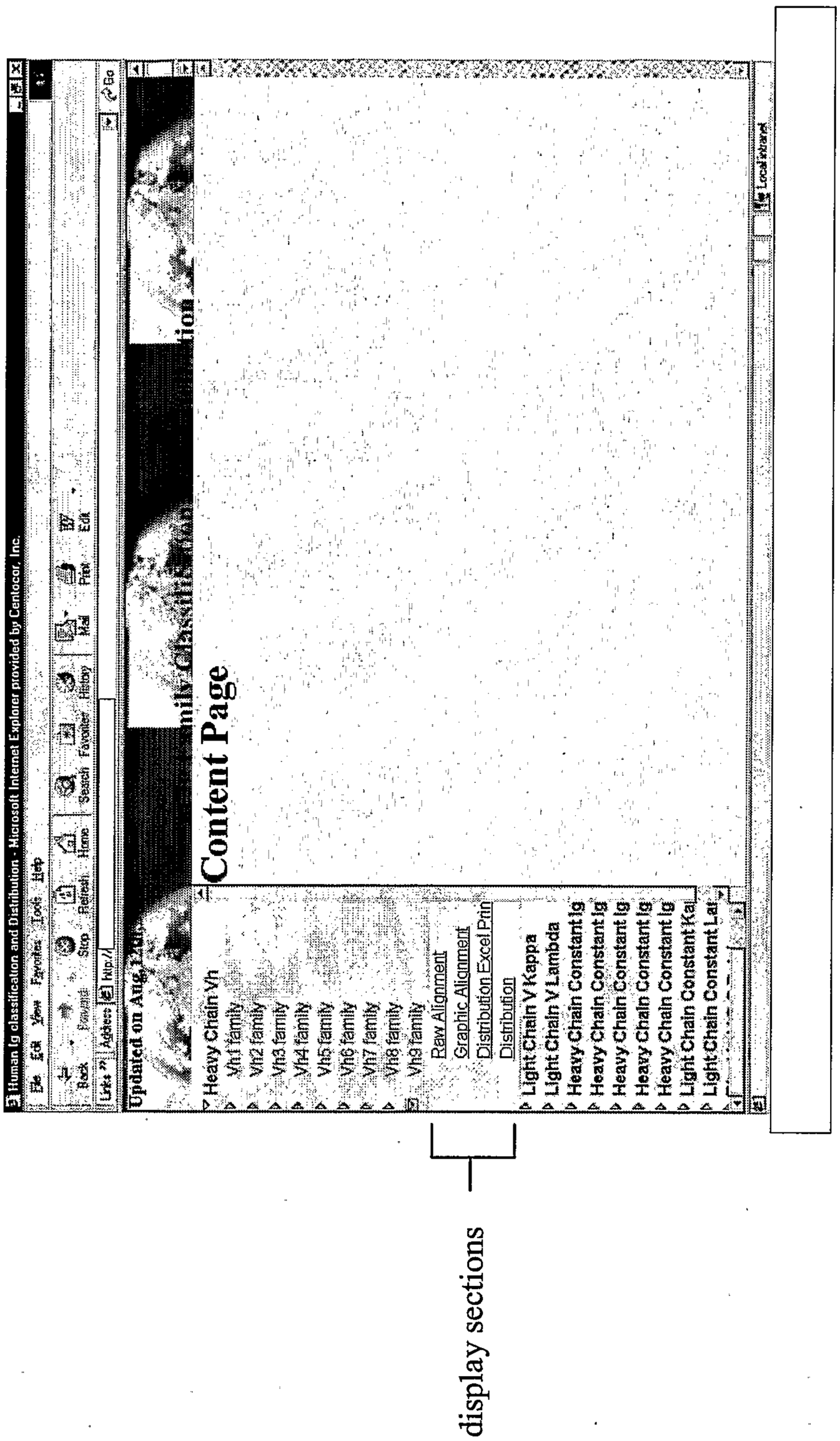

1
User input data initiation module
2
Parse module for aligned files
3
Examine each alignment position
4
Store all amino acids at each alignment position
5
Count and collect all the amino acid substitutions
6
Calculate and sort frequency of the substitutions
7
Determine a prototype amino acid at this position
No
A1
Calculate the gap frequency at this given position
B1
Remove the gap position
Yes
A2
Frequency >99%
No
B2
Next most frequently occurring amino acid
Yes=amino acid at the prototype position
8
Add the amino acid to a final prototype
9
Calculate and count the rest of amino acid substitutions
10
Data formulation module
11
Prototype sequence and substitutions

