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(54) Title: METHOD FOR EVALUATING AND COMPARING IMMUNOREPERTOIRES

(57) Abstract: Disclosed is a method for amplifying RNA and/or DNA from immune cell populations and using the amplified products to produce an immune response profile and 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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METHOD FOR EVALUATING AND COMPARING IMMUNOREPERTOIRES

Cross-Reference to Related Applications

[001] This application claims the benefit of priority of United States provisional patent application number 61/045,586, filed April 16, 2008.

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Field of the Invention

[002] The invention relates to methods for identifying biomarkers and to methods for identifying T-cell receptor, antibody, and MHC rearrangements in a population of cells.

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Background of the Invention

[003] 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.

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[004] 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, it has been estimated that approximately 2.5×10^7 possible antibodies or T cell receptors can be generated.

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[005] Although, at the germline level, human beings are capable of generating large numbers of diverse Igs and TRs, the number of available Igs and TRs for a particular individual is actually much smaller due to negative selection during B and T cell development. In some individuals, this process may not
5 remove some of the cells that would cross-react with the body's own tissues, and this may be the cause of some types of autoimmune diseases.

[006] 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
10 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 V_H Genes"). What is needed is a better method for evaluating changes in human immune response cells and associating those
15 changes with specific diseases.

Summary of the Invention

[007] The invention relates to a method for producing an immune status profile (ISP) for a human and/or animal. In one aspect of the invention, the
20 method comprises the steps of amplifying, in a first amplification reaction using target-specific primers, at least one RNA and/or DNA from a sample of white blood cells from at least one human or animal subject to produce at least one amplicon, at least a portion of the target-specific primers comprising additional nucleotides to incorporate into a resulting amplicon a binding site for a common
25 primer; rescuing the at least one amplicon from the first amplification reaction; amplifying, by the addition of common primers in a second amplification reaction,

the amplicons of the first amplification reaction having at least one binding site for a common primer; and sequencing the amplicons of the second amplification reaction to identify and quantify DNA sequences representing antibody and/or receptor rearrangements to create an immune status profile.

- 5 [008] In another aspect of the invention, the step of rescuing the at least one amplicon from the first amplification reaction may be omitted, and the first and second amplification reactions may occur without separation of the amplicons from the target-specific primers. Genomic DNA may also be amplified, and the step of amplifying DNA may be substituted for the step of amplifying RNA, especially in cases where analysis of an immune system component such as the major histocompatibility complex (MHC) is desired.

- [009] In aspects of the invention, subpopulations of white blood cells may be isolated 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 aspects of the method, 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, Delta, and/or Major Histocompatibility Complex (MHC) molecules I or II.

- [010] In another aspect of the invention, the method may also comprise compiling and comparing the immune cell profile for a population of normal individuals with the immune cell profile 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.

- [011] In another aspect of the invention, the method may comprise comparing the immune cell profile identified for a population of individuals to whom a vaccine has been administered with the immune cell profile for a

population of individuals to whom the vaccine was not administered to evaluate the efficacy of the vaccine in producing an immune response.

Brief Description of the Drawings

5 [012] Figure 1a and Figure 1b are photographs of gels illustrating the presence of amplification products obtained by the method of the invention using primers disclosed herein.

[013] Figure 2 illustrates distributions of domain usage for (a) Ig heavy chain in healthy control sample, (b) TCR beta chain in a blood sample from a
10 patient with colon cancer, (c) Ig kappa chain in a blood sample from a patient with Chronic Lymphocytic Leukemia (CLL), and (d) Ig lambda chain in a blood sample from a patient with Systemic lupus erythematosus (SLE). Empty spots indicate missing sequences associated with corresponding V-J combinations and the height of the column indicates the frequency of occurrence of a particular
15 sequence.

Detailed Description

[014] The inventor has developed a method for evaluating antibody and receptor rearrangements from a large number of cells, the method being useful
20 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
25 evaluating the efficacy of vaccines.

[015] The invention relates to a method for producing an immune status profile (ISP) for a human and/or animal. In one aspect of the invention, the method comprises the steps of amplifying, in a first amplification reaction using target-specific primers, at least one RNA from a sample of white blood cells from
5 at least one human or animal subject to produce at least one amplicon, at least a portion of the target-specific primers comprising additional nucleotides to incorporate into a resulting amplicon a binding site for a common primer; rescuing the at least one amplicon from the first amplification reaction; amplifying, by the addition of common primers in a second amplification reaction, the amplicons of
10 the first amplification reaction having at least one binding site for a common primer; and sequencing the amplicons of the second amplification reaction to identify and quantify DNA sequences representing antibody and/or receptor rearrangements to create an immune status profile.

[016] Where the term “comprising” is used herein, “consisting essentially
15 of” and “consisting of” may also be used. The term “immune status profile” is intended to mean a profile for an individual or population of individuals indicating the presence and/or absence of sequences representing specific rearrangements representing the diversity of B cells, T cells, and/or other cells of the human and/or animal immune system, as well as the frequency of their occurrence.
20 Where amplicons are referred to as “rescued” herein, it is to be understood that amplicon rescue may occur by the separation of amplicons from the primers which are used to create them, or may occur by dilution of the amplicon/primer mix so that, by virtue of the fact that there are significantly more amplicons than primers from a first amplification reaction, the effect of those primers is
25 minimized in a second amplification reaction using different primers. “Common primers” are those primers that may be used to amplify polynucleotides (e.g.,

amplicons from a first amplification produced by target-specific primers) having non-identical sequences in general, but sharing sequence similarities in that they contain binding sites for the same primers. Common primers are generally chosen for their efficiency at priming successful amplifications, so their use is effective for achieving higher levels of amplification in a non-target-specific manner in the method of the present invention. Common primer binding sites may be incorporated into amplicons resulting from a first amplification by attaching their sequences or their complementary sequences to the sequence of a target-specific primer. Common primers may be chosen by one of skill in the art by a variety of primer-design methods.

[017] Subpopulations of white blood cells may be isolated by flow cytometry to separate naïve B cells, mature B cells, memory B cells, naïve T cells, mature T cells, and memory T cells. Recombinations in these subpopulations of cells are generally rearrangements of B-cell immunoglobulin heavy chain (IgH), kappa and/or lambda light chains (IgK, IgL), T-cell receptor Beta, Gamma, Delta, and/or Major Histocompatibility Complex (MHC) molecules I or II.

