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(54) **Titre : PROCEDE D'EVALUATION D'UN IMMUNOREPERTOIRE**
(54) **Title: METHOD FOR EVALUATING AN IMMUNOREPERTOIRE**

(57) **Abrégé/Abstract:**
Disclosed is a method for amplifying RNA from T and B-cell populations and using the amplified RNA products to evaluate the possible correlation between a normal or abnormal immune response and the development of a disease such as an autoimmune disease, cancer, diabetes, or heart disease.

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(54) **Title**: METHOD FOR EVALUATING AN IMMUNOREPERTOIRE

(57) **Abstract**: Disclosed is a method for amplifying RNA from T and B-cell populations and using the amplified RNA products to evaluate the possible correlation between a normal or abnormal immune response and the development of a disease such as an autoimmune disease, cancer, diabetes, or heart disease.

METHOD FOR EVALUATING AN IMMUNOREPERTOIRE

[0001]

CROSS REFERENCE TO RELATED APPLICATION

[0002] This application claims priority to U.S. Provisional Application No. 61/763,451, entitled "Method for Evaluating an Immunorepertoire" and filed on February 11, 2013.

FIELD OF THE INVENTION

[0003] The invention relates to methods for identifying T-cell receptor antibody in a population of cells and methods for using that information to measure immune status of a patient and predict the likelihood of which disease the patient might have.

BACKGROUND OF THE INVENTION

[0004] Scientists have known for a number of years that certain diseases are associated with particular genes or genetic mutations. Genetic causation, however, accounts for only a portion of the diseases diagnosed in humans. Many diseases appear to be linked in some way to the immune system's response to infectious and environmental agents, but how the immune system plays a role in diseases such as cancer, Alzheimer's, costochondritis, fibromyalgia, lupus, and other diseases is still being determined.

[0005] The human genome comprises a total number of 567-588 IG (immunoglobulin) and TR (T cell receptor) genes (339-354 IG and 228-234 TR) per haploid genome, localized in the 7 major loci. They comprise 405-418 V, 32 D, 105-109 J and 25-29 C genes. The number of functional IG and TR genes is 321-353 per haploid genome. They comprise 187-216 V, 28 D, 86-88 J and 20-21 C genes (http://imgt.cines.fr). Through rearrangement of these genes, an estimated 2.5x10^7 possible antibodies or T cell receptors can be generated.

[0006] A few diseases to date have been associated with the body's reaction to a common antigen (Prinz, J. et al., Eur. J. Immunol. (1999) 29(10): 3360-3368, "Selection of Conserved TCR VDJ Rearrangements in Chronic Psoriatic Plaques Indicates a Common Antigen in Psoriasis Vulgaris) and/or to specific VDJ rearrangements (Tamaru, J. et al., Blood (1994) 84(3): 708-715, "Hodgkin's Disease with a B-cell Phenotype Often Shows a VDJ Rearrangement and Somatic Mutations in the VH Genes). What is needed is a better method for evaluating changes in human immune response cells and associating those changes with specific diseases.

SUMMARY OF THE INVENTION

[0007] The invention relates to a method for evaluating changes in immune response cell populations and associating those changes with a specific disease. In one aspect of the invention, the method comprises the steps of (a) isolating a subpopulation of white blood cells from at least one human or animal subject, (b) isolating RNA from the subpopulation of cells, (c) amplifying the RNA using RT-PCR in a first amplification reaction to produce amplicons using nested primers, at least a portion of the nested primers comprising additional nucleotides to incorporate into a resulting amplicon a binding site for a communal primer, (d) separating the amplicons from the first amplification reaction from one or more unused primers from the first amplification reaction, (e) amplifying, by the addition of communal primers in a second amplification reaction, the amplicons of the first amplification reaction having at least one binding site for a communal primer, and (f) sequencing the amplicons of the second amplification reaction to identify antibody and/or receptor rearrangements in the subpopulation of cells. In one embodiment, the subpopulation may comprise a whole blood population or another mixed population sample.

[0008] In one embodiment, the step of isolating a subpopulation of white blood cells may be performed by flow cytometry to separate naïve B cells, mature B cells, memory B cells, naïve T cells, mature T cells, and memory T cells. In various embodiments of the method, the recombinations in the subpopulation of cells are rearrangements of B-cell immunoglobulin heavy chain (IgH), kappa and/or lambda light chains (IgK, IgL), T-cell receptor Alpha, Beta, Gamma, Delta. In an additional embodiment,

[0009] In another aspect of the invention, the method may optionally comprise an additional step comprising (g) comparing the rearrangements identified for a population of individuals to whom a vaccine has been administered with the rearrangements identified for a population of individuals to whom the vaccine was not administered to evaluate the efficacy of the vaccine in producing an immune response.

[0010] The method may also optionally comprise the additional step of (g) comparing the rearrangements identified for a population of normal individuals with the rearrangements identified for a population of individuals who have been diagnosed with a disease to determine if there is a correlation between a specific rearrangement or set of rearrangements and the disease.

[0011] In various aspects, the method can produce semi-quantitative amplification of polynucleotides comprising complementarity determining region 3 (CDR3s), which result from genetic rearrangements within T or B cells and are responsible for the affinity and specificity of antibodies and/or T cell receptors for specific antigens. Semi-quantitative amplification provides a method to not only detect the presence of specific CDR3 sequences, but also determine the relative abundance of cells which have produced the necessary recombination events to produce those CDR3 sequences.

[0012] One aspect of the invention therefore relates to a method for analyzing semi-quantitative sequence information to provide one or more immune status reports for a human or animal. The method for producing an immune status report comprising the steps of (a) identifying one or more distinct CDR3 sequences that are shared between a subject's immunoprofile and a cumulative immunoprofile from a disease library stored in a database, summing a total number of a subject's detected sequences corresponding to those shared distinct CDR3 sequences, and computing the percentage of the total number of detected sequences in the subject's immunoprofile that are representative of those distinct CDR3s shared between the subject's immunoprofile and the disease library to create one or more original sharing indices; (b) randomly selecting sequences from a public library stored in a database to form a sub-library, the sub-library comprising a number of sequences that is approximately equal to the number of distinct CDR3 sequences in the disease library, identifying one or more distinct CDR3 sequences that are shared between the subject's immunoprofile and the sub-library, summing a total number of detected sequences corresponding to those shared CDR3 sequences, and calculating a percentage of the total number of detected sequences in the subject's immunoprofile that are shared between the subject's immunoprofile and the sub-library to create a sampling sharing index; (c) repeating step (b) at least 1000 or more times; and (d) estimating the P-value as the fraction of times the sampling sharing indices are greater than or equal to the original sharing index between a patient's immunoprofile and a disease library.

[0012a] In an embodiment, there is provided a computer-implemented method for analyzing semi-quantitative sequence information to identify and characterize the immunoprofile of a human or animal subject as normal or as being likely to indicate the presence of a disease, the method comprising the steps of: (a) identifying one or more distinct complementary determining region 3 (CDR3) sequences that are shared between the subject’s immunoprofile and a cumulative immunoprofile from a disease library representing a specific

disease stored in a database; (b) summing a total number of the subject's detected sequences corresponding to those shared distinct CDR3 sequences; (c) computing the percentage of the total number of detected sequences in the subject's immunoprofile that are representative of those distinct CDR3s shared between the subject's immunoprofile and the disease library to create one or more original sharing indices; (d) randomly selecting sequences from a public library stored in a database to form a sub-library, the sub-library comprising a number of distinct CDR3 sequences that is approximately equal to the number of distinct CDR3 sequences in the disease library; (e) identifying one or more distinct CDR3 sequences that are shared between the subject's immunoprofile and the sub-library; (f) summing a total number of detected sequences corresponding to those shared CDR3 sequences and calculating a percentage of the total number of detected sequences in the subject's immunoprofile that are shared between the subject's immunoprofile and the sub-library to create a sampling sharing index; (g) repeating steps (d)-(f) for a number of times sufficient to produce a result that is statistically significant for identifying and characterizing the immunoprofile of said subject as normal or as being likely to indicate the presence of a disease; (h) estimating the P-value as the fraction of times the sampling sharing indices are greater than or equal to the original sharing index between the immunoprofile of said subject and the disease library; and (i) characterizing the immunoprofile of the subject as normal if the estimated P-value is greater than 0.01, or characterizing the immunoprofile of the subject as being likely to indicate the presence of the disease represented by the disease library if the estimated P-value is less than 0.01.

[0012b] In an embodiment, there is provided a computer readable medium having recorded thereon computer executable instructions that when executed by a computer perform the method as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The disclosure can be better understood with reference to the following drawings. The elements of the drawings are not necessarily to scale relative to each other, emphasis instead being placed upon clearly illustrating the principles of the disclosure. Furthermore, like reference numerals designate corresponding parts throughout the several views.

[0014] Figure 1a and Figure 1b are photographs of a gel illustrating the presence of amplification products obtained by the method of the invention using primers disclosed herein.

[0015] Figure 2a and Figure 2b are cartoons representing the observed difference in diversity between an immunoprofile in an individual with a disease and an individual who is generally healthy, with each filled circle representing a distinct CDR3 sequence and the size of the circle representing the number of times that the distinct CDR3 sequence is found in the immunoprofile.

[0016] Figure 3 is a diagram illustrating the method for generating a public library.

[0017] Figure 4 is a diagram illustrating the method for generating a disease library.

[0018] Figure 5 illustrates results obtained by comparing a patient immunoprofile with a disease library, calculating a percentage for each distinct CDR3 in the patient immunoprofile that is shared between the two, and adding those percentages to produce a sum, or sharing index.

[0019] Figure 6 illustrates results obtained by comparing a patient immunoprofile with a subset of a public library, calculating a percentage for each distinct CDR3 that is shared between the two, and adding those percentages in the patient immunoprofile to produce a sum, or sharing index.

[0020] Figure 7 is a graph illustrating the method of the invention, where the area under the curve represents total sharing indices obtained for subsets of a public library (sub-libraries), a P-value is estimated, and sharing indices for comparisons of an individual's immunoprofile and one or more disease libraries are represented by vertical lines (DL_1, DL_2, etc.).

DETAILED DESCRIPTION

[0021] The inventors have developed methods for evaluating antibody and T cell receptor rearrangements from a large number of cells, the methods being useful for comparing rearrangements identified in populations of individuals to determine whether there is a correlation between a specific rearrangement or set of rearrangements and a disease, or certain symptoms of a disease. The method is also useful for establishing a history of the immune response of an individual or individuals in response to infectious and/or environmental agents, as well as for evaluating the efficacy of vaccines.

[0022] The invention relates to a method for evaluating changes in immune response cell populations and associating those changes with a specific disease. In one aspect of the invention, the method comprises the steps of (a) isolating a subpopulation of white blood cells from at least one human or animal subject, (b) isolating RNA from the subpopulation of cells, (c) amplifying the RNA using RT-PCR in a first amplification reaction to produce amplicons using nested primers, at least a portion of the nested primers comprising additional nucleotides to incorporate into a resulting amplicon a binding site for a communal primer, (d) separating the amplicons from the first amplification reaction from one or more unused primers from the first amplification reaction, (e) amplifying, by the addition of communal primers in a second amplification reaction, the amplicons of the first amplification reaction having at least one binding site for a communal primer, and (f) sequencing the amplicons of the second amplification reaction to identify antibody and/or receptor rearrangements in the subpopulation of cells. In one embodiment, the subpopulation may comprise a whole blood population or another mixed population sample.

[0023] In one embodiment, a peripheral blood sample is taken from a patient and the step of isolating a subpopulation of white blood cells may be performed by flow cytometry to separate naïve B cells, mature B cells, memory B cells, naïve T cells, mature T cells, and memory T cells. In various embodiments of the method, the recombinations in the subpopulation of cells are rearrangements of B-cell immunoglobulin heavy chain (IgH), kappa and/or lambda light chains (IgK, IgL), T-cell receptor Beta, Gamma, or Delta.

[0024] In a second aspect of the invention, the method may comprise an additional step (g) comparing the rearrangements identified for a population of normal individuals with the rearrangements identified for a population of individuals who have been diagnosed with a disease to determine if there is a correlation between a specific rearrangement or set of rearrangements and the disease.

[0025] In another aspect of the invention, the method may comprise an additional step comprising (g) comparing the rearrangements identified for a population of individuals to whom a vaccine has been administered with the rearrangements identified for a population of individuals to whom the vaccine was not administered to evaluate the efficacy of the vaccine in producing an immune response.

[0026] In some embodiments, the step of separating the amplicons from the first amplification reaction from one or more unused primers from the first amplification reaction may be omitted and the two amplification reactions may be performed in the same reaction tube.

[0027] The inventor previously developed a PCR method known as tem-PCR, which has been described in publication number WO2005/038039. More recently, the inventor has developed a method called arm-PCR, which was described in U.S. provisional patent application number 61/042,259. Also described is an apparatus for detecting target polynucleotides in a sample, the apparatus comprising a first amplification chamber for thermocycling to amplify one or more target polynucleotides to produce amplicons using nested primers, at least a portion of the nested primers comprising additional nucleotides to incorporate into a resulting amplicon a binding site for a communal primer; a means for separating the amplicons from the first amplification reaction from one or more unused primers from the first amplification reaction; and a second amplification chamber for thermocycling to amplify one or more amplicons produced during the first amplification reaction by the addition of communal primers in a second amplification reaction, the amplicons of the first amplification reaction having at least one binding site for at least one communal primer.

[0028] Also described is a PCR chip comprising a first PCR chamber fluidly connected to both a waste reservoir and a second PCR chamber, the waste reservoir and second PCR chamber each additionally comprising at least one electrode, the electrodes comprising a means for separating amplicons produced from the first PCR chamber. The second PCR chamber is fluidly connected to a hybridization and detection chamber, the hybridization and detection chamber comprising microspheres, or beads, arranged so that the physical position of the beads is an indication of a specific target polynucleotide's presence in the sampled analyzed by means of the chip.