Fig. 6


Human Ig classification and Distribution - Microsoft Internet Explorer provided by Centocor, Inc.
Human Ig classification and Distribution
Updated on Aug 12th
Heavy Chain Vh
Vh1 family
Vh2 family
Vh3 family
Vh4 family
Vh5 family
Vh6 family
Vh7 family
Vh8 family
Vh9 family
Raw Alignment
Graphic Alignment
Distribution Excel Prin
Distribution
Light Chain V Kappa
Light Chain V Lambda
Heavy Chain Constant Ig
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Light Chain Constant Ka
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Content Page
Links to data display sections

Fig. 7

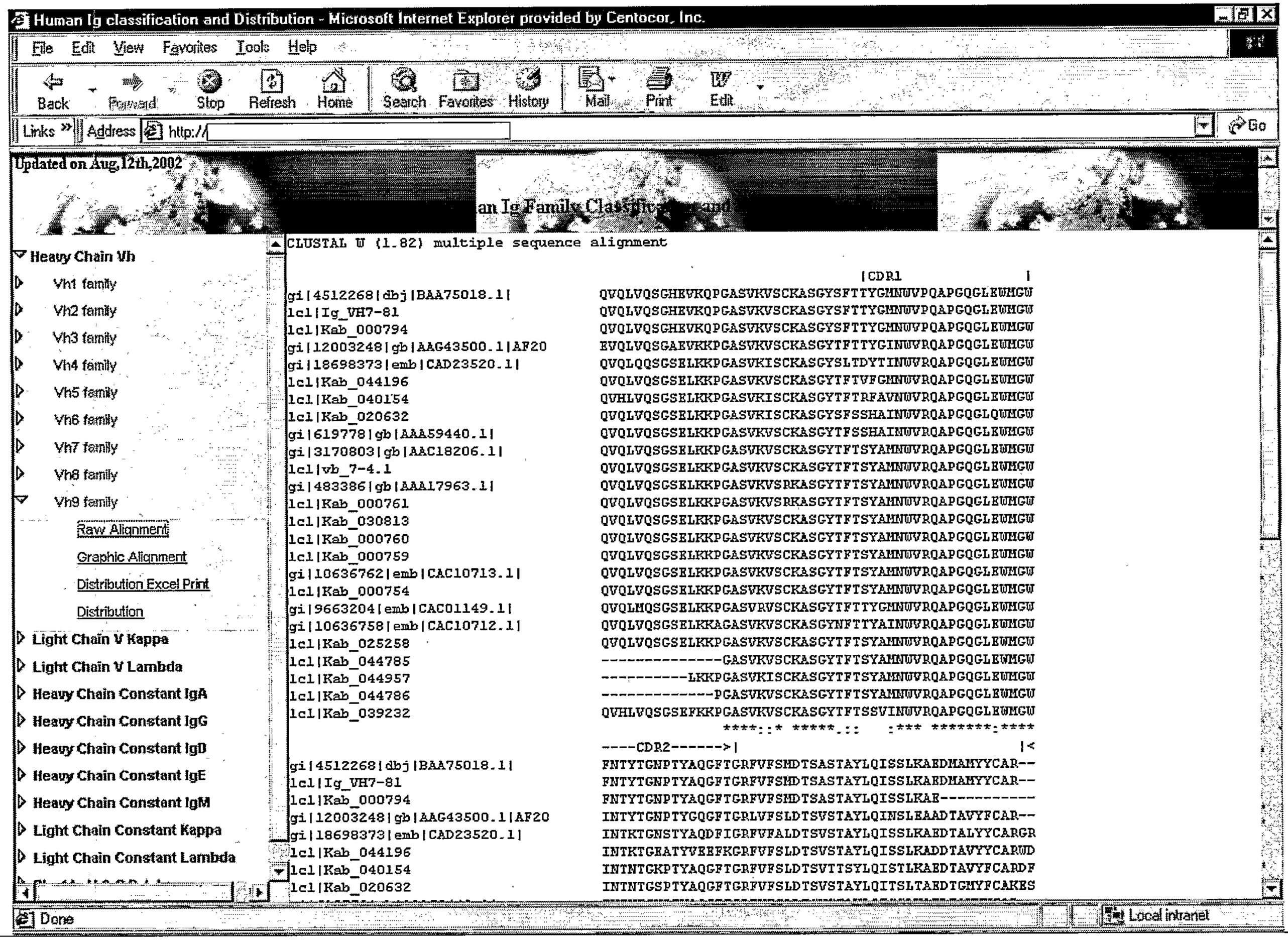

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Fig. 8

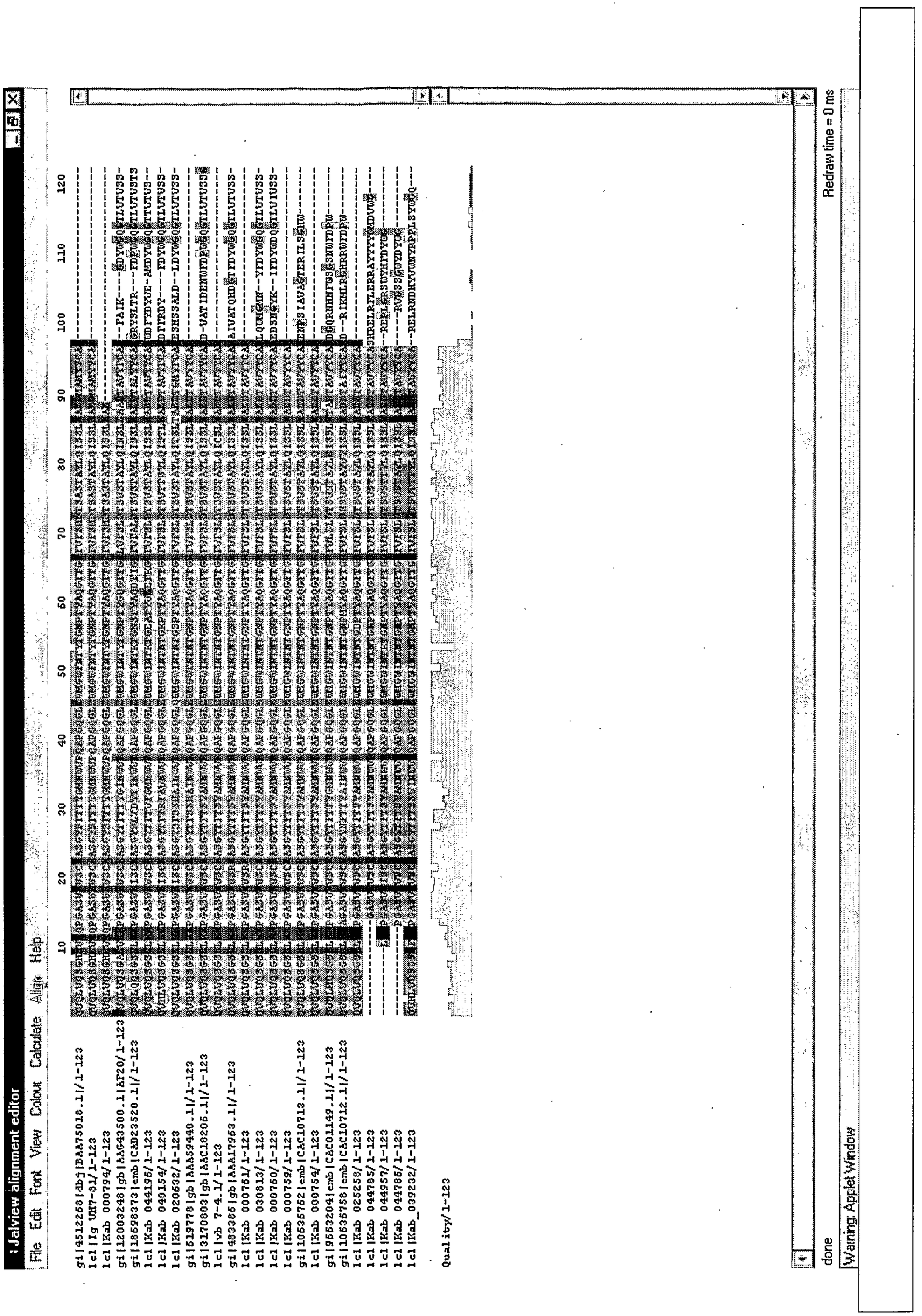
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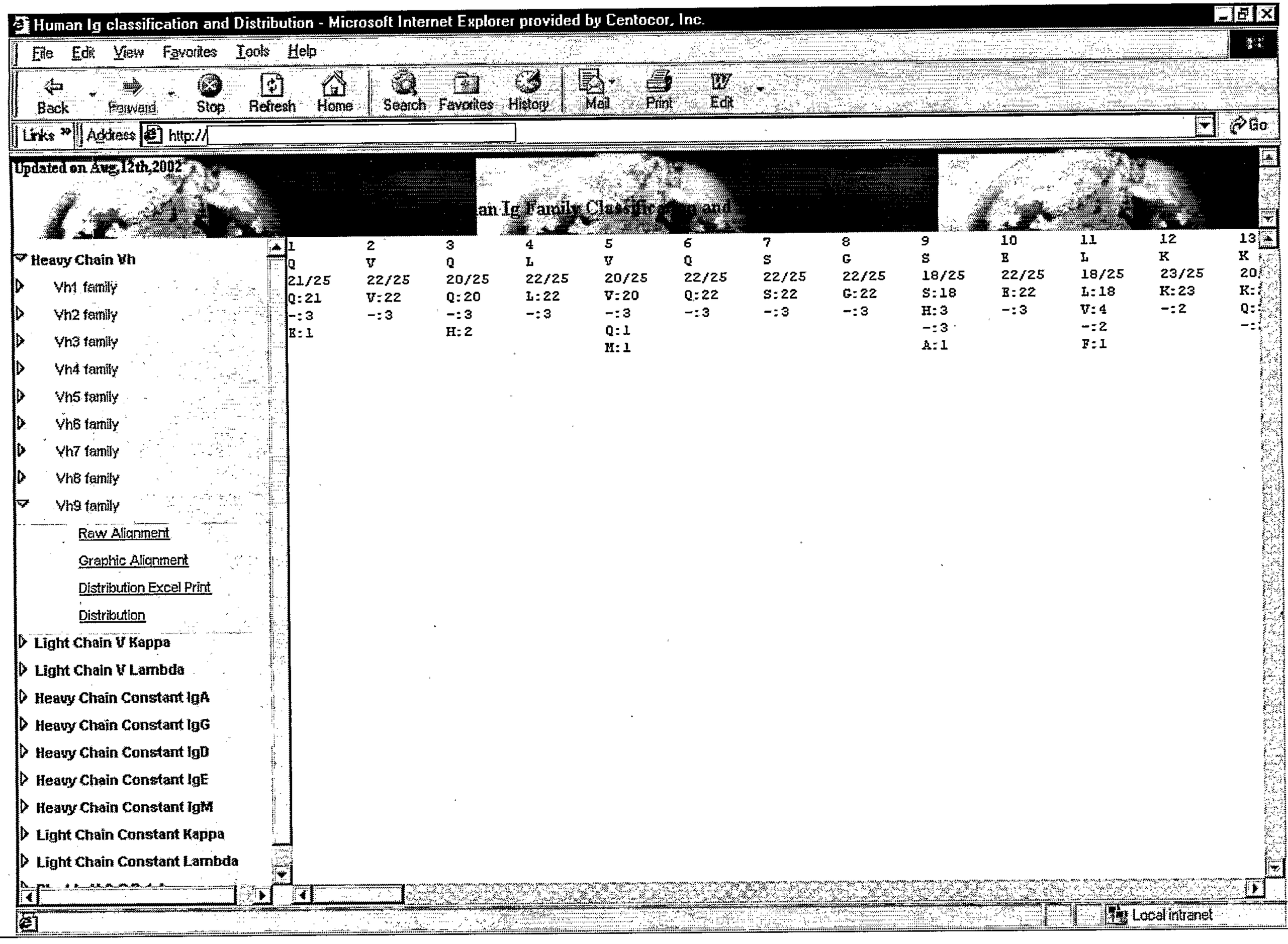
Fig. 9