[018] By performing an additional step, namely that of compiling and comparing the average immune status profile for a population of normal individuals with an average immune status profile for a population of individuals who have been diagnosed with a disease, it is possible to use the immune cell profile to determine if there is a correlation between a specific rearrangement or set of rearrangements and the disease.

[019] The invention also provides a method for evaluating vaccine efficacy, in terms of creating a change in the immune cell profile, by performing the steps of the method and comparing the immune cell profile identified for a population of individuals to whom a vaccine has been administered with the

immune cell profile for a population of individuals to whom the vaccine was not administered to evaluate the efficacy of the vaccine in producing an immune response.

[020] In one embodiment of the invention, a peripheral blood sample is
5 taken from a patient and isolation of 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, recombinations in the subpopulation of cells may comprise rearrangements of B-cell immunoglobulin heavy chain (IgH), kappa and/or
10 lambda light chains (IgK, IgL), T-cell receptor Beta, Gamma, Delta, or Major Histocompatibility Complex (MHC) molecules I or II.

[021] In some aspects, the step of rescuing the amplicons from the first amplification reaction may be omitted and the two amplification reactions may be performed in the same reaction tube without amplicon rescue or dilution of the
15 primers remaining from the first amplification reaction.

[022] 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 amplicon rescue multiplex polymerase chain reaction (arm-PCR), which is described in U.S.
20 PCT/US09/39552 and herein. Both the tem-PCR and 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.
25 The addition of a common primer binding site in the amplification reaction, and the subsequent amplification of target molecules using common 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

5 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

10 vaccine has achieved the desired immune response in individuals to whom the vaccine has been given.

[023] There are several commercially available high throughput sequencing technologies, such as Roche Life Sciences 454 Sequencing®. In this sequencing method, 454A and 454B primers are either linked onto PCR products

15 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 common 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

20 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

25 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.

[024] One aspect of the invention involves the development of a
5 database of immune status profiles, or “personal immunorepertoires” (PIRs), 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
10 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, many of which have as yet been unidentified. One particularly important use for the method of the invention enables studies of children to determine whether infectious disease, environmental agents, or
15 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 thimerosal in the vaccine, and studies have demonstrated that thimerosal does not appear to be a causative agent of the disease. There is still speculation that the
20 development of cocktail vaccines has correlated with the rise in the number of cases of autism, however, but 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
25 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.

[025] Imbalances of the PIR, 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 PIR diseases. There may be two PIR 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 PIR 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 PIR gained cells that produce Igs and TRs that normally should not be there. In the "loss of function" (LOF) PIR diseases, an individual does not have the appropriate functional B or T cells to fight a disease. His/her HLA typing determines that those cells are eliminated during the early stages of the immune cell maturation process, the cells generally being eliminated because they react too strongly to his/her own proteins.

[026] One aspect of the invention also comprises entering a patient immune cell profile into a database in combination with identifying information such as, 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.

[027] The described method includes a novel primer set that not only allows amplification of the entire immunorepertoire, but also allows multiplex

amplification that is semi-quantitative. Multiplex amplification requires that only a few PCR or RT-PCR reactions are needed. For example, all immunoglobulin (Ig) sequences present may be amplified in one reaction, or two or three reactions may be performed separately, using primers specific for IgH, IgL or IgK. Similarly,

5 the T-cell receptors (TRs) may be amplified in just one reaction, or may be amplified in a few reactions including the T-cell receptors designated TRA, TRB, TRD, and TRG. MHC genes may be amplified in just one PCR reaction. Semi-quantitative amplification allows all the targets in the multiplex reaction to be amplified independently, so that the end point analysis of the amplified products

10 will reflect the original internal ratio among the targets. Because this ratio is maintained, it is possible to produce an immune cell profile that indicates the presence or absence, as well as relative numbers, of various immune system cells. Amplification of RNA according to the method of the invention may be performed using any or all of the primers listed in Tables 1, 2, and/or 3. The

15 invention therefore provides a method for using at least one (which may, of course, more preferably include a least 2, at least 3, at least 4, etc.) primers chosen from among the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ

20 ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49,

25 SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59,

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10 ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118,
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20 ID NO: 295, SEQ ID NO: 296, SEQ ID NO: 297, SEQ ID NO: 298, SEQ ID NO: 299,
SEQ ID NO: 300, SEQ ID NO: 301, SEQ ID NO: 302, SEQ ID NO: 303, SEQ ID NO:
304, SEQ ID NO: 305, SEQ ID NO: 306, SEQ ID NO: 307, SEQ ID NO: 308, SEQ ID
NO: 309, SEQ ID NO: 310, SEQ ID NO: 311, SEQ ID NO: 312, and combinations
thereof, to perform a first amplification producing at least one first amplicon, at
25 least one of the target-specific primers containing additional base pairs so that
the amplification results in the addition of a binding sequence for at least one

common primer into the at least one first amplicon; amplifying the at least one first amplicon in a second amplification using at least one common primer to produce at least one second amplicon; and sequencing the at least one second amplicon to identify and quantify the sequences produced by the first and second

5 amplifications. The listed primers were designed by the inventor to provide efficient amplification of their respective RNA and/or DNA targets. Use of the entire group of primers is effective to produce a detailed immune status profile for an individual. Use of a subset may, however, be desired when specific populations of T or B cells, for example, are the subject of particular interest.

10 [028] 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 being used in the method of the invention to create a "baseline" immune status profile or personal immunorepertoire (PIR) from which future immune cell

15 profiles, created from blood samples taken during later years and analyzed by the method of the invention, may be compared. 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

20 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.

[029] The method of the invention may be especially useful for identifying commonalities between individuals with autoimmune diseases, for example, and

25 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.

[030] The method may also be useful for development of 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.

[031] 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.

[032] 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, antibody-specific primers are shown in Table 2, and HLA-specific primers are shown in Table 3. Therefore, the method may comprise using any combination of primers of Table 1, Table 2, and/or Table 3 to amplify RNA and/or DNA from a blood sample, and more particularly to identify antibodies, T-cell receptors, and HLA molecules within a population of cells. For example, an analysis of T-cell distribution might utilize all or a portion of the primers listed in Table 1 (SEQ ID NO: 1 through SEQ ID NO: 157). An analysis of Ig might utilize all or a portion of the primers listed in Table 2 (SEQ ID NO: 158 through SEQ ID NO: 225), and an analysis of HLA distribution might utilize all or a portion of the primers listed in Table 3 (SEQ ID NO: 159 through SEQ ID NO: 312).