[0029] The tem-PCR, and especially the arm-PCR, methods provide semi-quantitative amplification of multiple polynucleotides in one reaction. Additionally, arm-PCR provides added sensitivity. Both provide the ability to amplify multiple polynucleotides in one reaction, which is beneficial in the present method because the repertoire of various T and B cells, for example, is so large. The addition of a communal primer binding site in the amplification reaction, and the subsequent amplification of target molecules using communal primers, gives a quantitative, or semi-quantitative result—making it possible to determine the relative amounts of the cells comprising various rearrangements within a patient blood sample. Clonal expansion due to recognition of antigen results in a larger population of cells which recognize that antigen, and evaluating cells by their relative numbers provides a method for determining whether an antigen exposure has influenced expansion of antibody-producing B cells or receptor-bearing T cells. This is helpful for evaluating whether there may be a particular population of cells that is prevalent in individuals who have been diagnosed with a particular disease, for example, and may be especially helpful in evaluating whether or not a vaccine has achieved the desired immune response in individuals to whom the vaccine has been given.

[0030] There are several commercially available high throughput sequencing technologies, such as Roche Life Sciences's 454 sequencing. In the 454 sequencing method, 454A and 454B primers are linked onto PCR products either during PCR or ligated on after the PCR reaction. When done in conjunction with tem-PCR or arm-PCR, 454A and 454B primers may be used as communal primers in the amplification reactions. PCR products, usually a mixture of different sequences, are diluted to about 200 copies per µl. In an "emulsion PCR" reaction, (a semisolid gel like environment) the diluted PCR products are amplified by primers (454A or 454B) on the surface of the microbeads. Because the PCR templates are so dilute, usually only one bead is adjacent to one template, and confined in the semisolid environment, amplification only occurs on and around the beads. The beads are then eluted and put onto a plate with specially designed wells. Each well can only hold one bead. Reagents are then added into the wells to carry out pyrosequencing. A fiber-optic detector may be used to read the sequencing reaction from each well and the data is collected in parallel by a computer. One such high throughput reaction could generate up to 60 million reads (60 million beads) and each read can generate about 300bp sequences.

[0031] One aspect of the invention involves the development of a database of "personal immunorepertoires," or immunoprofiles, so that each individual may establish a baseline and follow the development of immune responses to antigens, both known and unknown, over a period of years. This information may, if information is gathered from a large number of individuals, provide an epidemiological database that will produce valuable information, particularly in regard to the development of those diseases, such as cancer and heart disease, which are thought to often arise from exposure to viral or other infectious agents or transformed cells, many of which have as yet been unidentified. One particularly important use for the method of the invention involves the evaluation of children to determine whether infectious disease, environmental agents, or vaccines may be the cause of autism. For example, many have postulated that vaccine administration may trigger the development of autism. However, many also attribute that potential correlation to the use of agents such as thimerosol in the vaccine, and studies have demonstrated that thimerosol does not appear to be a causative agent of the disease. There is still speculation that the development of cocktail vaccines has correlated with the rise in the number of cases of autism, however, gathering data to evaluate a potential causal connection for multiple antigens is extremely difficult. The method of the present invention simplifies that process and may provide key information for a better understanding of autism and other diseases in which the immune response of different individuals may provide an explanation for the differential development of disease in some individuals exposed to an agent or a group of agents, while others similarly exposed do not develop the disease.

[0032] Imbalances of the immunoprofile, triggered by infection, may lead to many diseases, including cancers, leukemia, neuronal diseases (Alzheimer's, Multiple Sclerosis, Parkinson's, autism etc.), autoimmune diseases, and metabolic diseases. These diseases may be called immunoprofile diseases. There may be two immunoprofile disease forms. (1) a “loss of function” form, and (2) a “gain of function” form. In the “loss of function” form, a person is susceptible to a disease because his/her restricted and/or limited immunoprofile lacks the cells that produce the most efficient and necessary IGs and TRs. In the “gain of function” form, a person is susceptible to a disease because his/her immunoprofile gained cells that produce IGs and TRs that normally should not be there. In the “loss of a function” (LOF) immunoprofile diseases, an individual does not have the appropriate functional B or T cells to fight a disease. His/her HLA typing has determined that those cells are eliminated during the early stages of the immune cell maturation process, the cells generally being eliminated because they react to strongly to his/her own proteins.

[0033] One aspect of the invention also provides a method comprising (a) amplifying and sequencing one or more RNAs from the T cells and/or B cells from one or more individuals, (b) inputting the sequences into a database to provide data which may be stored on a computer, server, or other electronic storage device, (c) inputting identifying information and characteristics for an individual corresponding to the sequences of the one or more RNAs as data which may also be stored on a computer, server, or other electronic storage device, and (d) evaluating the data of step (b) and step (e) for one or more individuals to determine whether a correlation exists between the one or more RNA sequences and one or more characteristics of the individual corresponding to the sequence(s). Identifying information may include, for example, a patient identification number, a code comprising the patient's HLA type, a disease code comprising one or more clinical diagnoses that may have been made, a "staging code" comprising the date of the sample, a cell type code comprising the type of cell subpopulation from which the RNA was amplified and sequenced, and one or more sequence codes comprising the sequences identified for the sample.

[0034] The described method includes a novel primer design that not only allows amplification of the entire immunorepertoire, but also allows amplification in a highly multiplex fashion and semiquantitatively. Multiplex amplification requires that only a few PCR or RT-PCR reactions will be needed. For example, all IGs may be amplified in one reaction, or it could be divided into two or three reactions for IgH, IgL or IgK. Similarly, the T-cell receptors (TRs) may be amplified in just one reaction, or may be amplified in a few reactions including TRA, TRB, TRD, and TRG. Semi-quantitative amplification means that all the targets in the multiplex reaction will be amplified independently, so that the end point analysis of the amplified products will reflect the original internal ratio among the targets.

[0035] In various aspects, the method can produce semi-quantitative amplification of polynucleotides comprising complementarity determining regions (CDRs), which result from genetic rearrangements within T or B cells and are responsible for the affinity and specificity of antibodies and/or T cell receptors for specific antigens. Semi-quantitative amplification provides a method to not only detect the presence of specific CDR3 sequences, but also determine the relative numbers of cells which have produced the necessary recombination events to produce those CDR3 sequences.

[0036] One aspect of the invention therefore relates to a method for analyzing semi-quantitative sequence information to provide one or more immune status reports for a human or animal. The method for producing an immune status report comprising the steps of (a) identifying one or more distinct CDR3 sequences that are shared between a subject's immunoprofile and a disease library stored in a database, summing the total of those shared CDR3 sequences and computing the percentage of the total number of sequences in the subject's immunoprofile that are shared between the subject's immunoprofile and the disease library to create one or more original sharing indices; (b) randomly selecting sequences from a public library stored in a database to form a sub-library, the sub-library comprising a number of sequences that is approximately equal to the number of distinct sequences in the disease library, identifying one or more distinct CDR3 sequences that are shared between the subject's immunoprofile and the sub-library, summing the total of those shared CDR3 sequences and calculating the percentage of the total number of sequences in the subject's immunoprofile that are shared between the subject's immunoprofile and the sub-library to create a sampling sharing index; (c) repeating step (b) at least 1000 or more times; and (d) estimating the P-value as the fraction of times the sampling sharing indices are greater than or equal to the original sharing index between a patient's immunoprofile and a disease library.

[0037] The inventors have discovered that the immunoprofile of individuals who have certain diseases, such as, for example, cancer, autoimmune disease, etc., may be characterized by a lack of diversity in one or more immune cell population(s). Figure 1 is a cartoon illustrating the difference that may be observed between, for example, the distinct type and number of T-cells present in a blood sample from a cancer patient (Fig. 1a) and a healthy patient (Fig. 1b), where each circle represents a distinct type of T-cell, as represented by an amplified and sequenced recombined cDNA of the complementarity determining region of the T-cell receptor (e.g., CDR3), and the relative number of cells which are determined, by PCR amplification and sequencing, to share the same CDR3 sequence. As Fig. 1a indicates, there may be fewer distinct cells of different specificities, but larger numbers of cells of certain specificities, as represented by the CDR3 sequences. Fig. 1b illustrates a normal profile of more different cells, but fewer numbers of each type of cell sharing the same CDR3 sequence.

[0038] The list of each distinct CDR3-expressing cell, and the numbers of such cells represented within a blood or tissue sample from a human or animal, can constitute an immunoprofile for that human or animal. Compiling the immunoprofiles from a group of humans, for example, the group comprising both healthy individuals and individuals with various different diseases may provide a “public library” that is representative of the type of diversity found in a normal population (Fig. 2). Similarly, compiling the immunoprofiles of a group of individuals who have been clinically diagnosed with a particular disease may provide a “disease library” that is representative of the lack of diversity, the specific CDR3s of the expanded populations of cells, etc. (Fig. 3). These immunoprofiles may be stored in a database, accessible via computer access to the internet, for example, so that the information may be used in the method of the invention to analyze the immune status of a patient.

[0039] An immunoprofile, comprising a listing of distinct CDR3-expressing cells (“distinct CDR3s”, those cells sharing a unique CDR3 sequence) and the numbers of each distinct CDR3 present in a blood or tissue sample from an individual may be produced for an individual patient. The patient's immunoprofile is compared to the combined immunoprofiles of a group of patients who have been diagnosed with a particular disease (a disease library, stored in a database). This can be done for a series of disease libraries, and shown in Fig. 4.

[0040] Millions of possible combinations are possible for the public library, the immune systems of most of those individuals generally exhibiting increased diversity over that of a group of individuals who have been diagnosed with a specific disease. Therefore, the inventors determined that an accurate assessment and comparison for the method of the invention would be facilitated by the step of preparing sub-libraries by randomly sampling/selecting from the lists of distinct CDR3s and their numbers in the public library. The number of distinct CDR3s, represented by unique peptide sequence of CDR3 fragments, should be approximately equal to the number of distinct CDR3s identified in the disease library, or an average calculated from more than one disease library. Producing a significant number of sub-libraries, such as, for example, 1000 or more sub-libraries, produced by randomly sampling from the public library, increases the presence of a variety of distinct CDR3s and produces a result that is statistically significant effective for identifying and characterizing an individual patient's immunoprofile as normal ("healthy") or characterized by the presence of a type and number of cells that have been associated with a particular disease.

[0041] In the method of the invention, a patient supplies a clinical sample comprising, for example, blood or tissue, from which distinct CDR3s are semi-quantitatively amplified and sequenced. This provides the identity and the relative abundance of each CDR3 for all distinct CDR3s. This information may be entered into a program which accesses a database containing at least one public library and one or more disease libraries. Software used for data entry and/or analysis may be accessed via internet access to the database, or may be located on an individual personal computer, with internet access to the sequence information in the database. Comparisons are obtained between the individual immunoprofile and the various libraries and sub-libraries, and results are generated as generally illustrated in Fig. 4 and Fig. 5, where specific CDR3 sequences are detected, the numbers of those distinct CDR3 sequences detected are counted, and a determination is made as to whether or not that specific distinct CDR3 is present in both the individual's immunoprofile and a specific library (i.e., that specific distinct CDR3 is "shared" between the individual and the library). The percentages representing numbers of those CDR3s that are determined to be shared are added together to produce a sum comprising the fraction of the total that comprises CDR3s in the individual's immunoprofile shared between the individual's immunoprofile and the specific library (i.e., a "sharing index"). From the results obtained for the sub-libraries, a P-value is calculated as the probability that a random percentage would be greater than or equal to the percentage noted for a particular disease library, and a significant result is noted when the fraction of times the sampling sharing indices exceeds the original sharing index for a particular library is less than 0.01, for instance. If that sharing index represents the relationship between the individual's immunoprofile and a disease library, the individual may then be informed of the likelihood that the individual/patient has the disease represented by the specific disease library. If P-values computed against all disease libraries is greater than 0.01, the individual's report may indicate that the immune profile looks normal and the disease state has not been detected.

[0042] As sequence data is compiled and stored in one or more databases for multiple populations of individuals, it may additionally be possible to associate certain sharing indexes with libraries representing populations with pre-conditions or predispositions to certain diseases. The immune system is both proactive and reactive, and changes in the immune system, reflected in the immunoprofile, may provide the first—and sometimes the only—signal that a predisposition, a precondition, or even an established disease is present. The inventors have utilized the method to demonstrate that certain types of cancers, inflammatory bowel disease, and certain viral infections may be detected by determining the sharing index between a patient and an established disease library, obtained by sequencing CDR3s using the ARM-PCR method to produce a subset of the immunorepertoire representing the CDR3s present.

[0043] The results are even more reliable when a filter is applied to the sequence data. For example, the inventors have developed a “SMART” filter for the sequence data that aids in the generation of significantly more reliable results. This is described further in the Examples.

[0044] By way of further explanation, the following example may be illustrative of the methods of the invention. Blood samples may be taken from children prior to administration of any vaccines, those blood samples for each child establishing a “baseline” from which future samples may be evaluated. For each child, the future samples may be utilized to determine whether there has been an exposure to an agent which has expanded a population of cells known to be correlated with a disease, and this may serve as a “marker” for the risk of development of the disease in the future. Individuals so identified may then be more closely monitored so that early detection is possible, and any available treatment options may be provided at an earlier stage in the disease process.

[0045] By means of providing another example, blood samples may be taken from children prior to administration of any vaccines, those blood samples from each child establishing a “baseline” from which future samples may be evaluated. For each child and for the entire population of children in the study, those baselines may be compared to the results of RNA sequencing of T and B cells using target-specific primers to amplify antibody and T-cell receptor, after vaccine administration. The comparison may further involve the evaluation of data regarding symptoms, diagnosed diseases, and other information associated for each individual with the corresponding antibody, and T-cell receptor sequences. If a relationship exists between the administration of a vaccine and the development of a particular disease, individuals who exhibit symptoms of that disease may also share a corresponding antibody or T-cell receptor, for example, or a set of corresponding antibodies or T-cell receptors.

[0046] The method of the invention may be especially useful for identifying commonalities between individuals with autoimmune diseases, for example, and may provide epidemiological data that will better describe the correlation between infectious and environmental factors and diseases such as heart disease, atherosclerosis, diabetes, and cancer—providing "biomarkers" that signal either the presence of a disease, or the tendency to develop disease.