Human Ig classification and Distribution - Microsoft Internet Explorer provided by Centocor, Inc.
File Edit View Favorites Tools Help
Back Forward Stop Refresh Home Search Favorites History Mail Print Edit
Links Address http://
Go
Updated on Aug.12th,2002
an Ig Family Classific
and
Heavy Chain Vh
Vh1 family
Vh2 family
Vh3 family
Vh4 family
Vh5 family
Vh6 family
Vh7 family
Vh8 family
Vh9 family
Raw Alignment
Graphic Alignment
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Light Chain V Lambda
Heavy Chain Constant IgA
Heavy Chain Constant IgG
Heavy Chain Constant IgD
Heavy Chain Constant IgE
Heavy Chain Constant IgM
Light Chain Constant Kappa
Light Chain Constant Lambda
1 2 3 4 5 6 7 8 9 10 11 12 13
Q V Q L V Q S G S E L K K
21/25 22/25 20/25 22/25 20/25 22/25 22/25 22/25 18/25 22/25 18/25 23/25 20/
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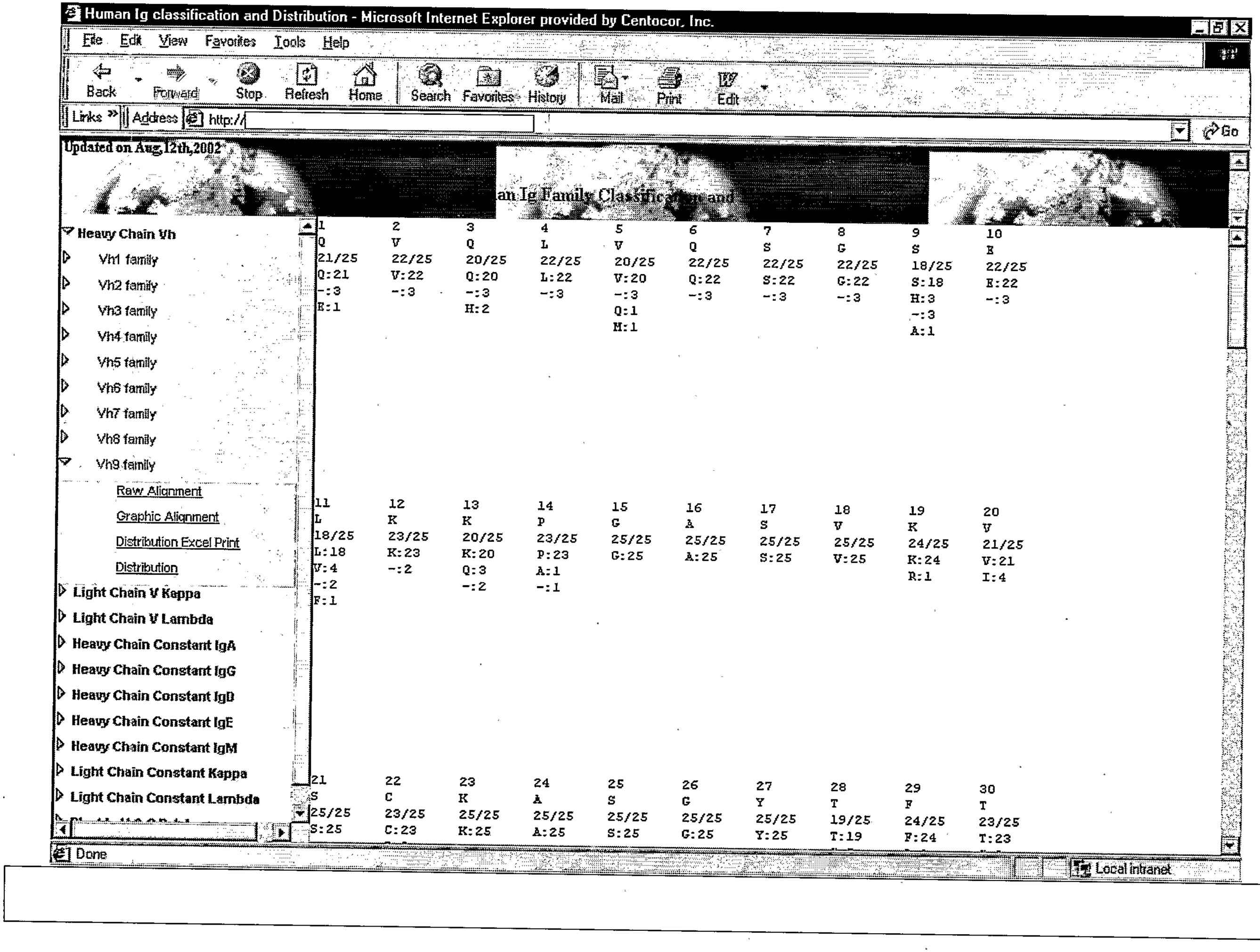
Local intranet

Fig. 10



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