[033] 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.

[034] To amplify the genes coding for immunoglobulin superfamily molecules, the inventor designed nested primers based on sequence information available 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 genes

within the same family. For the MHC gene locus, the intent is to amplify genomic DNA.

The invention may be further described by means of the following non-limiting examples.

5

Examples

Amplification of T or B Cell Rearrangement Sites

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

[036] 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
15 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/uL. 454A and 454B were at a concentration of 30 pmol/μL.

[037] Three different RNA samples were ordered from ALLCELLS
20 (www.allcells.com). All samples were diluted down to a final concentration of 4 ng/uL. The samples used were: ALL-PB-MNC (from a patient with acute lymphoblastic leukemia), NPB-Pan T Cells (normal T cells) and NPB-B Cells (normal B cells).

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

10 μL of Qiagen® Buffer

- 2 μ L of DNTP's
 2 μ L of Enzyme
 23.5 μ L of dH₂O
 10 μ L of the appropriate primer mix
 5 2.5 μ L of the appropriate template (10ng of RNA total)

[039] The samples were run using the following cycling conditions:

- 50°C for 30 minutes
 95°C for 15 minutes
 10 94°C for 30 seconds
 15 cycles of
 55°C for 1 minute
 72°C for 1 minute
 94°C for 15 seconds
 15 6 cycles of
 70°C for 1 minute 30 seconds
 94°C for 15 seconds
 30 cycles of
 55°C for 15 seconds
 20 72°C for 15 seconds
 72°C for 3 minutes
 4°C Hold

[040] The order of samples placed in the gel shown in Fig. 1a was: (1)

- 25 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
 30 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.

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

[042] 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 μ L 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 F_i and R_i primers were used (5 pmol each) to amplify only one specific PCR product. The following cycling conditions were used to amplify the samples:

10 95°C for 5 minutes
 30 cycles of
 94°C for 30 seconds
 72°C for 1 minute
 72°C for 3 minutes
 15 4°C hold

[043] A Qiagen PCR kit was used to amplify the products. The Master Mix used for the PCR contained the following: 5 μ L 10x PCR Buffer, 1 μ L dNTP, 0.25 μ L HotStartTaq Plus, and 39.75 μ L H₂O. (For a mix for 12 reactions: 60 μ L 10x PCR Buffer, 12 μ L dNTP, 3 μ L HotStartTaq Plus, and 477 μ L H₂O.)

[044] The photograph of the gel in Fig. 1b shows the PCR products of the following reactions: (1) Ladder; (2) TRAV1F_i+TRACR_i with alpha delta Pan T PCR product; (3) TRAV2F_i+TRACR_i 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_0 are “forward outer” primers, with R_i and R_0 indicating “reverse inner” and “reverse outer” primers, respectively.

[045] As illustrated by Fig. 1b, a single PCR product was generated
5 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
10 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. In every case, a single clone was obtained, and a specific T cell receptor V gene that matched the F_i primer was identified.

15 *Sequencing of Immune Cell RNA Using Primers of SEQ ID NO: 1 – SEQ ID NO: 312*

[046] Pan-T, pan-B, and neutrophil isolation was performed using super-paramagnetic polystyrene beads coated with monoclonal antibody specific for
20 certain cell types (Dynabeads®, Invitrogen Corp., Carlsbad, California) following manufacturer’s instructions. Anti-CD3 beads were used to isolate pan-T cells, anti-CD19 beads for pan-B cells, and anti-CD15 beads for neutrophils. Isolated cells were resuspended in 300 μ l RNeasy Protect® (Qiagen) reagent and counted using a hemacytometer.

25 [047] T cell subpopulations were isolated from a normal patient 48-year-old Asian male. PMBCs were obtained from 40 ml of whole blood collected in sodium heparin by density centrifugation over Ficoll Prep Plus Reagent. Pan-T cells were isolated from the mononuclear layer using a magnetic bead isolation

kit (Miltenyi Biotec, Auburn, California), following manufacturer's instructions.

Anti-CD4 and anti-CD25 beads were used to isolate regulatory T cells, anti-CD56 for NKT cells, anti-CD8 for cytotoxic T cells, and anti-CD4 and anti-CD294 for Th2 cells. Th1 cells were isolated via negative selection. A separate 40 ml sample of whole blood collected in sodium heparin was used to obtain naive, activated, and memory T cell subpopulations. Anti-CD45RA beads were used to isolate naive T cells, anti-CD69 for activated T cells and anti-CD45RO beads for memory T cells. Isolated cells were re-suspended in 300 µl of RNeasy Protect® reagent (Qiagen). Cells were counted using a hemacytometer.

10 [048] DNA extraction from the isolated neutrophils was performed using a QIAmp® DNA mini kit (Qiagen) using the protocol provided by the manufacturer. RNA was extracted from T cell subsets using an RNeasy® kit (Qiagen) according to the protocol provided by the manufacturer. The concentrations of extracted DNA and RNA were measured using Nanodrop® technology (Nanodrop Technologies, 15 Wilmington, Delaware). Samples were stored at -80 °C.

[049] RT-PCR was performed according to the method of the invention using nested PCR to amplify multiple targets and target-specific primers to incorporate a common primer binding sequence into the resulting amplicons in a first amplification reaction. Common primers were then used in a second 20 amplification reaction to exponentially amplify the amplicons rescued from the first amplification reaction while preserving the relative ratios of each amplicon. PCR was performed using a One-Step RT-PCR kit (Qiagen). DNA amplification for HLA typing was similarly performed, but with a multiplex PCR kit (Qiagen). Each amplicon mixture was subjected to high-throughput sequencing with the Roche 25 454 sequencing platform.