[0047] The method may also be useful for development passive immunity therapies. For example, following exposure to an infectious agent, certain antibody-producing B cells and/or T cells are expanded. The method of the invention enables the identification of protective antibodies, for example, and those antibodies may be utilized to provide passive immunity therapies in situations where such therapy is needed.

[0048] The method of the invention may also provide the ability to accomplish targeted removal of cells with undesirable rearrangements, the method providing a means by which such cells rearrangements may be identified.

[0049] The inventor has identified and developed target-specific primers for use in the method of the invention. T-cell-specific primers are shown in Table 1, and antibody-specific primers are shown in Table 2. An additional embodiment of the invention is a method of using any one or a combination of primers of Table 1 or Table 2, to amplify RNA from a blood sample, and more particularly to identify antibodies, T-cell receptors, and HLA molecules within a population of cells.

[0050] Arm-PCR or tem-PCR may be used to amplify genes coding for the immunoglobulin superfamily molecules in am amplification method described previously by the inventor (Han et al., 2006. Simultaneous Amplification and Identification of 25 Human Papillomavirus Types with Templex Technology. J. Clin. Micro. 44(11). 4157-4162). In a tem-PCR reaction, nested gene-specific primers are designed to enrich the targets during initial PCR cycling. Later, universal "Super" primers are used to amplify all targets. Primers are designated as F_o (forward out), F_i (forward in), R_i (reverse in), R_o (reverse out), FS (forward super primer) and RS,(reverse super primer), with super primers being common to a variety of the molecules due to the addition of a binding site for those primers at the end of a target-specific primer. The gene-specific primers (F_o, F_i, R_i, and R_o) are used at extremely low concentrations. Different primers are involved in the tem-PCR process at each of the three major stages. First, at the “enrichment” stage, low-concentration gene-specific primers are given enough time to find the templates. For each intended target, depending on which primers are used, four possible products may be generated: F_o/R_o, F_i/R_o, F_i/R_i, and F_o/R_i. The enrichment stage is typically carried out for 10 cycles. In the second, or “tagging” stage, the annealing temperature is raised to 72°C, and only the long 40-nucleotide inside primers (F_i and R_i) will work. After 10 cycles of this tagging stage, all PCR products are “tagged” with the universal super primer sequences. Then, at the third “amplification” stage, high-concentration super primers work efficiently to amplify all targets and label the PCR products with biotin during the process. Specific probes may be covalently linked with Luminex color-coated beads.

[0051] To amplify the genes coding for immunoglobulin superfamily molecules, the inventor designed nested primers based on sequence information in the public domain. For studying B and T cell VDJ rearrangement, the inventor designed primers to amplify rearranged and expressed RNAs. Generally, a pair of nested forward primers is designed from the V genes and a set of reverse nested primers are designed from the J or C genes. The average amplicon size is 250-350bp. For the IgHV genes, for example, there are 123 genes that can be classified into 7 different families, and the present primers are designed to be family specific. However, if sequencing the amplified cDNA sequences, there are enough sequence diversities to allow further differentiation among the gene within the same family. For the MHC gene locus, the intent is to amplify genomic DNA.

EXAMPLES

Calculation of Sharing Index

[0052] Assuming that **S** is a subject's immunoprofile (IP), which is represented by **N** unique CDR3 sequences $CDR3_1$, $CDR3_2$, ... $CDR3_n$, each CDR3 has its own frequency $\mathbf{s}_1$, $\mathbf{s}_2$, ... $\mathbf{s}_n$.

[0053] **D** is a disease library, which is the sum of a certain number of patients' immunoprofile with **M** unique CDR3s. All patients in the disease library were diagnosed to have the same disease.

[0054] **P** is a public library, which is the sum of a large number of control's immunoprofile.

[0055] The **S**haring **I**ndex is defined as the sum of $\mathbf{s}_x$, $\mathbf{s}_y$, ... $\mathbf{s}_z$, where $CDR3_x$, $CDR3_y$, ... $CDR3_z$ are shared in the subject's immunoprofile and a library. Note that $\mathbf{s}_x$, $\mathbf{s}_y$, ... $\mathbf{s}_z$ is the frequency of CDR3s in the subject's immunoprofile, not in the library.

[0056] Assuming that there are always more unique CDR3s in a public library (P) than in a disease library (D), **M** unique CDR3s in the public library are randomly selected and used to create a sub-library P1 and the sharing index ($\mathbf{SI}_{p1}$) between the subject and the sub-library computed according to above formula. The sampling procedure is repeated 1000 or more times and 1000 or more $\mathbf{SI}_{px}$ are computed.

[0057] The sharing index $\mathbf{SI}_d$ between the subject and the disease library are computed in the same manner. The **P-value** is defined as the fraction of all **SI**s ($\mathbf{SI}_{p1}$, $\mathbf{SI}_{p2}$, ... $\mathbf{SI}_{px}$, $\mathbf{SI}_d$, (Note that $\mathbf{SI}_d$ is included), which is equal to or greater than $\mathbf{SI}_d$. Note that, when sampling CDR3s in the public library, CDR3s found in **x** control's immunoprofiles are given **x** times of chances to be sampled.

Amplification of T or B Cell Rearrangement Sites

[0058] All oligos were resuspended using 1x TE. All oligos except 454A and 454B were resuspended to a concentration of 100pmol/µL. 454A and 454B were resuspended to a concentration of 1000pmol/µL 454A and 454B are functionally the same as the communal primers described previously, the different sequences were used for follow up high throughput sequencing procedures.

[0059] Three different primer mixes were made. An Alpha Delta primer mix included 82 primers (all of TRAV-C + TRDV-C), a Beta Gamma primer mix included 79 primers (all of TRBVC and TRGV-C) and a B cell primer mix that included a total of 70 primers. F_o, F_i, and R_i primers were at a concentration of 1pmol/µL. R_o primers were at a concentration of 5 pmol/µL. 454A and 454B were at a concentration of 30 pmol/µL.

[0060] Three different RNA samples were ordered from ALLCELLS (www.allcells.com). All samples were diluted down to a final concentration of 4 ng/uL. The samples ordered were:

Cell type:	**Source:**
ALL-PB-MNC	A patient with acute lymphoblastic leukemia
NPB-Pan T Cells	Normal T cells
NPB-B Cells	Normal B cells

[0061] RT-PCR was performed using a Qiagen One-Step RT-PCR kit. Each sample contained the following:

10 µL of Qiagen Buffer
2 µL of DNTP's
2 µl of Enzyme
23.5 µL of dH_2O
10 µL of the appropriate primer mix
2.5 µL of the appropriate template (10ng of RNA total)

The samples were run using the following cycling conditions:

50°C for 30 minutes

95°C for 15 minutes
94°C for 30 seconds
15 cycles of
55°C for 1 minute
72°C for 1 minute
94°C for 15 seconds
6 cycles of
70°C for 1 minute 30 seconds
94°C for 15 seconds
30 cycles of
55°C for 15 seconds
72°C for 15 seconds
72°C for 3 minutes
4°C Hold

[0062] The order of samples placed in the gel shown in Fig. 1a was: (1) Ladder (500bp being the largest working down in steps of 20bp, the middle bright band in Fig. 1a is 200bp); (2) α + δ primer mix with 10ng Pan T Cells Template; (3) β + γ primer mix with 10ng Pan T Cells Template; (4) B Cell primer mix with 10ng B Cells Template; (5) B Cell primer mix with 10ng ALL Cells Template; (6) α + δ primer mix with 10ng ALL Cells Template; (7) β + γ primer mix with 10ng ALL Cells Template; 8. α + δ primer mix blank; (9) β + γ primer mix blank; (10) B Cell primer mix blank; (11)Running buffer blank. These samples were run on a pre-cast ClearPAGE® SDS 10% gel using 1X ClearPAGE® DNA native running buffer.

[0063] The initial experiment showed that a smear is generated from PCR reactions where templates were included. The smears indicate different sizes of PCR products were generated that represented a mixture of different VDJ rearrangements. There is some background amplification from the B cell reaction. Further improvement on that primer mix was required to clean up the reaction.

[0064] To determine whether the PCR products indeed include different VDJ rearrangements, it was necessary to isolate and sequence the single clones. Instead of using the routine cloning procedures, the inventor used a different strategy. PCR products generated from the Alpha Delta mix and the Beta Gamma mix (lanes 2 and 3 in Fig. 1a) were diluted 1:1000 and a 2 µl aliquot used as PCR template in the following reaction. Then, instead of using a mixture of primers that targeting the entire repertoire, one pair of specific Fi and Ri primers were used (5 pmol each) to amplify only one specific PCR product. The following cycling conditions were used to amplify the samples:

95°C for 5 minutes
30 cycles of
94°C for 30 seconds
72°C for 1 minute
72°C for 3 minutes
4°C hold

[0065] A Qiagen PCR kit was used to amplify the products. The Master Mix used for the PCR contained the following:

	Per Reaction	Master Mix x 12
10x PCR Buffer	5μL	60μL
dNTP	1μL	12μL
HotStartTaq Plus	0.25μL	3μL
H_2O	39.75 μL	477 μL

[0066] The photograph of the gel in Fig. 1b shows the PCR products of the following reactions: (1) Ladder; (2) TRAV1Fi+TRACRi with alpha delta Pan T PCR product; (3) TRAV2Fi+TRACRi with alpha delta Pan T PCR product; (4) TRAV3F$_i$+TRACR$_i$ with alpha delta Pan T PCR product; (5) TRAV4F$_i$+TRACR$_i$ with alpha delta Pan T PCR product; (6) TRAV5F$_i$+TRACR$_i$ with alpha delta Pan T PCR product; (7) TRAV1F$_i$+TRACR$_i$ with alpha delta Pan T PCR product; (8) TRAV2F$_i$+TRACR$_i$ with alpha delta Pan T PCR product; (9) TRAV3F$_i$+TRACR$_i$ with alpha delta Pan T PCR product; (10) TRAV4F$_i$+TRACR$_i$ with alpha delta Pan T PCR product; (11) TRAV5F$_i$+TRACR$_i$ with alpha delta Pan T PCR product; (12) PCR Blank. Primers listed as F$_1$ are “forward inner” primers and primers listed as F$_o$ are “forward outer” primers, with R$_i$ and R$_o$ indicating “reverse inner” and “reverse outer” primers, respectively.

[0067] As illustrated by Fig. 1b, a single PCR product was generated from each reaction. Different size bands were generated from different reactions. This PCR cloning approach is successful for two major reasons--(1) The PCR templates used in this reaction were diluted PCR products (1:1000) of previous reactions that used primer mixes to amplify all possible VDJ rearrangements (for example, a primer mix was used that included total of 82 primers to amplify T cell receptor Alpha and Delta genes) and (2) Only one pair of PCR primers, targeting a specific V gene, are used in each reaction during this “cloning” experiment. Some of these products were gel purified and sequenced. The following are example sequences obtained from the protocol described above. In every case, a single clone was obtained, and a specific T cell receptor V gene that matched the Fi primer was identified.

TRAV1 template + 454A as sequencing primer:
NNNNNNNNNNCNTANTCGGTCTAAGGGTACNGNTACCTCCTTTTGAAGGAGCT
CCAGATGAAAGACTCTGCCTCTTACCTCTGTGCTGTGAGAGATANCAACNATCA
CTTAATCTTGGGCGCTGGGAGCAGACTAATTATAATGCCAGATATCCACAACCC
TGACCCTGCCGCGTACCAGCTGAAAGACTATGAACAGGATGGGGAGGCAGNAG
NAGNAG (SEQ ID NO. 1)

TRAV1 template + 454A as sequencing primer:
NNNNNNNNNNGNANGNNCAGGGTTCTGGATATTTGGTTTNACAATTAGCTTGGT
CCCTGCTCCAAAGATTAATTTGTAGTTGCTATCCCTCACAGCACAGAGGTAAGA
GGAAGAGTATTTCTTCTGGAGCTCCTTCAACAGGAGGAAACTGTACCCTTTATA
CCTACTAAGGAATGAAGA (SEQ ID NO. 2)

TRAV2 template + 454A as sequencing primer:
NNNNNNNNNNNNTNNCGGTTCTCTTNNTCGCTGCTCATCCTCCAGGTGCGGGA
GGCAGATGCTGCTGTTTACTACTGTGCTGTGNANNANGGCANNGACAACAACCT
CNTCTTTGGTGGAGGNACCCTACTNNTGGTTATNCCNAATANCCANAACCCTGA
CCCTGCCGAGNAGCAGCANAAAAACTNNNAGGGGGGTGGAGAAGNANNNNN
(SEQ ID NO. 3)

TRAV3 template + 454A as sequencing primer:
NNNNNNNNNNNNNNGGNNNGGNAGCTATGGCTTTGAAGCTGAATTTAACAAGA
GCCAAACCTCCTTCCACCTGAAGAAACCATCTGCCCTTGTGAGCGACTCCGCTT
TGTACTTCTGTGCTGTGAGAGACATCAACGCTGCCGGCAACAACCTAACTTTTG
GAGGAAGAACCATGGTGCTAGTTAAACCAAATATCCATAACCCTGACGCTGCCG
TGTACCAGCTGAAAGACTCTGAGGGGGCTGGAGAGGNAGGNG (SEQ ID NO. 4)

TRAV4 template + 454A as sequencing primer:
NNNNNANNGGNNNNNGTTTATCCCTGCCGACAGAAAGTCCAGCACTCTGAGCC
TGCCCCGGGTTTCCCTGAGCGACACTGCTGTGTACTACTGCCTCGTGGGTGAC
CGGTCTGGAAACAGCGATGAAATTTTCATCTTAGGAAGAAGAACGCTTCTAGTC
ATCCANCCCAACATCCACAACCCTGCCGCGGAGNAGCACCAGAAAAAAGATGA
TGAGGGGGANGNAGNAGNANNNN (SEQ ID NO. 5)