[050] More than 1.6 million effective sequences were generated for one single individual (normal 48-year-old Asian male) by sampling different subpopulations of lymphocytes in peripheral blood at different time points. Additionally, 170,734 effective sequences were generated for the colon cancer, CLL, SLE, and a second healthy patient (a 32-year-old Caucasian male). The number of unique reads generated in this study was compared to the number of unique reads existing in public databases in Table 1. The public sequence data set was compiled by searching Genbank nucleotide database with terms of 'human[orgn] AND (immunoglobulin[titl] OR T-cell receptor[titl]) AND mRNA[titl]'. In addition, the annotated IMGT/LIGM-DB (Brezinschek *et al*, 1995) cDNA sequences were gathered with a Python script. The two data sets were merged, and one copy was kept for any redundant sequences.

[051] Biased usage of V, and J gene segments in a healthy control, CLL, colon cancer, and SLE sample was analyzed. The bias of domain usage was particularly outstanding for TCR beta chain in the colon cancer sample and SLE sample, while in the healthy control sample the domain usage is quite normal without significant bias to any particular domain. It was evident that colon cancer and SLE profiles not only show clonal expansion, but demonstrate the loss of overall diversity, as well.

[052] The distribution of functional germline V, J gene segments seen in the pan-T and pan-B populations from normal patient indicated that 87.2% of potential combinations have sequences observed. Only IGHV3-d was not observed in this investigation, while TRBV4-3, IGHV3-d, IGHV4-30-4 and IGHV4-31 and IGHL3-22 were observed in other samples with extremely low frequency. Previous research did not reveal any cDNA sequence data related to IGHV3-d, which suggests that IGHV3-d may be used infrequently. Some sequences were present

in high (e.g. 1000) numbers, while others were present in significantly lower numbers. The inventor believes that higher numbers represent lymphocyte clonal expansions, reflecting the real immune responses in the subject. Studies of VH gene distribution in normal individuals have previously found the frequency of

5 usage in general to be similar to the germline complexity, while many immune responses show some level of bias in the usage of V, D and J gene segments.

Table 1

Primer	Sequence	Sequence ID Number
TRAV1Fo	5' - TGCACGTACCAGACATCTGG - 3'	SEQ ID NO: 1
TRAV1Fi	AGGTCGTTTTTCTTCATTCC	SEQ ID NO: 2
TRAV2Fo	TCTGTAATCACTCTGTGTCC	SEQ ID NO: 3
TRAV2Fi	AGGGACGATACAACATGACC	SEQ ID NO: 4
TRAV3Fo	CTATTCACTCTCTGGAAACC	SEQ ID NO: 5
TRAV3Fi	ATACATCACAGGGGATAACC	SEQ ID NO: 6
TRAV4Fo	TGTAGCCACAACAACATTGC	SEQ ID NO: 7
TRAV4Fi	AAAGTTACAAACGAAGTGGC	SEQ ID NO: 8
TRAV5Fo	GCACTTACACAGACAGCTCC	SEQ ID NO: 9
TRAV5Fi	TATGGACATGAAACAAGACC	SEQ ID NO: 10
TRAV6Fo	GCAACTATACAACTATTCC	SEQ ID NO: 11
TRAV6Fi	GTTTTCTTGCTACTCATACG	SEQ ID NO: 12
TRAV7Fo	TGCACGTACTCTGTCACTCG	SEQ ID NO: 13
TRAV7Fi	GGATATGAGAAGCAGAAAGG	SEQ ID NO: 14
TRAV8Fo	AATCTCTTCTGGTATGTSCA	SEQ ID NO: 15
TRAV8Fi	GGYTTTGAGGCTGAATTTA	SEQ ID NO: 16
TRAV9Fo	GTCCAATATCCTGGAGAAGG	SEQ ID NO: 17
TRAV9Fi	AACCACTTCTTTCCACTTGG	SEQ ID NO: 18
TRAV10Fo	AATGCAATTATACAGTGAGC	SEQ ID NO: 19
TRAV10Fi	TGAGAACACAAAGTCGAACG	SEQ ID NO: 20
TRAV11Fo	TCTTAATTGTACTTATCAGG	SEQ ID NO: 21
TRAV11Fi	TCAATCAAGCCAGAAGGAGC	SEQ ID NO: 22
TRAV12Fo	TCAGTGTTCCAGAGGGAGCC	SEQ ID NO: 23
TRAV12Fi	ATGGAAGGTTTACAGCACAG	SEQ ID NO: 24
TRAV13Fo	ACCCTGAGTGTCCAGGAGGG	SEQ ID NO: 25
TRAV13Fi	TTATAGACATTCGTTCAAAT	SEQ ID NO: 26