TRAV5 template + 454A as sequencing primer:
NNNNNNNNNNNNNNNNTCNCTGNTCTATTGAATAAAAAGGATAAACATCTGTCT
CTGCGCATTGCAGACACCCAGACTGGGGACTCAGCTATCTACTTCTGTGCAGA
GAGCCCCGGTGGCGGCAGCAACTTCTTCTTTGGTGGAGGAGCANTACTACTAG
TCGTTCTACATANCCACAACCATGATNCCGCCGAGTACNTGCTGAAAAAATATG
ATGAGGATGGAGAAGAAGNAGCATNAN (SEQ ID NO. 6)

TRBV19Fi template + 454A as sequencing primer:
NNNNNNNNCTGAGGGTANNCGTCTCTCGGGAGAAGAAGGAATCCTTTCCTCTC
ACTGTGACATCGGCCCAAAAGAACCCGACAGCTTTCTATCTCTGTGCCAGTAGT
ATGGGGGGGGGGGCCTACAATGAGNACGGCGGCGGGGGAGGGACNNTGCTC
GTCGTGGAGGAGGACATGAAGGTCTTGCCCGCNNCNGAGGAAGNTGNANANG
AACCATAAAAATGCGCTGGCTGAANNN (SEQ ID NO. 7)

TRBV20Fi template + 454A as sequencing primer:
NNNNNNNNNNNGCTCNNNNNNNCNCATACGAGCAAGGCGTCGAGAAGGACAAG
TTTCTCACAACCATGCAAGCCTGACCTTGTCCACTCTGACAGTGACCAGTGCCC
ATCCTGAAGACAGCAGCTTCTACATCTGCAGTGCTAGAGGGGGGGGGGGGGA
CGACTACTACTACTTCGGCGGGGGGGGCATGCTGATCGTGGAGGAGGAGGAC
ATGNAGCTCCTCCCCGCCGCCGAGGTTGTTGTGTNTNNANCATCATACTGNTG
GTGGAGNAGNAGNAGCN (SEQ ID NO. 8)

TRBV21Fi template + 454A as sequencing primer:
NNNNNNNNNNNNNNNGNNNNNNNNNNNNTACTTTCNGAATGAAGAACTTATTCA
GAAAGCAGAAATAATCAATGAGCGATTTTTAGCCCAATGCTCCAAAAACTCATCC
TGTACCTTGGAGTTCCAGTCCACGGAGTCAGGGGACACAGCACTGTATTTCTGT
GCCAGCAGCA (SEQ ID NO. 9)

TRBV23Fi template + 454A as sequencing primer:
NNNGNNNNNNNANNGGANANGCACAAGAAGCGATTCTCATCTCAATGCCCCAA
GAACGCACCCTGCAGCCTGGCAATCCTGTCCTCAGAACCGGGAGACACGGCAC
TGTATCTCTGCGCCAGCAGTCAATCGGGGGGGGGGGGGAGGGCCGTCCGCAG
CGGGGGGGGGGGGGGCCGGGGGACGGTCCCAAAGAGAAAGAAAACCTGCCC
CCCGCGCTCGGGCGGTGTGATTGAGCGAAACAGACAGGAAGGNAAGNAAAAAA
NNNNANCNNCNCTCNN (SEQ ID NO. 10)

TRBV24Fi template + 454A as sequencing primer:
NNNNNNNNGNNANNNTCTGATGGANACAGTGTCTCTCGACAGGCACAGGCTAA
ATTCTCCCTGTCCCTAGAGTCTGCCATCCCCAACCAGACAGCTCTTTACTTCTGT
GCCACCAGTGANGCGGGGGGCGGGGACCACTACTTCGGGGGGGGGAGGCGG
ACCAGGGTGCTGGTCGACGAGAAAAAGGAGCTCCCCCCCGCCGCCGCTGTGG
TTGTTGCTTCATAATAATCAGGNNGGNGAGGNAGNAGNAANN (SEQ ID NO. 11)

[0068] To investigate the impact of artifacts on the overall repertoire analysis of the TCRβ transcriptome, the inventors conducted control experiments using chemically synthesized TCRβ CDR3 templates. For this, the inventors chemically synthesized four distinct clones, clonally purified each clone, and prepared different mixes of the four constructs as templates for amplicon rescue multiplex (ARM)-PCR. Two different reaction mixtures were subjected to two independent ARM-PCR reactions, and the pooled PCR products were sequenced at a length of 100bp from both ends using the Illimuna HiSeq2000®. The inventors first joined together paired-end reads through overlapping alignment with a modified Needleman-Wunsch algorithm, and then mapped the merged sequences to germline V, D and J reference sequences.

[0069] Without cleaning, the inventors obtained a total of 5,729,613 sequences from template mix I that could be mapped to TCRβ V, D and J segments. Surprisingly, the sequence reads purportedly represented a total of 36,439 unique CDR3 variants. Therefore, given that only four distinct CDR3 variants were present in the template mixtures, virtually all of the identified CDR3 variants must be non-authentic. Similar results were obtained for the second template mix, in which a total of 9,131,681 VDJ-mapped sequences were identified that mimicked the existence of 50,354 unique TCRβ CDR3 variants. The inventors' independent sequencing experiments show that only a few distinct CDR3 template variants can create artifactual repertoire diversities that far outweigh the real template diversity, and thus the inventors set out to eliminate these artifacts.

[0070] The quality of 3' end Illumina sequencing reads is generally considered to be low. In the context of repertoire sequencing, this is troublesome because PCR primers need to be positioned distal enough from the hypervariable V(D)J junctions to avoid negative effects due to primer-template mismatching. As a consequence, the CDR3 segments of interest are generally "shifted" closer to the 3' end of the sequencing reads, the region with increased sequencing error rates. Another technical issue that deserves attention is the observation that sequencing errors are context-specific and consequently strand-specific. Therefore, it is realistic to assume that the probability that a sequencing error in a forward read coincides with that in the corresponding reverse read is rare.

[0071] Considering this, the inventors devised a paired-end strategy that affords double-strand sequencing of complete TCR CDR3 segments on the basis of the

Illumina® technology. In this approach, forward and reverse sequencing primers are positioned at the framework region 3 and at the TCR J region or the 5' end of the C region, respectively. Taking into account the average length of Illumina sequence reads (currently 100-150 bp) this design enables the complete sequencing of both strands that define a CDR3 segment. In a second step, the forward and reverse reads are then analyzed for sequence mismatches and CDR3 sequences that exhibit non-identity of both strands are eliminated using a newly developed paired-end filtering algorithm.

[0072] Applying this sequencing error filter to the 5,729,613 CDR3 sequences obtained for template mix I, the inventors identified a total of 2,751,131 (48%) CDR3 sequences that contained conflicting sequence information on their opposite strands. Discarding of these sequences resulted in the elimination of 35,455 (97.2%) distinct artifactual CDR3 variants. Consistent with this, the paired-end filter removed 4,308,020 (47%) CDR3 sequences from template mix II, leading to the elimination of 49,063 (97.4%) artifactual CDR3 variants. A total of 973 and 1271 unique CDR3 variants, respectively, passed through the filter. These results indicate that paired-end sequencing and filtering reduces the total number of non-authentic unique CDR3 sequences by almost two orders of magnitude.

[0073] Detailed analysis of the frequency distribution of the non-authentic CDR3 variants after the sequencing error filter revealed that in both mixtures approximately 50% of all artifacts were single-copy sequences. About 10% of these artifactual CDR3s displayed >100 copy numbers and accounted for > 80% of all artifactual CDR3 variants. Given that variable TCR genes do not undergo somatic hypermutation, the inventors developed a reference algorithm that identifies and removes CDR3 sequence reads that display nucleotide mismatches relative to the mapped germline V, D and J reference sequences, as these must be artifacts generated at the level of PCR amplification or sequencing.

[0074] Applying this filtering algorithm to the "paired-end filtered" sequences of template mix I, a total of 29,804 sequences, which corresponded to 609 unique CDR3 variants, were removed. For template mix II, 54,516 artifactual sequences (831 unique CDR3 variants) were identified. Thus, the use of the reference sequence filter leads to a 60% reduction of non-authentic distinct CDR3 sequences. The reference filter is ineffective at the V-J and D-J junctions because the randomly added nucleotides in these regions during somatic recombination cannot be mapped. Therefore, the inventors implemented a PCR filter after computational simulation experiments to better understand four variables: the impact of the initial template number, the replication efficiency of each cycle, the cycle number (n), and the DNA polymerase error rate (μ) on the total end-point error rate. In contrast, the inventors noted that the PCR polymerase error rate has a pronounced effect on the number of accumulated errors

[0075] In the inventors' control sequencing experiments, PCR amplification was performed with 15 cycles and 45 cycles in the first and second reaction, using Taq polymerase. To simulate error accumulation during the ARM-PCR reactions more realistically, the PCR efficiency was set to decreased 5% per cycle for the first 25 cycles and 10% per cycle for the remaining cycles. The PCR efficiency was reset to 1.0 for each fresh PCR reaction. Furthermore, the inventors allowed mutation at the second position. Published substitution error rates for Taq enzyme, expressed as errors per bp per cycle, range from 0.023 x10^{-4} to 2.1 x10^{-4}. In the simulation experiments, the substitution error rate was set at 2.7 x10^{-5}, and the insertion-deletion (indel) error rate was set as 1.0 x10^{-6}. Taq polymerase is known to have a much higher insertion-and-deletion (indel) mutation rate in homopolymeric region of templates. For a homopolymeric region, indel mutation in any position of this region generates identical pattern. Therefore, the indel error rate in a homopolymeric region was set as n x μ, where n is the length of the homopolymeric region and μ is 1.0 x 10^{-6}.

[0076] Because the impact of the initial template number and the PCR efficiency on the endpoint error rate is small, it should be safe to apply the same end-point error rate estimated from the simulation experiments to molecules with different initial number and different replication efficiencies in a multiplex PCR reaction. The cutoff error rates (μ) were empirically set as error rates at the 9999th 10000-quantiles point for each category. For two similar CDR3 sequences, A and B, of frequency NA and NB (NA >> NB) that differ in less than three positions, if NA * μ ≥ NB, where μ is the corresponding cutoff error rate, CDR3 sequence B will be excluded. Applying this filtering algorithm to the “reference filtered” sequences of template mix I, a total of 22,369 sequences, which corresponded to 281 unique CDR3 variants, were removed. For template mix II, 39,920 artifactual sequences (348 unique CDR3 variants) were identified (Table 1). Thus, the use of the PCR amplification error filter leads to a further reduction of non-authentic distinct CDR3 sequences by around 80%.

[0077] In the pool of sequences that had passed through the above filters, the inventors identified several high-abundance CDR3 variants, which differed from their most similar input template sequences at multiple positions. Because the occurrence of PCR substitution and/or indel mutation at multiple positions of CDR3 fragments is extremely rare according to simulation experiments, those CDR3 variants must arise from other source of artifacts. Intriguingly, the inventors noted that some of these sequences were composed of the fragments of two distinct input templates and exhibited clear breakpoints, which identified them as chimeras. Chimeric sequences are PCR artifacts that arise from incomplete primer extension or template switching during PCR and form mosaic-like structures. In light of this unexpected PCR artifact, the inventors developed a computational “mosaic filter.” Using this filtering algorithm, the inventors identified a total of 17 and 15 chimeric sequences in template mixtures I and II, respectively. Of note, some of these CDR3 chimeras displayed sequence copy numbers >1000, indicating that the inventors’ algorithm for the filter is capable of identifying high-abundance chimeric CDR3 sequences.

[0078] Application of the filtering algorithms resulted in the elimination of 99.8% of the non-authentic unique CDR3 sequences generated by high-throughput sequencing of only four defined TCR CDR3 templates. Only 62 and 73 artifactual CDR3 sequences, respectively, passed through all filters. Among these, the two most abundant CDR3 sequences were identical in both mixing experiments. Most likely they represent chimeric artifacts which escaped filtering because of a single nucleotide substitution located exactly at the breakpoint. Among the remaining erroneous CDR3, 85% (n=53) and 75% (n=55) were single reads, respectively. To eliminate this minor fraction of artifacts, the inventors propose that high-stringency data analysis of TCR immune repertoires should include an additional filter that removes single copy CDR3 reads (frequency threshold filter).