TRAV14Fo	TGGACTGCACATATGACACC	SEQ ID NO: 27
TRAV14Fi	CAGCAAAATGCAACAGAAGG	SEQ ID NO: 28
TRAV16Fo	AGCTGAAGTGCAACTATTCC	SEQ ID NO: 29
TRAV16Fi	TCTAGAGAGAGCATCAAAGG	SEQ ID NO: 30
TRAV17Fo	AATGCCACCATGAACTGCAG	SEQ ID NO: 31
TRAV17Fi	GAAAGAGAGAAACACAGTGG	SEQ ID NO: 32
TRAV18Fo	GCTCTGACATTAACTGCAC	SEQ ID NO: 33
TRAV18Fi	CAGGAGACGGACAGCAGAGG	SEQ ID NO: 34
TRAV19Fo	ATGTGACCTTGGACTGTGTG	SEQ ID NO: 35
TRAV19Fi	GAGCAAAATGAAATAAGTGG	SEQ ID NO: 36
TRAV20Fo	ACTGCAGTTACACAGTCAGC	SEQ ID NO: 37
TRAV20Fi	AGAAAGAAAGGCTAAAGCC	SEQ ID NO: 38
TRAV21Fo	ACTGCAGTTTCACTGATAGC	SEQ ID NO: 39
TRAV21Fi	CAAGTGGAAGACTTAATGCC	SEQ ID NO: 40
TRAV22Fo	GGGAGCCAATTCCACGCTGC	SEQ ID NO: 41
TRAV22Fi	ATGGAAGATTAAGCGCCACG	SEQ ID NO: 42
TRAV23Fo	ATTTCAATTATAAACTGTGC	SEQ ID NO: 43
TRAV23Fi	AAGGAAGATTCACAATCTCC	SEQ ID NO: 44
TRAV24Fo	GCACCAATTTACCTGCAGC	SEQ ID NO: 45
TRAV24Fi	AGGACGAATAAGTGCCACTC	SEQ ID NO: 46
TRAV25Fo	TCACCACGTACTGCAATTCC	SEQ ID NO: 47
TRAV25Fi	AGACTGACATTTTCAGTTTGG	SEQ ID NO: 48
TRAV26Fo	TCGACAGATTCMCTCCCAGG	SEQ ID NO: 49
TRAV26Fi	GTCCAGYACCTTGATCCTGC	SEQ ID NO: 50
TRAV27Fo	CCTCAAGTGTTTTTTTCCAGC	SEQ ID NO: 51
TRAV27Fi	GTGACAGTAGTTACGGGTGG	SEQ ID NO: 52
TRAV29Fo	CAGCATGTTTGATTATTTCC	SEQ ID NO: 53
TRAV29Fi	ATCTATAAGTTCCATTAAGG	SEQ ID NO: 54
TRAV30Fo	CTCCAAGGCTTTTATATTCTG	SEQ ID NO: 55
TRAV30Fi	ATGATATTACTGAAGGGTGG	SEQ ID NO: 56
TRAV34Fo	ACTGCACGTCATCAAAGACG	SEQ ID NO: 57
TRAV34Fi	TTGATGATGCTACAGAAAGG	SEQ ID NO: 58
TRAV35Fo	TGAACTGCACTTCTTCAAGC	SEQ ID NO: 59
TRAV35Fi	CTTGATAGCCTTATATAAGG	SEQ ID NO: 60
TRAV36Fo	TCAATTGCAGTTATGAAGTG	SEQ ID NO: 61
TRAV36Fi	TTTATGCTAACTTCAAGTGG	SEQ ID NO: 62
TRAV38Fo	GCACATATGACACCAGTGAG	SEQ ID NO: 63
TRAV38Fi	TCGCCAAGAAGCTTATAAGC	SEQ ID NO: 64
TRAV39Fo	TCTACTGCAATTATTCAACC	SEQ ID NO: 65
TRAV39Fi	CAGGAGGGACGATTAATGGC	SEQ ID NO: 66
TRAV40Fo	TGAACTGCACATACACATCC	SEQ ID NO: 67
TRAV40Fi	ACAGCAAAAACCTTCGGAGGC	SEQ ID NO: 68
TRAV41Fo	AACTGCAGTTACTCGGTAGG	SEQ ID NO: 69

TRAV41Fi	AAGCATGGAAGATTAATTGC	SEQ ID NO: 70
TRACRo	GCAGACAGACTTGTCACTGG	SEQ ID NO: 71
TRACRi	AGTCTCTCAGCTGGTACACG	SEQ ID NO: 72
TRBV1Fo	AATGAAACGTGAGCATCTGG	SEQ ID NO: 73
TRBV1Fi	CATTGAAAACAAGACTGTGC	SEQ ID NO: 74
TRBV2Fo	GTGTCCCCATCTCTAATCAC	SEQ ID NO: 75
TRBV2Fi	TGAAATCTCAGAGAAGTCTG	SEQ ID NO: 76
TRBV3Fo	TATGTATTGGTATAAACAGG	SEQ ID NO: 77
TRBV3Fi	CTCTAAGAAATTTCTGAAGA	SEQ ID NO: 78
TRBV4Fo	GTCTTTGAAATGTGAACAAC	SEQ ID NO: 79
TRBV4Fi	GGAGCTCATGTTTGTCTACA	SEQ ID NO: 80
TRBV5Fo	GATCAAAACGAGAGGACAGC	SEQ ID NO: 81
TRBV5aFi	CAGGGGCCCCAGTTTATCTT	SEQ ID NO: 82
TRBV5bFi	GAAACARAGGAACTTCCCT	SEQ ID NO: 83
TRBV6aFo	GTGTGCCCAGGATATGAACC	SEQ ID NO: 84
TRBV6bFo	CAGGATATGAGACATAATGC	SEQ ID NO: 85
TRBV6aFi	GGTATCGACAAGACCCAGGC	SEQ ID NO: 86
TRBV6bFi	TAGACAAGATCTAGGACTGG	SEQ ID NO: 87
TRBV7Fo	CTCAGGTGTGATCCAATTTTC	SEQ ID NO: 88
TRBV7aFi	TCTAATTTACTTCCAAGGCA	SEQ ID NO: 89
TRBV7bFi	TCCCAGAGTGATGCTCAACG	SEQ ID NO: 90
TRBV7cFi	ACTTACTTCAATTATGAAGC	SEQ ID NO: 91
TRBV7dFi	CCAGAATGAAGCTCAACTAG	SEQ ID NO: 92
TRBV9Fo	GAGACCTCTCTGTGTACTGG	SEQ ID NO: 93
TRBV9Fi	CTCATTCAGTATTATAATGG	SEQ ID NO: 94
TRBV10Fo	GGAATCACCCAGAGCCCAAG	SEQ ID NO: 95
TRBV10Fi	GACATGGGCTGAGGCTGATC	SEQ ID NO: 96
TRBV11Fo	CCTAAGGATCGATTTTCTGC	SEQ ID NO: 97
TRBV11Fi	ACTCTCAAGATCCAGCCTGC	SEQ ID NO: 98
TRBV12Fo	AGGTGACAGAGATGGGACAA	SEQ ID NO: 99
TRBV12aFi	TGCAGGGACTGGAATTGCTG	SEQ ID NO: 100
TRBV12bFi	GTACAGACAGACCATGATGC	SEQ ID NO: 101
TRBV13Fo	CTATCCTATCCCTAGACACG	SEQ ID NO: 102
TRBV13Fi	AAGATGCAGAGCGATAAAGG	SEQ ID NO: 103
TRBV14Fo	AGATGTGACCCAATTTCTGG	SEQ ID NO: 104
TRBV14Fi	AGTCTAAACAGGATGAGTCC	SEQ ID NO: 105
TRBV15Fo	TCAGACTTTGAACCATAACG	SEQ ID NO: 106
TRBV15Fi	AAAGATTTTAACAATGAAGC	SEQ ID NO: 107
TRBV16Fo	TATTGTGCCCCAATAAAAGG	SEQ ID NO: 108
TRBV16Fi	AATGTCTTTGATGAAACAGG	SEQ ID NO: 109
TRBV17Fo	ATCCATCTTCTGGTCACATG	SEQ ID NO: 110
TRBV17Fi	AACATTGCAGTTGATTTCAGG	SEQ ID NO: 111