Table 1

Locus	Primer Name	Sequence	SEQ ID NO.	Sequence	SEQ ID NO.
TRAV-C	TRAV1Fo	TGCACGTACCAGACATCTGG	12	TGCACGTACCAGACATCTGG	12
	TRAV1Fi	AGGTCGTTTTTCTTCATTCC	13	GCCTCCCTCGCGCCATCAGAGGTCGTTTTTCTTCATTCC	14
	TRAV2Fo	TCTGTAATCACTCTGTGTCC	15	TCTGTAATCACTCTGTGTCC	15
	TRAV2Fi	AGGGACGATACAACATGACC	16	GCCTCCCTCGCGCCATCAGAGGGACGATACAACATGACC	17
	TRAV3Fo	CTATTCAGTCTCTGGAAACC	18	CTATTCAGTCTCTGGAAACC	18
	TRAV3Fi	ATACATCACAGGGGATAACC	19	GCCTCCCTCGCGCCATCAGATACATCACAGGGGATAACC	20
	TRAV4Fo	TGTAGCCACAACAACATTGC	21	TGTAGCCACAACAACATTGC	21
	TRAV4Fi	AAAGTTACAAACGAAGTGGC	22	GCCTCCCTCGCGCCATCAGAAAGTTACAAACGAAGTGGC	23
	TRAV5Fo	GCACTTACACAGACAGCTCC	24	GCACTTACACAGACAGCTCC	24
	TRAV5Fi	TATGGACATGAAACAAGACC	25	GCCTCCCTCGCGCCATCAGTATGGACATGAAACAAGACC	26
	TRAV6Fo	GCAACTATACAAACTATTCC	27	GCAACTATACAAACTATTCC	27
	TRAV6Fi	GTTTTCTTGCTACTCATACG	28	GCCTCCCTCGCGCCATCAGGTTTTCTTGCTACTCATACG	29
	TRAV7Fo	TGCACGTACTCTGTCAGTCG	30	TGCACGTACTCTGTCAGTCG	30
	TRAV7Fi	GGATATGAGAAGCAGAAAGG	31	GCCTCCCTCGCGCCATCAGGGATATGAGAAGCAGAAAGG	32
	TRAV8Fo	AATCTCTTCTGGTATGTSCA	33	AATCTCTTCTGGTATGTSCA	33
	TRAV8Fi	GGYTTTGAGGCTGAATTTA	34	GCCTCCCTCGCGCCATCAGGGY	35

Locus	Primer Name	Sequence	SEQ ID NO.	Sequence	SEQ ID NO.
				TTTGAGGCTGAATTTA	
	TRAV9Fo	GTCCAATATCCTGGAGAAGG	36	GTCCAATATCCTGGAGAAGG	36
	TRAV9Fi	AACCACTTCTTTCCACTTGG	37	GCCTCCCTCGCGCCATCAGAACCACTTCTTTCCACTTGG	38
	TRAV10Fo	AATGCAATTATACAGTGAGC	39	AATGCAATTATACAGTGAGC	39
	TRAV10Fi	TGAGAACACAAAGTCGAACG	40	GCCTCCCTCGCGCCATCAGTGAGAACACAAAGTCGAACG	41
	TRAV11Fo	TCTTAATTGTACTTATCAGG	42	TCTTAATTGTACTTATCAGG	42
	TRAV11Fi	TCAATCAAGCCAGAAGGAGC	43	GCCTCCCTCGCGCCATCAGTCAATCAAGCCAGAAGGAGC	44
	TRAV12Fo	TCAGTGTTCCAGAGGGAGCC	45	TCAGTGTTCCAGAGGGAGCC	45
	TRAV12Fi	ATGGAAGGTTTACAGCACAG	46	GCCTCCCTCGCGCCATCAGATGGAAGGTTTACAGCACAG	47
	TRAV13Fo	ACCCTGAGTGTCCAGGAGGG	48	ACCCTGAGTGTCCAGGAGGG	48
	TRAV13Fi	TTATAGACATTCGTTCAAAT	49	GCCTCCCTCGCGCCATCAGTTATAGACATTCGTTCAAAT	50
	TRAV14Fo	TGGACTGCACATATGACACC	51	TGGACTGCACATATGACACC	51
	TRAV14Fi	CAGCAAAATGCAACAGAAGG	52	GCCTCCCTCGCGCCATCAGCAGCAAAATGCAACAGAAGG	53
	TRAV16Fo	AGCTGAAGTGCAACTATTCC	54	AGCTGAAGTGCAACTATTCC	54
	TRAV16Fi	TCTAGAGAGAGCATCAAAGG	55	GCCTCCCTCGCGCCATCAGTCTAGAGAGAGCATCAAAGG	56
	TRAV17Fo	AATGCCACCATGAACTGCAG	57	AATGCCACCATGAACTGCAG	57
	TRAV17Fi	GAAAGAGAGAAACACAGTGG	58	GCCTCCCTCGCGCCATCAGGAA	59

Locus	Primer Name	Sequence	SEQ ID NO.	Sequence	SEQ ID NO.
				AGAGAGAAACACAGTGG	
	TRAV18Fo	GCTCTGACATTAAACTGCAC	60	GCTCTGACATTAAACTGCAC	60
	TRAV18Fi	CAGGAGACGGACAGCAGAGG	61	GCCTCCCTCGCGCCATCAGCAGGAGACGGACAGCAGAGG	62
	TRAV19Fo	ATGTGACCTTGGACTGTGTG	63	ATGTGACCTTGGACTGTGTG	63
	TRAV19Fi	GAGCAAAATGAAATAAGTGG	64	GCCTCCCTCGCGCCATCAGGAGCAAAATGAAATAAGTGG	65
	TRAV20Fo	ACTGCAGTTACACAGTCAGC	66	ACTGCAGTTACACAGTCAGC	66
	TRAV20Fi	AGAAAGAAAGGCTAAAAGCC	67	GCCTCCCTCGCGCCATCAGAGAAAGAAAGGCTAAAAGCC	68
	TRAV21Fo	ACTGCAGTTTCACTGATAGC	69	ACTGCAGTTTCACTGATAGC	69
	TRAV21Fi	CAAGTGGAAGACTTAATGCC	70	GCCTCCCTCGCGCCATCAGCAAGTGGAAGACTTAATGCC	71
	TRAV22Fo	GGGAGCCAATTCCACGCTGC	72	GGGAGCCAATTCCACGCTGC	72
	TRAV22Fi	ATGGAAGATTAAGCGCCACG	73	GCCTCCCTCGCGCCATCAGATGGAAGATTAAGCGCCACG	74
	TRAV23Fo	ATTTCAATTATAAACTGTGC	75	ATTTCAATTATAAACTGTGC	75
	TRAV23Fi	AAGGAAGATTCACAATCTCC	76	GCCTCCCTCGCGCCATCAGAAGGAAGATTCACAATCTCC	77
	TRAV24Fo	GCACCAATTTCACCTGCAGC	78	GCACCAATTTCACCTGCAGC	78
	TRAV24Fi	AGGACGAATAAGTGCCACTC	79	GCCTCCCTCGCGCCATCAGAGGACGAATAAGTGCCACTC	80
	TRAV25Fo	TCACCACGTACTGCAATTCC	81	TCACCACGTACTGCAATTCC	81
	TRAV25Fi	AGACTGACATTTCAGTTTGG	82	GCCTCCCTCGCGCCATCAGAGACTGACATTTCAGTTTGG	83
	TRAV26Fo	TCGACAGATT	84	TCGACAGATTC	84

Locus	Primer Name	Sequence	SEQ ID NO.	Sequence	SEQ ID NO.
		CMCTCCCAGG		MCTCCCAGG	
	TRAV26Fi	GTCCAGYACCTTGATCCTGC	85	GCCTCCCTCGCGCCATCAGGTCCAGYACCTTGATCCTGC	86
	TRAV27Fo	CCTCAAGTGTTTTTTCCAGC	87	CCTCAAGTGTTTTTTCCAGC	87
	TRAV27Fi	GTGACAGTAGTTACGGGTGG	88	GCCTCCCTCGCGCCATCAGGTGACAGTAGTTACGGGTGG	89
	TRAV29Fo	CAGCATGTTTGATTATTTCC	90	CAGCATGTTTGATTATTTCC	90
	TRAV29Fi	ATCTATAAGTTCCATTAAGG	91	GCCTCCCTCGCGCCATCAGATCTATAAGTTCCATTAAGG	92
	TRAV30Fo	CTCCAAGGCTTTATATTCTG	93	CTCCAAGGCTTTATATTCTG	93
	TRAV30Fi	ATGATATTACTGAAGGGTGG	94	GCCTCCCTCGCGCCATCAGATGATATTACTGAAGGGTGG	95
	TRAV34Fo	ACTGCACGTCATCAAAGACG	96	ACTGCACGTCATCAAAGACG	96
	TRAV34Fi	TTGATGATGCTACAGAAAGG	97	GCCTCCCTCGCGCCATCAGTTGATGATGCTACAGAAAGG	98
	TRAV35Fo	TGAACTGCACTTCTTCAAGC	99	TGAACTGCACTTCTTCAAGC	99
	TRAV35Fi	CTTGATAGCCTTATATAAGG	100	GCCTCCCTCGCGCCATCAGCTTGATAGCCTTATATAAGG	101
	TRAV36Fo	TCAATTGCAGTTATGAAGTG	102	TCAATTGCAGTTATGAAGTG	102
	TRAV36Fi	TTTATGCTAACTTCAAGTGG	103	GCCTCCCTCGCGCCATCAGTTTATGCTAACTTCAAGTGG	104
	TRAV38Fo	GCACATATGACACCAGTGAG	105	GCACATATGACACCAGTGAG	105
	TRAV38Fi	TCGCCAAGAAGCTTATAAGC	106	GCCTCCCTCGCGCCATCAGTCGCCAAGAAGCTTATAAGC	107
	TRAV39Fo	TCTACTGCAATTATTCAACC	108	TCTACTGCAATTATTCAACC	108
	TRAV39Fi	CAGGAGGGA	109	GCCTCCCTCGC	110

Locus	Primer Name	Sequence	SEQ ID NO.	Sequence	SEQ ID NO.
		CGATTAATGGC		GCCATCAGCAGGAGGGACGATTAATGGC	
	TRAV40Fo	TGAACTGCACATACACATCC	111	TGAACTGCACATACACATCC	111
	TRAV40Fi	ACAGCAAAAACTTCGGAGGC	112	GCCTCCCTCGCGCCATCAGACAGCAAAAACTTCGGAGGC	113
	TRAV41Fo	AACTGCAGTTACTCGGTAGG	114	AACTGCAGTTACTCGGTAGG	114
	TRAV41Fi	AAGCATGGAAGATTAATTGC	115	GCCTCCCTCGCGCCATCAGAAGCATGGAAGATTAATTGC	116
	TRACRo	GCAGACAGACTTGTCACTGG	117	GCAGACAGACTTGTCACTGG	117
	TRACRi	AGTCTCTCAGCTGGTACACG	118	GCCTTGCCAGCCCGCTCAGAGTCTCTCAGCTGGTACACG	119
TRBV-C	TRBV1Fo	AATGAAACGTGAGCATCTGG	120	AATGAAACGTGAGCATCTGG	120
	TRBV1Fi	CATTGAAAACAAGACTGTGC	121	GCCTCCCTCGCGCCATCAGCATTGAAAACAAGACTGTGC	122
	TRBV2Fo	GTGTCCCCATCTCTAATCAC	123	GTGTCCCCATCTCTAATCAC	123
	TRBV2Fi	TGAAATCTCAGAGAAGTCTG	124	GCCTCCCTCGCGCCATCAGTGAAATCTCAGAGAAGTCTG	125
	TRBV3Fo	TATGTATTGGTATAAACAGG	126	TATGTATTGGTATAAACAGG	126
	TRBV3Fi	CTCTAAGAAATTTCTGAAGA	127	GCCTCCCTCGCGCCATCAGCTCTAAGAAATTTCTGAAGA	128
	TRBV4Fo	GTCTTTGAAATGTGAACAAC	129	GTCTTTGAAATGTGAACAAC	129
	TRBV4Fi	GGAGCTCATGTTTGTCTACA	130	GCCTCCCTCGCGCCATCAGGGAGCTCATGTTTGTCTACA	131
	TRBV5Fo	GATCAAAACGAGAGGACAGC	132	GATCAAAACGAGAGGACAGC	132

Locus	Primer Name	Sequence	SEQ ID NO.	Sequence	SEQ ID NO.
	TRBV5aFi	CAGGGGCCCCAGTTTATCTT	133	GCCTCCCTCGCGCCATCAGCAGGGGCCCCAGTTTATCTT	134
	TRBV5bFi	GAAACARAGGAAACTTCCCT	135	GCCTCCCTCGCGCCATCAGGAAACARAGGAAACTTCCCT	136
	TRBV6aFo	GTGTGCCCAGGATATGAACC	137	GTGTGCCCAGGATATGAACC	137
	TRBV6bFo	CAGGATATGAGACATAATGC	138	CAGGATATGAGACATAATGC	138
	TRBV6aFi	GGTATCGACAAGACCCAGGC	139	GCCTCCCTCGCGCCATCAGGGTATCGACAAGACCCAGGC	140
	TRBV6bFi	TAGACAAGATCTAGGACTGG	141	GCCTCCCTCGCGCCATCAGTAGACAAGATCTAGGACTGG	142
	TRBV7Fo	CTCAGGTGTGATCCAATTTC	143	CTCAGGTGTGATCCAATTTC	143
	TRBV7aFi	TCTAATTTACTTCCAAGGCA	144	GCCTCCCTCGCGCCATCAGTCTAATTTACTTCCAAGGCA	145
	TRBV7bFi	TCCCAGAGTGATGCTCAACG	146	GCCTCCCTCGCGCCATCAGTCCCAGAGTGATGCTCAACG	147
	TRBV7cFi	ACTTACTTCAATTATGAAGC	148	GCCTCCCTCGCGCCATCAGACTTACTTCAATTATGAAGC	149
	TRBV7dFi	CCAGAATGAAGCTCAACTAG	150	GCCTCCCTCGCGCCATCAGCCAGAATGAAGCTCAACTAG	151
	TRBV9Fo	GAGACCTCTCTGTGTACTGG	152	GAGACCTCTCTGTGTACTGG	152
	TRBV9Fi	CTCATTCAGTATTATAATGG	153	GCCTCCCTCGCGCCATCAGCTCATTCAGTATTATAATGG	154
	TRBV10Fo	GGAATCACCCAGAGCCCAAG	155	GGAATCACCCAGAGCCCAAG	155
	TRBV10Fi	GACATGGGCTGAGGCTGATC	156	GCCTCCCTCGCGCCATCAGGACATGGGCTGAGGCTGATC	157
	TRBV11Fo	CCTAAGGATC	158	CCTAAGGATCG	158