TRBV18Fo	GCAGCCCAATGAAAGGACAC	SEQ ID NO: 112
TRBV18Fi	AATATCATAGATGAGTCAGG	SEQ ID NO: 113
TRBV19Fo	TGAACAGAATTTGAACCACG	SEQ ID NO: 114
TRBV19Fi	TTTCAGAAAGGAGATATAGC	SEQ ID NO: 115
TRBV20Fo	TCGAGTGCCGTTCCCTGGAC	SEQ ID NO: 116
TRBV20Fi	GATGGCAACTTCCAATGAGG	SEQ ID NO: 117
TRBV21Fo	GCAAAGATGGATTGTGTTCC	SEQ ID NO: 118
TRBV21Fi	CGCTGGAAGAAGAGCTCAAG	SEQ ID NO: 119
TRBV23Fo	CATTTGGTCAAAGGAAAAGG	SEQ ID NO: 120
TRBV23Fi	GAATGAACAAGTTCTTCAAG	SEQ ID NO: 121
TRBV24Fo	ATGCTGGAATGTTCTCAGAC	SEQ ID NO: 122
TRBV24Fi	GTCAAAGATATAAACAAAGG	SEQ ID NO: 123
TRBV25Fo	CTCTGGAATGTTCTCAAACC	SEQ ID NO: 124
TRBV25Fi	TAATTCCACAGAGAAGGGAG	SEQ ID NO: 125
TRBV26Fo	CCCAGAATATGAATCATGTT	SEQ ID NO: 126
TRBV26Fi	ATTCACCTGGCACTGGGAGC	SEQ ID NO: 127
TRBV27Fo	TTGTTCTCAGAATATGAACC	SEQ ID NO: 128
TRBV27Fi	TGAGGTGACTGATAAGGGAG	SEQ ID NO: 129
TRBV28Fo	ATGTGTCCAGGATATGGACC	SEQ ID NO: 130
TRBV28Fi	AAAAGGAGATATTCTTGAGG	SEQ ID NO: 131
TRBV29Fo	TCACCATGATGTTCTGGTAC	SEQ ID NO: 132
TRBV29Fi	CTGGACAGAGCCTGACACTG	SEQ ID NO: 133
TRBV30Fo	TGTGGAGGGAACATCAAACC	SEQ ID NO: 134
TRBV30Fi	TTCTACTCCGTTGGTATTGG	SEQ ID NO: 135
TRBCRo	GTGTGGCCTTTTGGGTGTGG	SEQ ID NO: 136
TRBCRi	TCTGATGGCTCAAACACAGC	SEQ ID NO: 137
TRDV1Fo	TGTATGAAACAAGTTGGTGG	SEQ ID NO: 138
TRDV1Fi	CAGAATGCAAAAAGTGGTCG	SEQ ID NO: 139
TRDV2Fo	ATGAAAGGAGAAGCGATCGG	SEQ ID NO: 140
TRDV2Fi	TGGTTTCAAAGACAATTTCC	SEQ ID NO: 141
TRDV3Fo	GACACTGTATATTCAAATCC	SEQ ID NO: 142
TRDV3Fi	GCAGATTTTACTCAAGGACG	SEQ ID NO: 143
TRDCRo	AGACAAGCGACATTTGTTCC	SEQ ID NO: 144
TRDCRi	ACGGATGGTTTGGTATGAGG	SEQ ID NO: 145
TRGV1-5Fo	GGGTCATCTGCTGAAATCAC	SEQ ID NO: 146
TRGV1-5,8Fi	AGGAGGGGAAGGCCCCACAG	SEQ ID NO: 147
TRGV8Fo	GGGTCATCAGCTGTAATCAC	SEQ ID NO: 148
TRGV5pFi	AGGAGGGGAAGACCCACAG	SEQ ID NO: 149

TRGV9Fo	AGCCCGCCTGGAATGTGTGG	SEQ ID NO: 150
TRGV9Fi	GCACTGTCAGAAAGGAATCC	SEQ ID NO: 151
TRGV10Fo	AAGAAAAGTATTGACATACC	SEQ ID NO: 152
TRGV10Fi	ATATTGTCTCAACAAAATCC	SEQ ID NO: 153
TRGV11Fo	AGAGTGCCACATATCTTGG	SEQ ID NO: 154
TRGV11Fi	GCTCAAGATTGCTCAGGTGG	SEQ ID NO: 155
TRGCRo	GGATCCCAGAATCGTGTTGC	SEQ ID NO: 156
TRGCRi	GGTATGTTCCAGCCTTCTGG	SEQ ID NO: 157

Table 2

Primer	Sequence	Sequence ID Number
IgHV1aFo	AGTGAAGGTCTCCTGCAAGG	SEQ ID NO: 158
IgHV1bFo	AGTGAAGGTTTCCTGCAAGG	SEQ ID NO: 159
IgHV1aFi	AGTTCCAGGGCAGAGTCAC	SEQ ID NO: 160
IgHV1bFi	AGTTTCAGGGCAGGGTCAC	SEQ ID NO: 161
IgHV1cFi	AGTTCCAGGAAAGAGTCAC	SEQ ID NO: 162
IgHV1dFi	AATTCAGGACAGAGTCAC	SEQ ID NO: 163
IgHV2Fo	TCTCTGGGTCTCTCACTCAGC	SEQ ID NO: 164
IgHV2Fi	AAGGCCCTGGAGTGGCTTGC	SEQ ID NO: 165
IgHV3aFo	TCCCTGAGACTCTCCTGTGC	SEQ ID NO: 166
IgHV3bFo	CTCTCCTGTGCAGCCTCTGG	SEQ ID NO: 167
IgHV3cFo	GGTCCCTGAGACTCTCCTGT	SEQ ID NO: 168
IgHV3dFo	CTGAGACTCTCCTGTGTAGC	SEQ ID NO: 169
IgHV3aFi	CTCCAGGGAAGGGGCTGG	SEQ ID NO: 170
IgHV3bFi	GGCTCCAGGCAAGGGGCT	SEQ ID NO: 171
IgHV3cFi	ACTGGGTCCGCCAGGCTCC	SEQ ID NO: 172
IgHV3dFi	GAAGGGGCTGGAGTGGGT	SEQ ID NO: 173
IgHV3eFi	AAAAGGTCTGGAGTGGGT	SEQ ID NO: 174
IgHV4Fo	AGACCCTGTCCCTCACCTGC	SEQ ID NO: 175
IgHV4Fi	AGGGVCTGGAGTGGATTGGG	SEQ ID NO: 176
IgHV5Fo	GCGCCAGATGCCCGGAAAG	SEQ ID NO: 177
IgHV5Fi	GGCCASGTCACCATCTCAGC	SEQ ID NO: 178
IgHV6Fo	CCGGGGACAGTGTCTCTAGC	SEQ ID NO: 179
IgHV6Fi	GCCTTGAGTGGCTGGGAAGG	SEQ ID NO: 180
IgHV7Fo	GTTTCCTGCAAGGCTTCTGG	SEQ ID NO: 181
IgHV7Fi	GGCTTGAGTGGATGGGATGG	SEQ ID NO: 182
IgHJRo	ACCTGAGGAGACGGTGACC	SEQ ID NO: 183
IgHJ1Ri	CAGTGCTGGAAGTATTGAGC	SEQ ID NO: 184
IgHJ2Ri	AGAGATCGAAGTACCAGTAG	SEQ ID NO: 185

IgHJ3Ri	CCCCAGATATCAAAAGCATC	SEQ ID NO: 186
IgHJ4Ri	GGCCCCAGTAGTCAAAGTAG	SEQ ID NO: 187
IgHJ5Ri	CCCAGGGGTCGAACCAAGTTG	SEQ ID NO: 188
IgHJ6Ri	CCCAGACGTCCATGTAGTAG	SEQ ID NO: 189
IgKV1Fo	TAGGAGACAGAGTCACCATC	SEQ ID NO: 190
IgKV1Fi	TTCAGYGRCAAGTGGATCTGG	SEQ ID NO: 191
IgKV2Fo	GGAGAGCCGGCCTCCATCTC	SEQ ID NO: 192
IgKV2aFi	TGGTACCTGCAGAAGCCAGG	SEQ ID NO: 193
IgKV2bFi	CTTCAGCAGAGGCCAGGCCA	SEQ ID NO: 194
IgKV3-7Fo	GCCTGGTACCAGCAGAAACC	SEQ ID NO: 195
IgKV3Fi	GCCAGGTTCAAGTGGCAGTGG	SEQ ID NO: 196
IgKV6-7Fi	TCGAGGTTCAAGTGGCAGTGG	SEQ ID NO: 197
IgKV4-5Fi	GACCGATTCAAGTGGCAGCGG	SEQ ID NO: 198
IgKCRo	TTCAACTGCTCATCAGATGG	SEQ ID NO: 199
IgKCRi	ATGAAGACAGATGGTGCAGC	SEQ ID NO: 200
IgLV1aFo	GGGCAGAGGGTCACCATCTC	SEQ ID NO: 201
IgLV1bFo	GGACAGAAGGTCAACCATCTC	SEQ ID NO: 202
IgLV1aFi	TGGTACCAGCAGCTCCCAGG	SEQ ID NO: 203
IgLV1bFi	TGGTACCAGCAGCTTCCAGG	SEQ ID NO: 204
IgLV2Fo	CTGCACTGGAACCAGCAGTG	SEQ ID NO: 205
IgLV2Fi	TCTCTGGCTCCAAGTCTGGC	SEQ ID NO: 206
IgLV3aFo	ACCAGCAGAAGCCAGGCCAG	SEQ ID NO: 207
IgLV3bFo	GAAGCCAGGACAGGCCCTG	SEQ ID NO: 208
IgLV3aFi	CTGAGCGATTCTCTGGCTCC	SEQ ID NO: 209
IgLV3bFi	TTCTCTGGGTCCACCTCAGG	SEQ ID NO: 210
IgLV3cFi	TTCTCTGGGTCCAGCTCAGG	SEQ ID NO: 211
IgLV4Fo	TCGGTCAAGCTCACCTGCAC	SEQ ID NO: 212
IgLV4Fi	GGGCTGACCGCTACCTCACC	SEQ ID NO: 213
IgLV5Fo	CAGCCTGTGCTGACTCAGCC	SEQ ID NO: 214
IgLV5Fi	CCAGCCGCTTCTCTGGATCC	SEQ ID NO: 215
IgLV6Fo	CCATCTCCTGCACCCGAGC	SEQ ID NO: 216
IgLV7-8Fo	TCCCCWGGAGGGACAGTCAC	SEQ ID NO: 217
IgLV9, 11Fo	CTCMCTGCACCCTGAGCAG	SEQ ID NO: 218
IgLV10Fo	AGACCGCCACACTCACCTGC	SEQ ID NO: 219
IgLV6, 8Fi	CTGATCGSTTCTCTGGCTCC	SEQ ID NO: 220
IgLV7Fi	CTGCCCCGGTTCTCAGGCTCC	SEQ ID NO: 221
IgLV9Fi	ATCCAGGAAGAGGATGAGAG	SEQ ID NO: 222
IgLV10- 11Fi	CTCCAGCCTGAGGACGAGGC	SEQ ID NO: 223