Locus	Primer Name	Sequence	SEQ ID NO.	Sequence	SEQ ID NO.
		GATTTTCTGC		ATTTTCTGC	
	TRBV11Fi	ACTCTCAAGATCCAGCCTGC	159	GCCTCCCTCGCGCCATCAGACTCTCAAGATCCAGCCTGC	160
	TRBV12Fo	AGGTGACAGAGATGGGACAA	161	AGGTGACAGAGATGGGACAA	161
	TRBV12aFi	TGCAGGGACTGGAATTGCTG	162	GCCTCCCTCGCGCCATCAGTGCAGGGACTGGAATTGCTG	163
	TRBV12bFi	GTACAGACAGACCATGATGC	164	GCCTCCCTCGCGCCATCAGGTACAGACAGACCATGATGC	165
	TRBV13Fo	CTATCCTATCCCTAGACACG	166	CTATCCTATCCCTAGACACG	166
	TRBV13Fi	AAGATGCAGAGCGATAAAGG	167	GCCTCCCTCGCGCCATCAGAAGATGCAGAGCGATAAAGG	168
	TRBV14Fo	AGATGTGACCCAATTTCTGG	169	AGATGTGACCCAATTTCTGG	169
	TRBV14Fi	AGTCTAAACAGGATGAGTCC	170	GCCTCCCTCGCGCCATCAGAGTCTAAACAGGATGAGTCC	171
	TRBV15Fo	TCAGACTTTGAACCATAACG	172	TCAGACTTTGAACCATAACG	172
	TRBV15Fi	AAAGATTTTAACAATGAAGC	173	GCCTCCCTCGCGCCATCAGAAAGATTTTAACAATGAAGC	174
	TRBV16Fo	TATTGTGCCCCAATAAAAGG	175	TATTGTGCCCCAATAAAAGG	175
	TRBV16Fi	AATGTCTTTGATGAAACAGG	176	GCCTCCCTCGCGCCATCAGAATGTCTTTGATGAAACAGG	177
	TRBV17Fo	ATCCATCTTCTGGTCACATG	178	ATCCATCTTCTGGTCACATG	178
	TRBV17Fi	AACATTGCAGTTGATTCAGG	179	GCCTCCCTCGCGCCATCAGAACATTGCAGTTGATTCAGG	180
	TRBV18Fo	GCAGCCCAATGAAAGGACAC	181	GCAGCCCAATGAAAGGACAC	181
	TRBV18Fi	AATATCATAGATGAGTCAGG	182	GCCTCCCTCGCGCCATCAGAATATCATAGATGAGTCAGG	183

Locus	Primer Name	Sequence	SEQ ID NO.	Sequence	SEQ ID NO.
	TRBV19Fo	TGAACAGAATTTGAACCACG	184	TGAACAGAATTTGAACCACG	184
	TRBV19Fi	TTTCAGAAAGGAGATATAGC	185	GCCTCCCTCGCGCCATCAGTTTCAGAAAGGAGATATAGC	186
	TRBV20Fo	TCGAGTGCCGTTCCCTGGAC	187	TCGAGTGCCGTTCCCTGGAC	187
	TRBV20Fi	GATGGCAACTTCCAATGAGG	188	GCCTCCCTCGCGCCATCAGGATGGCAACTTCCAATGAGG	189
	TRBV21Fo	GCAAAGATGGATTGTGTTCC	190	GCAAAGATGGATTGTGTTCC	190
	TRBV21Fi	CGCTGGAAGAAGAGCTCAAG	191	GCCTCCCTCGCGCCATCAGCGCTGGAAGAAGAGCTCAAG	192
	TRBV23Fo	CATTTGGTCAAAGGAAAAGG	193	CATTTGGTCAAAGGAAAAGG	193
	TRBV23Fi	GAATGAACAAGTTCTTCAAG	194	GCCTCCCTCGCGCCATCAGGAATGAACAAGTTCTTCAAG	195
	TRBV24Fo	ATGCTGGAATGTTCTCAGAC	196	ATGCTGGAATGTTCTCAGAC	196
	TRBV24Fi	GTCAAAGATATAAACAAAGG	197	GCCTCCCTCGCGCCATCAGGTCAAAGATATAAACAAAGG	198
	TRBV25Fo	CTCTGGAATGTTCTCAAACC	199	CTCTGGAATGTTCTCAAACC	199
	TRBV25Fi	TAATTCCACAGAGAAGGGAG	200	GCCTCCCTCGCGCCATCAGTAATTCCACAGAGAAGGGAG	201
	TRBV26Fo	CCCAGAATATGAATCATGTT	202	CCCAGAATATGAATCATGTT	202
	TRBV26Fi	ATTCACCTGGCACTGGGAGC	203	GCCTCCCTCGCGCCATCAGATTCACCTGGCACTGGGAGC	204
	TRBV27Fo	TTGTTCTCAGAATATGAACC	205	TTGTTCTCAGAATATGAACC	205
	TRBV27Fi	TGAGGTGACTGATAAGGGAG	206	GCCTCCCTCGCGCCATCAGTGAGGTGACTGATAAGGGAG	207
	TRBV28Fo	ATGTGTCCAGGATATGGACC	208	ATGTGTCCAGGATATGGACC	208
	TRBV28Fi	AAAAGGAGAT	209	GCCTCCCTCGC	210

Locus	Primer Name	Sequence	SEQ ID NO.	Sequence	SEQ ID NO.
		ATTCCTGAGG		GCCATCAGAAAAGGAGATATTCCTGAGG	
	TRBV29Fo	TCACCATGATGTTCTGGTAC	211	TCACCATGATGTTCTGGTAC	211
	TRBV29Fi	CTGGACAGAGCCTGACACTG	212	GCCTCCCTCGCGCCATCAGCTGGACAGAGCCTGACACTG	213
	TRBV30Fo	TGTGGAGGGAACATCAAACC	214	TGTGGAGGGAACATCAAACC	214
	TRBV30Fi	TTCTACTCCGTTGGTATTGG	215	GCCTCCCTCGCGCCATCAGTTCTACTCCGTTGGTATTGG	216
	TRBCRo	GTGTGGCCTTTTGGGTGTGG	217	GTGTGGCCTTTTGGGTGTGG	217
	TRBCRi	TCTGATGGCTCAAACACAGC	218	GCCTTGCCAGCCCGCTCAGTCTGATGGCTCAAACACAGC	219
TRDV-C	TRDV1Fo	TGTATGAAACAAGTTGGTGG	220	TGTATGAAACAAGTTGGTGG	220
	TRDV1Fi	CAGAATGCAAAAAGTGGTCG	221	GCCTCCCTCGCGCCATCAGCAGAATGCAAAAAGTGGTCG	222
	TRDV2Fo	ATGAAAGGAGAAGCGATCGG	223	ATGAAAGGAGAAGCGATCGG	223
	TRDV2Fi	TGGTTTCAAAGACAATTTCC	224	GCCTCCCTCGCGCCATCAGTGGTTTCAAAGACAATTTCC	225
	TRDV3Fo	GACACTGTATATTCAAATCC	226	GACACTGTATATTCAAATCC	226
	TRDV3Fi	GCAGATTTTACTCAAGGACG	227	GCCTCCCTCGCGCCATCAGGCAGATTTTACTCAAGGACG	228
	TRDCRo	AGACAAGCGACATTTGTTCC	229	AGACAAGCGACATTTGTTCC	229
	TRDCRi	ACGGATGGTTTGGTATGAGG	230	GCCTTGCCAGCCCGCTCAGACGGATGGTTTGGTATGAGG	231

Locus	Primer Name	Sequence	SEQ ID NO.	Sequence	SEQ ID NO.
TRGV-C	TRGV1-5Fo	GGGTCATCTGCTGAAATCAC	232	GGGTCATCTGCTGAAATCAC	232
	TRGV1-5,8Fi	AGGAGGGGAAGGCCCCACAG	233	GCCTCCCTCGCGCCATCAGAGGAGGGGAAGGCCCCACAG	234
	TRGV8Fo	GGGTCATCAGCTGTAATCAC	235	GGGTCATCAGCTGTAATCAC	235
	TRGV5pFi	AGGAGGGGAAGACCCCACAG	236	GCCTCCCTCGCGCCATCAGAGGAGGGGAAGACCCCACAG	237
	TRGV9Fo	AGCCCGCCTGGAATGTGTGG	238	AGCCCGCCTGGAATGTGTGG	238
	TRGV9Fi	GCACTGTCAGAAAGGAATCC	239	GCCTCCCTCGCGCCATCAGGCACTGTCAGAAAGGAATCC	240
	TRGV10Fo	AAGAAAAGTATTGACATACC	241	AAGAAAAGTATTGACATACC	241
	TRGV10Fi	ATATTGTCTCAACAAAATCC	242	GCCTCCCTCGCGCCATCAGATATTGTCTCAACAAAATCC	243
	TRGV11Fo	AGAGTGCCCACATATCTTGG	244	AGAGTGCCCACATATCTTGG	244
	TRGV11Fi	GCTCAAGATTGCTCAGGTGG	245	GCCTCCCTCGCGCCATCAGGCTCAAGATTGCTCAGGTGG	246
	TRGCRo	GGATCCCAGAATCGTGTTGC	247	GGATCCCAGAATCGTGTTGC	247
	TRGCRi	GGTATGTTCCAGCCTTCTGG	248	GCCTTGCCAGCCCGCTCAGGGTATGTTCCAGCCTTCTGG	249

Table 2

Locus	Primer Name	Sequence	SEQ ID NO.	Ordered	SEQ ID NO.
IgHV-J	IgHV1aFo	AGTGAAGGTCTCCTGCAAGG	250	AGTGAAGGTCTCCTGCAAGG	250
	IgHV1bFo	AGTGAAGGTTTCCTGCAAGG	251	AGTGAAGGTTTCCTGCAAGG	251
	IgHV1aFi	AGTTCCAGGGCAGAGTCAC	252	GCCTCCCTCGCGCCATCAGAGTTCCAGGGCAGAGTCAC	253
	IgHV1bFi	AGTTTCAGGGCAGGGTCAC	254	GCCTCCCTCGCGCCATCAGAGTTTCAGGGCAGGGTCAC	255
	IgHV1cFi	AGTTCCAGGAAAGAGTCAC	256	GCCTCCCTCGCGCCATCAGAGTTCCAGGAAAGAGTCAC	257
	IgHV1dFi	AATTCCAGGACAGAGTCAC	258	GCCTCCCTCGCGCCATCAGAATTCCAGGACAGAGTCAC	259
	IgHV2Fo	TCTCTGGGTTCTCACTCAGC	260	TCTCTGGGTTCTCACTCAGC	260
	IgHV2Fi	AAGGCCCTGGAGTGGCTTGC	261	GCCTCCCTCGCGCCATCAGAAGGCCCTGGAGTGGCTTGC	262
	IgHV3aFo	TCCCTGAGACTCTCCTGTGC	263	TCCCTGAGACTCTCCTGTGC	263
	IgHV3bFo	CTCTCCTGTGCAGCCTCTGG	264	CTCTCCTGTGCAGCCTCTGG	264
	IgHV3cFo	GGTCCCTGAGACTCTCCTGT	265	GGTCCCTGAGACTCTCCTGT	265
	IgHV3dFo	CTGAGACTCTCCTGTGTAGC	266	CTGAGACTCTCCTGTGTAGC	266
	IgHV3aFi	CTCCAGGGAAGGGGCTGG	267	GCCTCCCTCGCGCCATCAGCTCCAGGGAAGGGGCTGG	268
	IgHV3bFi	GGCTCCAGGCAAGGGGCT	269	GCCTCCCTCGCGCCATCAGGGCTCCAGGCAAGGGGCT	270
	IgHV3cFi	ACTGGGTCCGCCAGGCTCC	271	GCCTCCCTCGCGCCATCAGACTGGGTCCGCCAGGCTCC	272
	IgHV3dFi	GAAGGGGCTGGAGTGGGT	273	GCCTCCCTCGCGCCATCAGGAAGGGGCTGGAGTGGGT	274

Locus	Primer Name	Sequence	SEQ ID NO.	Ordered	SEQ ID NO.
	IgHV3eFi	AAAAGGTCTGGAGTGGGT	275	GCCTCCCTCGCGCCATCAGAAAAGGTCTGGAGTGGGT	276
	IgHV4Fo	AGACCCTGTCCCTCACCTGC	277	AGACCCTGTCCCTCACCTGC	277
	IgHV4Fi	AGGGVCTGGAGTGGATTGGG	278	GCCTCCCTCGCGCCATCAGAGGGVCTGGAGTGGATTGGG	279
	IgHV5Fo	GCGCCAGATGCCCGGGAAAG	280	GCGCCAGATGCCCGGGAAAG	280
	IgHV5Fi	GGCCASGTCACCATCTCAGC	281	GCCTCCCTCGCGCCATCAGGGCCASGTCACCATCTCAGC	282
	IgHV6Fo	CCGGGGACAGTGTCTCTAGC	283	CCGGGGACAGTGTCTCTAGC	283
	IgHV6Fi	GCCTTGAGTGGCTGGGAAGG	284	GCCTCCCTCGCGCCATCAGGCCTTGAGTGGCTGGGAAGG	285
	IgHV7Fo	GTTTCCTGCAAGGCTTCTGG	286	GTTTCCTGCAAGGCTTCTGG	286
	IgHV7Fi	GGCTTGAGTGGATGGGATGG	287	GCCTCCCTCGCGCCATCAGGGCTTGAGTGGATGGGATGG	288
	IgHJRo	ACCTGAGGAGACGGTGACC	289	ACCTGAGGAGACGGTGACC	289
	IgHJ1Ri	CAGTGCTGGAAGTATTCAGC	290	GCCTTGCCAGCCCGCTCAGCAGTGCTGGAAGTATTCAGC	291
	IgHJ2Ri	AGAGATCGAAGTACCAGTAG	292	GCCTTGCCAGCCCGCTCAGAGAGATCGAAGTACCAGTAG	293
	IgHJ3Ri	CCCCAGATATCAAAAGCATC	294	GCCTTGCCAGCCCGCTCAGCCCCAGATATCAAAAGCATC	295
	IgHJ4Ri	GGCCCCAGTAGTCAAAGTAG	296	GCCTTGCCAGCCCGCTCAGGGCCCCAGTAGTCAAAGTAG	297
	IgHJ5Ri	CCCAGGGGTCGAACCAGTTG	298	GCCTTGCCAGCCCGCTCAGCCCAGGGGTCGAACCAGTTG	299

Locus	Primer Name	Sequence	SEQ ID NO.	Ordered	SEQ ID NO.
	IgHJ6Ri	CCCAGACGTCCATGTAGTAG	300	GCCTTGCCAGCCCGCTCAGCCCAGACGTCCATGTAGTAG	301
IgKV-C	IgKV1Fo	TAGGAGACAGAGTCACCATC	302	TAGGAGACAGAGTCACCATC	302
	IgKV1Fi	TTCAGYGRCAGTGGATCTGG	303	GCCTCCCTCGCGCCATCAGTTCAGYGRCAGTGGATCTGG	304
	IgKV2Fo	GGAGAGCCGGCCTCCATCTC	305	GGAGAGCCGGCCTCCATCTC	305
	IgKV2aFi	TGGTACCTGCAGAAGCCAGG	306	GCCTCCCTCGCGCCATCAGTGGTACCTGCAGAAGCCAGG	307
	IgKV2bFi	CTTCAGCAGAGGCCAGGCCA	308	GCCTCCCTCGCGCCATCAGCTTCAGCAGAGGCCAGGCCA	309
	IgKV3-7Fo	GCCTGGTACCAGCAGAAACC	310	GCCTGGTACCAGCAGAAACC	310
	IgKV3Fi	GCCAGGTTCAGTGGCAGTGG	311	GCCTCCCTCGCGCCATCAGGCCAGGTTCAGTGGCAGTGG	312
	IgKV6-7Fi	TCGAGGTTCAGTGGCAGTGG	313	GCCTCCCTCGCGCCATCAGTCGAGGTTCAGTGGCAGTGG	314
	IgKV4-5Fi	GACCGATTCAGTGGCAGCGG	315	GCCTCCCTCGCGCCATCAGGACCGATTCAGTGGCAGCGG	316
	IgKCRo	TTCAACTGCTCATCAGATGG	317	TTCAACTGCTCATCAGATGG	317
	IgKCRi	ATGAAGACAGATGGTGCAGC	318	GCCTTGCCAGCCCGCTCAGATGAAGACAGATGGTGCAGC	319
IgLV-C	IgLV1aFo	GGGCAGAGGGTCACCATCTC	320	GGGCAGAGGGTCACCATCTC	320
	IgLV1bFo	GGACAGAAGGTCACCATCTC	321	GGACAGAAGGTCACCATCTC	321
	IgLV1aFi	TGGTACCAGCAGCTCCCAGG	322	GCCTCCCTCGCGCCATCAGTGGTACCAGCAGCTCCCAGG	323

Locus	Primer Name	Sequence	SEQ ID NO.	Ordered	SEQ ID NO.
	IgLV1bFi	TGGTACCAGCAGCTTCCAGG	324	GCCTCCCTCGCGCCATCAGTGGTACCAGCAGCTTCCAGG	325
	IgLV2Fo	CTGCACTGGAACCAGCAGTG	326	CTGCACTGGAACCAGCAGTG	326
	IgLV2Fi	TCTCTGGCTCCAAGTCTGGC	327	GCCTCCCTCGCGCCATCAGTCTCTGGCTCCAAGTCTGGC	328
	IgLV3aFo	ACCAGCAGAAGCCAGGCCAG	329	ACCAGCAGAAGCCAGGCCAG	329
	IgLV3bFo	GAAGCCAGGACAGGCCCCTG	330	GAAGCCAGGACAGGCCCCTG	330
	IgLV3aFi	CTGAGCGATTCTCTGGCTCC	331	GCCTCCCTCGCGCCATCAGCTGAGCGATTCTCTGGCTCC	332
	IgLV3bFi	TTCTCTGGGTCCACCTCAGG	333	GCCTCCCTCGCGCCATCAGTTCTCTGGGTCCACCTCAGG	334
	IgLV3cFi	TTCTCTGGCTCCAGCTCAGG	335	GCCTCCCTCGCGCCATCAGTTCTCTGGCTCCAGCTCAGG	336
	IgLV4Fo	TCGGTCAAGCTCACCTGCAC	337	TCGGTCAAGCTCACCTGCAC	337
	IgLV4Fi	GGGCTGACCGCTACCTCACC	358	GCCTCCCTCGCGCCATCAGGGGCTGACCGCTACCTCACC	338
	IgLV5Fo	CAGCCTGTGCTGACTCAGCC	339	CAGCCTGTGCTGACTCAGCC	339
	IgLV5Fi	CCAGCCGCTTCTCTGGATCC	340	GCCTCCCTCGCGCCATCAGCCAGCCGCTTCTCTGGATCC	341
	IgLV6Fo	CCATCTCCTGCACCCGCAGC	342	CCATCTCCTGCACCCGCAGC	342
	IgLV7-8Fo	TCCCCWGGAGGGACAGTCAC	343	TCCCCWGGAGGGACAGTCAC	343
	IgLV9,11Fo	CTCMCCTGCACCCTGAGCAG	344	CTCMCCTGCACCCTGAGCAG	344
	IgLV10Fo	AGACCGCCACACTCACCTGC	345	AGACCGCCACACTCACCTGC	345
	IgLV6,8Fi	CTGATCGSTTCTCTGGCTCC	346	GCCTCCCTCGCGCCATCAGCTGATCGSTTCTCTGGCTCC	347
	IgLV7Fi	CTGCCCGGTTCT	348	CTGCCCGGTTCT	348

Locus	Primer Name	Sequence	SEQ ID NO.	Ordered	SEQ ID NO.
		CAGGCTCC		CAGGCTCC	
	IgLV9Fi	ATCCAGGAAGAGGATGAGAG	349	GCCTCCCTCGCGCCATCAGATCCAGGAAGAGGATGAGAG	350
	IgLV10-11Fi	CTCCAGCCTGAGGACGAGGC	351	GCCTCCCTCGCGCCATCAGCTCCAGCCTGAGGACGAGGC	352
	IgLC1-7Ro	GCTCCCGGGTAGAAGTCACT	353	GCTCCCGGGTAGAAGTCACT	353
	IgLC1-7Ri	AGTGTGGCCTTGTTGGCTTG	354	GCCTTGCCAGCCCGCTCAGAGTGTGGCCTTGTTGGCTTG	355
	454A	GCCTCCCTCGCGCCATCAG	356	GCCTCCCTCGCGCCATCAG	356
	454B	GCCTTGCCAGCCCGCTCAG	357	GCCTTGCCAGCCCGCTCAG	357

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 54995-3 Seq 21-AUG-15 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

CLAIMS:

1. A computer-implemented method for analyzing semi-quantitative sequence information to identify and characterize the immunoprofile of a human or animal subject as normal or as being likely to indicate the presence of a disease, the method comprising the steps of:

(a) identifying one or more distinct complementary determining region 3 (CDR3) sequences that are shared between the subject's immunoprofile and a cumulative immunoprofile from a disease library representing a specific disease stored in a database;

(b) summing a total number of the subject's detected sequences corresponding to those shared distinct CDR3 sequences;

(c) computing the percentage of the total number of detected sequences in the subject's immunoprofile that are representative of those distinct CDR3s shared between the subject's immunoprofile and the disease library to create one or more original sharing indices;

(d) randomly selecting sequences from a public library stored in a database to form a sub-library, the sub-library comprising a number of distinct CDR3 sequences that is approximately equal to the number of distinct CDR3 sequences in the disease library;

(e) identifying one or more distinct CDR3 sequences that are shared between the subject's immunoprofile and the sub-library;

(f) summing a total number of detected sequences corresponding to those shared CDR3 sequences and calculating a percentage of the total number of detected sequences in the subject's immunoprofile that are shared between the subject's immunoprofile and the sub-library to create a sampling sharing index;

(g) repeating steps (d)-(f) for a number of times sufficient to produce a result that is statistically significant for identifying and characterizing the immunoprofile of said subject as normal or as being likely to indicate the presence of a disease;

(h) estimating the P-value as the fraction of times the sampling sharing indices are greater than or equal to the original sharing index between the immunoprofile of said subject and the disease library; and

(i) characterizing the immunoprofile of the subject as normal if the estimated P-value is greater than 0.01, or characterizing the immunoprofile of the subject as being likely to indicate the presence of the disease represented by the disease library if the estimated P-value is less than 0.01;

wherein step (a) is preceded by semi-quantitative amplification and sequencing of distinct CDR3s from said subject, wherein said semi-quantitative amplification and sequencing comprises the steps of:

(i) isolating a subpopulation of white blood cells from said subject;

(ii) isolating RNA from the subpopulation of cells;

(iii) amplifying the RNA using RT-PCR in a first amplification reaction to produce amplicons using nested primers, at least a portion of the nested primers comprising additional nucleotides to incorporate into a resulting amplicon a binding site for a communal primer;

(iv) separating the amplicons from the first amplification reaction from one or more unused primers from the first amplification reaction;

(v) amplifying, by the addition of communal primers in a second amplification reaction, the amplicons of the first amplification reaction having at least one binding site for a communal primer, wherein the product of the second amplification reaction is polynucleotides comprising distinct CDR3s; and

(vi) sequencing the amplicons of the second amplification reaction to identify antibody and/or receptor rearrangements in the subpopulation of cells;

wherein the amplification and sequencing steps (v) and (iv) provide semi-quantitative sequence information for distinct CDR3s from the subject.

2. The method of claim 1, wherein step (g) comprises repeating steps (d) – (f) at least 1000 or more times.

3. The method of claim 1, wherein the step of isolating a subpopulation of white blood cells is performed by flow cytometry.

4. The method of claim 1, wherein the subpopulation of white blood cells comprises T cells.

5. The method of claim 4, wherein the T cells are selected from the group consisting of naïve T cells, mature T cells, and memory T cells.

6. The method of claim 1, wherein the subpopulation of white blood cells comprises B cells.

7. The method of claim 6, wherein the B cells are selected from the group consisting of naïve B cells, mature B cells, and memory B cells.

8. The method of claim 1, wherein the rearrangements in the subpopulations of cells are selected from the group consisting of rearrangements of B-cell immunoglobulin heavy chain (IgH), B-cell kappa, B-cell lambda light chains, T-cell receptor Beta, T-cell Gamma, and T-cell Delta.

9. A computer readable medium having recorded thereon computer executable instructions that when executed by a computer perform the method of claim 1.

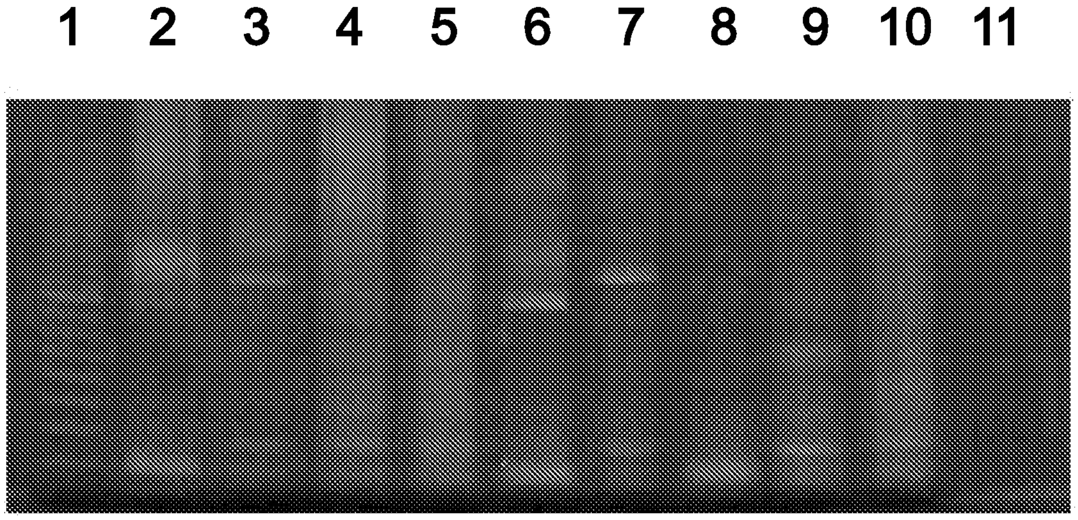


FIG. 1a

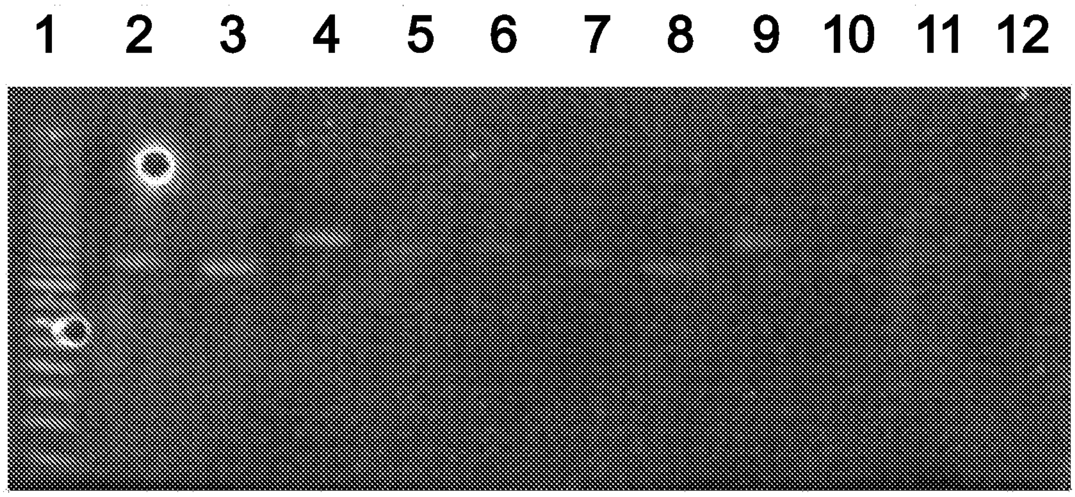


FIG. 1b

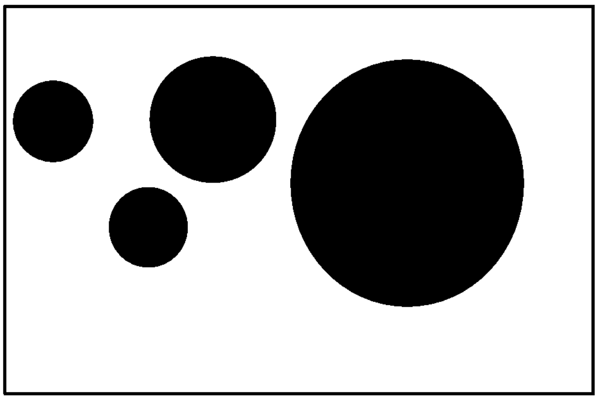


FIG. 2a

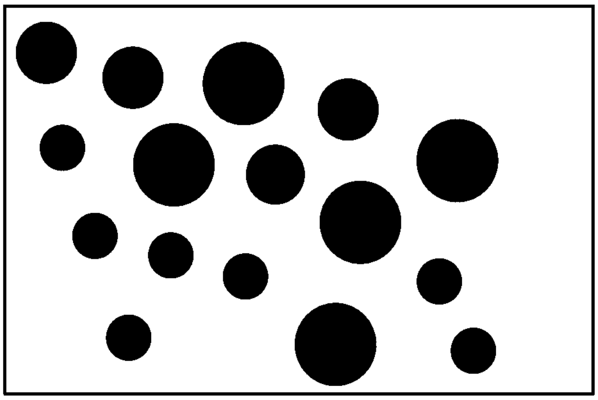


FIG. 2b

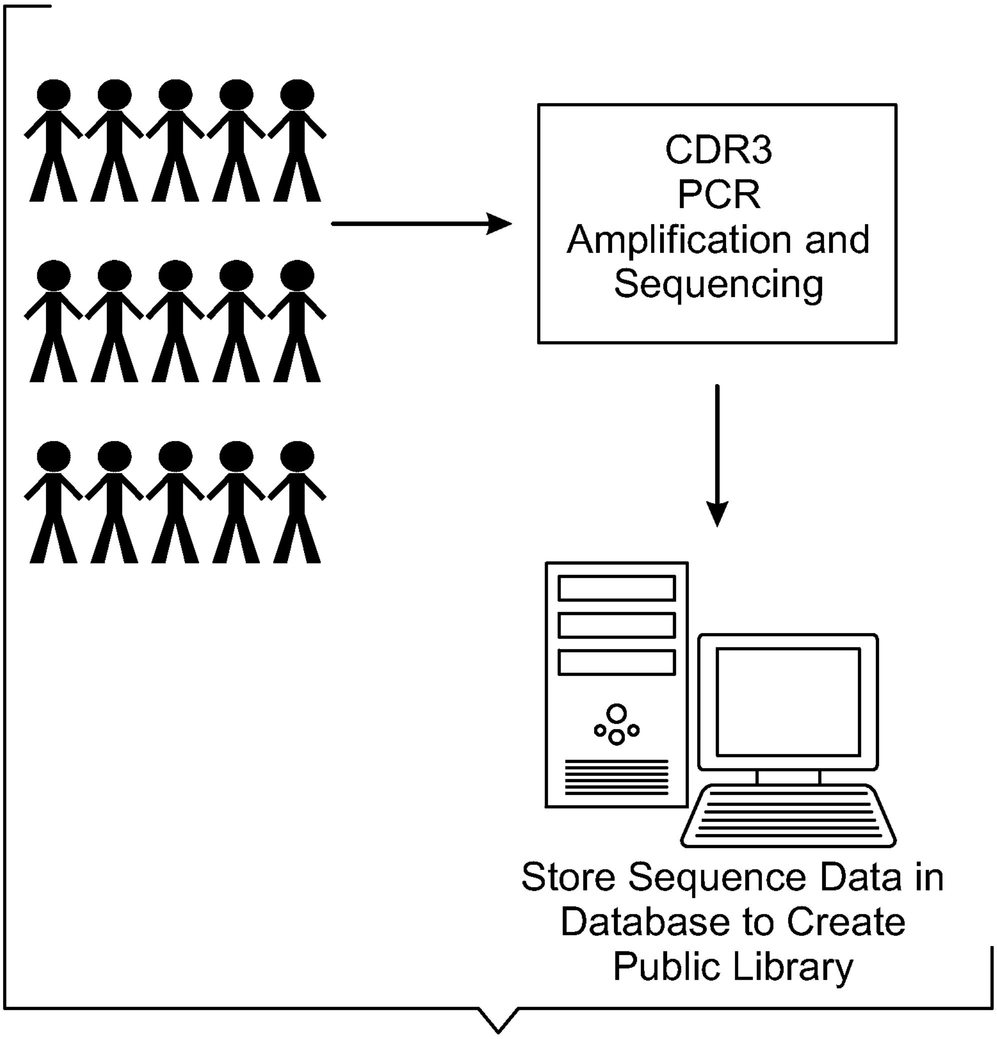

CDR3
PCR
Amplification and
Sequencing
Store Sequence Data in
Database to Create
Public Library

FIG. 3

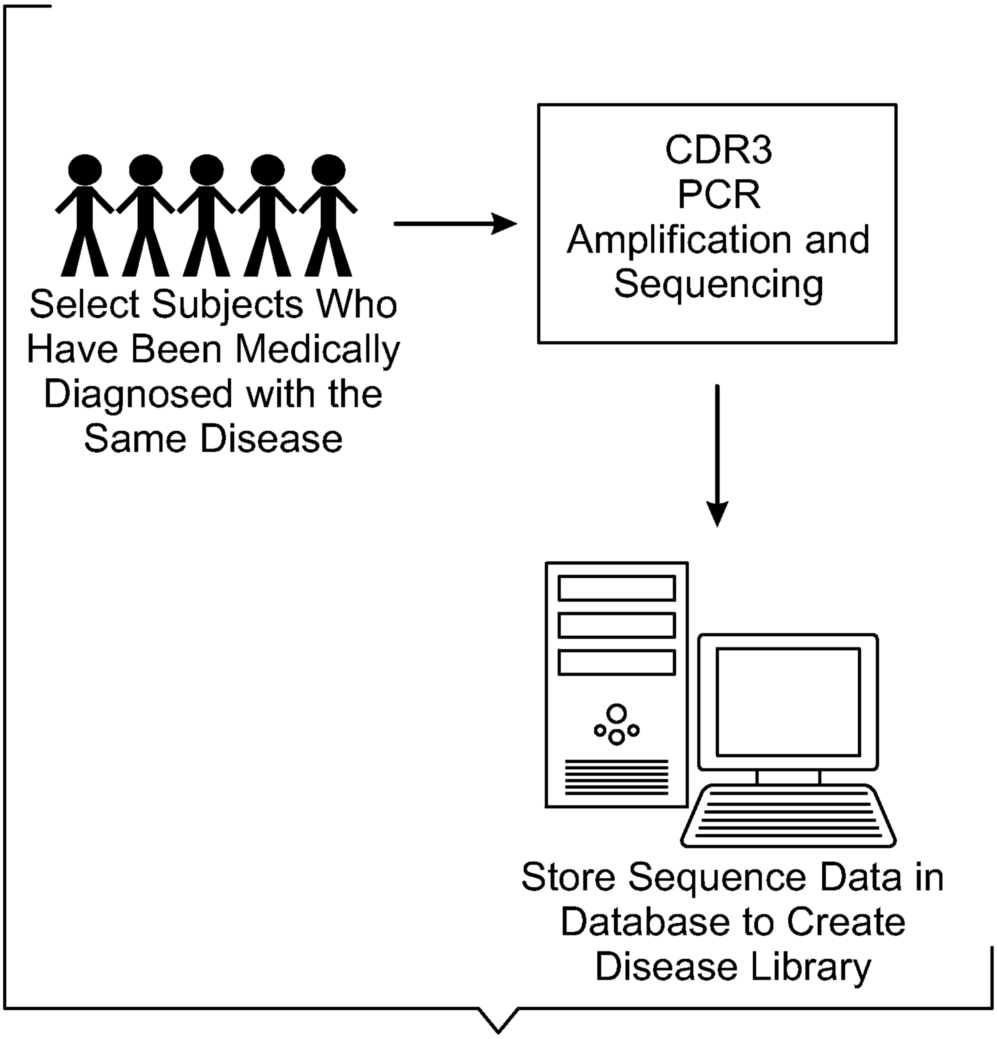

Select Subjects Who Have Been Medically Diagnosed with the Same Disease
CDR3 PCR Amplification and Sequencing
Store Sequence Data in Database to Create Disease Library

FIG. 4

CDR3	**Read count**	**Percentage**	**Shared?**
CDR3$_1$	120345	0.0602%	**Yes**
CDR3$_2$	1542	0.0008%	No
CDR3$_3$	4530	0.0023%	No
CDR3$_4$	8762	0.0044%	**Yes**
CDR3$_5$	689	0.0003%	No
CDR3$_6$	325	0.0002%	No
CDR3$_7$	8452	0.0042%	**Yes**
CDR3$_8$	23540	0.0118%	**Yes**
CDR3$_9$	3841	0.0019%	No
CDR3$_n$	20	0.0000%	No
Sum			0.0805495%

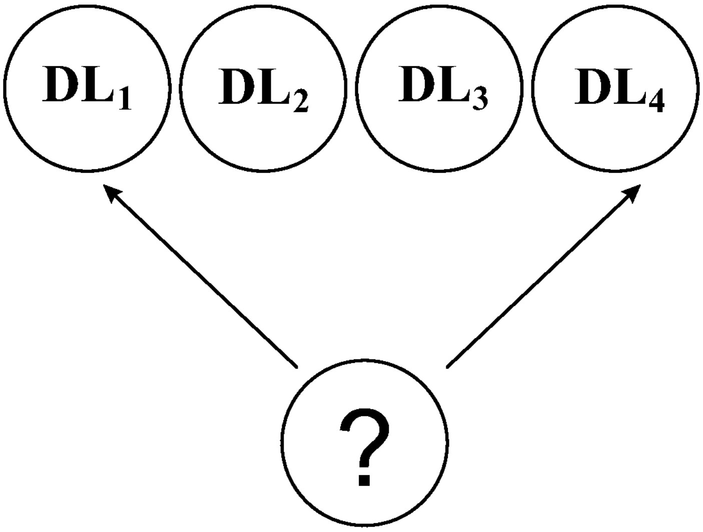
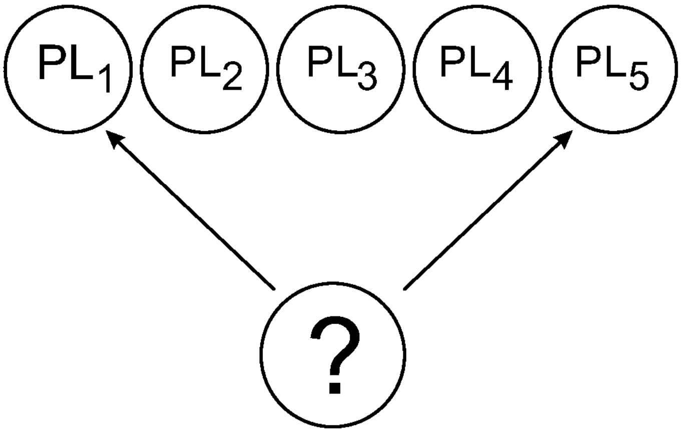


FIG. 5



CDR3	**Read count**	**Percentage**	**Shared?**
$CDR3_1$	120345	0.0602%	N
$CDR3_2$	1542	0.0008%	**Y**
$CDR3_3$	4530	0.0023%	**Y**
$CDR3_4$	8762	0.0044%	N
$CDR3_5$	689	0.0003%	**Y**
$CDR3_6$	325	0.0002%	**Y**
$CDR3_7$	8452	0.0042%	N
$CDR3_8$	23540	0.0118%	N
$CDR3_9$	3841	0.0019%	N
$CDR3_n$	20	0.0000%	**Y**
Sum			0.003553%

FIG. 6

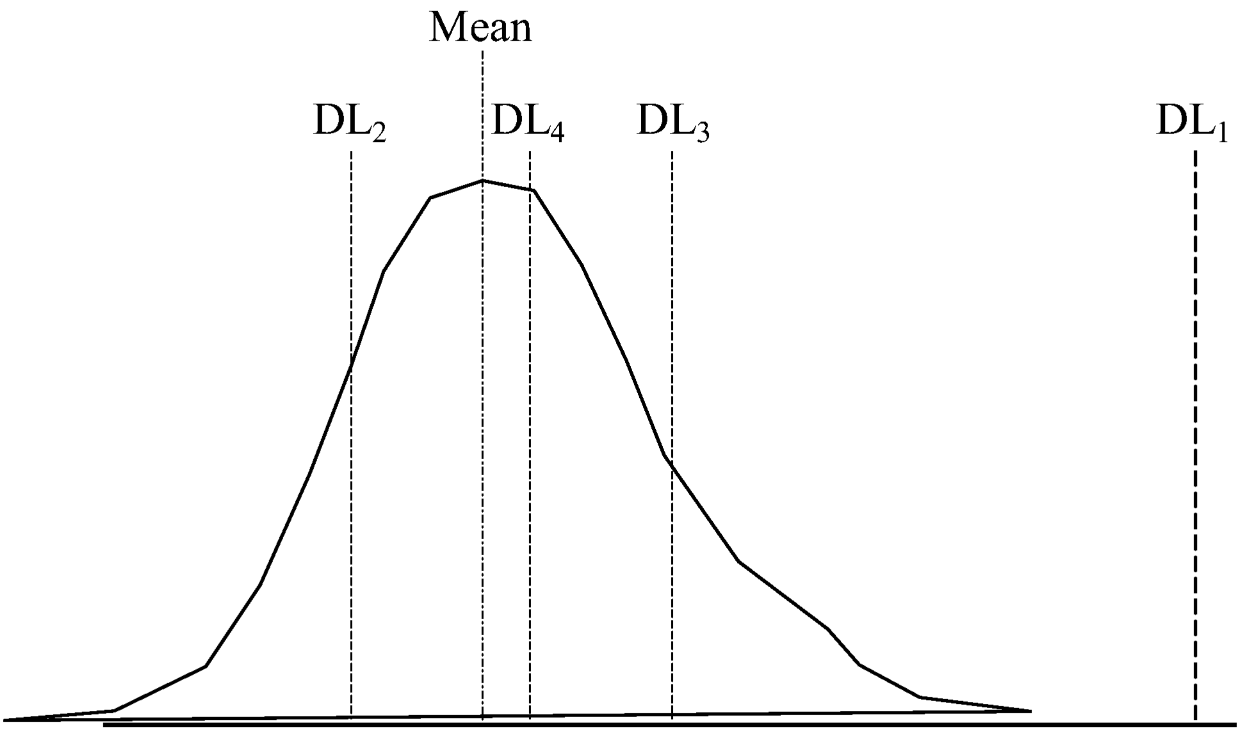


FIG. 7