IgLC1-7Ro	GCTCCCGGGTAGAAGTCACT	SEQ ID NO: 224
IgLC1-7Ri	AGTGTGGCCTTGTTGGCTTG	SEQ ID NO: 225

Table 3

5

Primer	Sequence	Sequence ID Number
HLAI Fo11	CCCACTCCATGAGGTATTTTC	SEQ ID NO: 226
HLAI Fo12	CCTACTCCATGAGGTATTTTC	SEQ ID NO: 227
HLAI Fo31	GCGGGGAGCCCCGCTTCATC	SEQ ID NO: 228
HLAI Fo32	GCGGGAAGCCCCGCTTCATC	SEQ ID NO: 229
HLAI Fo33	GTGGAGAGCCCCGCTTCATC	SEQ ID NO: 230
HLAI Fo34	GCGGAAAGCCCCGCTTCATC	SEQ ID NO: 231
HLAI Fo35	GCGGAAAGCCCCACTTCATC	SEQ ID NO: 232
HLAI Fo36	GCGGGAAGCCCCACTTCATC	SEQ ID NO: 233
HLAI Fi11	GTGGGCTACGTGGACGACAC	SEQ ID NO: 234
HLAI Fi12	GTGGGCTACGTGGACGGCAC	SEQ ID NO: 235
HLAI- Fi21	GTTCGTGCGGTTTCGACAGCG	SEQ ID NO: 236
HLAI- Fi22	GTTCGTGCGGTTTGACAGCG	SEQ ID NO: 237
HLAI- Fi23	GTTCGTGAGGTTTCGACAGCG	SEQ ID NO: 238
HLAI Ri11	TAATCCTTGCCGTCGTAGGC	SEQ ID NO: 239
HLAI Ri12	TAATCCTTGCCGTCGTAAGC	SEQ ID NO: 240
HLAI Ri13	TAATCTTTGCCGTCGTAGGC	SEQ ID NO: 241
HLAI Ro11	GGTCCTCGTTCAGGGCGATG	SEQ ID NO: 242
HLAI Ro12	GGTCCTCTTTCAGGGCGATG	SEQ ID NO: 243
HLAI Ro13	GGTCCTCGTTCAAGGCGATG	SEQ ID NO: 244
HLAI Ro14	GATCCTCGTTCAGGGCGATG	SEQ ID NO: 245

HLAI Ro15	GGTCCTCATTCAGGGCGATG	SEQ ID NO: 246
HLAI- Fo41	GCCTACGACGGCAAGGATTA	SEQ ID NO: 247
HLAI- Fo42	GCTTACGACGGCAAGGATTA	SEQ ID NO: 248
HLAI- Fo43	GCCTACGACGGCAAAGATTA	SEQ ID NO: 249
HLAI- Fi31	CATCGCCCTGAACGAGGACC	SEQ ID NO: 250
HLAI- Fi32	CATCGCCCTGAAAGAGGACC	SEQ ID NO: 251
HLAI- Fi33	CATCGCCTTGAACGAGGACC	SEQ ID NO: 252
HLAI- Fi34	CATCGCCCTGAACGAGGATC	SEQ ID NO: 253
HLAI- Fi35	CATCGCCCTGAATGAGGACC	SEQ ID NO: 254
HLAI- Ri21	GGTATCTGCGGAGCCCGTCC	SEQ ID NO: 255
HLAI- Ri22	GGTATCTGCGGAGCCACTCC	SEQ ID NO: 256
HLAI- Ri23	GGTGTCTGCGGAGCCACTCC	SEQ ID NO: 257
HLAI- Ri24	GGTATCCGCGGAGCCACTCC	SEQ ID NO: 258
HLAI- Ro21	GCAGCGTCTCCTTCCCGTTC	SEQ ID NO: 259
HLAI- Ro22	CCAGCTTGTCCTTCCCGTTC	SEQ ID NO: 260
HLAI- Ro23	CCAGCGTGTCTTCCCGTTC	SEQ ID NO: 261
HLAI- Ro24	GCAGCGTCTCCTTCCCATTTC	SEQ ID NO: 262
HLAI- Ro25	GCAGCGTCTCCTTCKKGTTTC	SEQ ID NO: 263
DRB17 Fo11	TGTCATTTCTTCAATGGGAC	SEQ ID NO: 264
DRB1 Fo12	AGTGTCAATTTCTTCAACGGG	SEQ ID NO: 265
DRB1 Fo13	GTGTTATTTCTTCAATGGGA	SEQ ID NO: 266
DRB1 Fo14	GTGTCAATTCTTCAATGGGA	SEQ ID NO: 267
DRB4 Fo	GTGTCATTTCTTCAATGGGA	SEQ ID NO: 268
DRB1 Fi11	GGAGCGGGTGCGGTTGCTGG	SEQ ID NO: 269
DRB1	GGAGCGGGTGCGGTACCTGG	SEQ ID NO: 270

Fi12		
DRB1 Fi13	GGAGCGGGTGCGGTTCTGG	SEQ ID NO: 271
DRB1 Fi14	GGAGCGGGTGCGATTCTGG	SEQ ID NO: 272
DRB1 Fi15	GGAGCGGGTGCGGTATCTGC	SEQ ID NO: 273
DRB1 Fi16	GGAGCGGGTGCGGTTACTGG	SEQ ID NO: 274
DRB45 Fi	ACATCTATAACCAAGAGGAG	SEQ ID NO: 275
DRB6 Fi	ACATCCATAAACGGGAGGAG	SEQ ID NO: 276
DRB7 Fi	TATAACCAAGAGGAGTACGT	SEQ ID NO: 277
DRB Ri11	AACCCCGTAGTTGTGTCTGC	SEQ ID NO: 278
DRB4 Ri	AACCCCGTAGTTGTATCTGC	SEQ ID NO: 279
DRB6 Ri	CGTAATTGTATCTGCAGTAG	SEQ ID NO: 280
DRB7 Ri	TAGTTGTCCACTTCGGCCCG	SEQ ID NO: 281
DRB Ro1	CGCTGCACTGTGAAGCTCTC	SEQ ID NO: 282
DRB Ro2	CGCTGCACCGTGAAGCTCTC	SEQ ID NO: 283
DRA Fo	CCTGTGGAAGTGAAGAGGCC	SEQ ID NO: 284
DRA Fi	CAACGTCTCATCTGTTTCA	SEQ ID NO: 285
DRA Ri	CTGCTGCATTGCTTTTGCGC	SEQ ID NO: 286
DRA Ro	TTACAGAGGCCCTGCGTT	SEQ ID NO: 287
DPB Fo	GTCCAGGGCAGGGCCACTCC	SEQ ID NO: 288
DPB Fi11	AATTACGTGTACCAGGGACG	SEQ ID NO: 289
DPB Fi12	AATTACCTTTTCCAGGGACG	SEQ ID NO: 290
DPB Fi13	AATTACGTGTACCAGTTACG	SEQ ID NO: 291
DPB Fi14	AATTACGCGTACCAGTTACG	SEQ ID NO: 292
DPB Ri11	CGGCCTCGTCCAGCTCGTAG	SEQ ID NO: 293
DPB Ri12	TGGGCCCCGCCAGCTCGTAG	SEQ ID NO: 294
DPB Ri13	TGGGCCCCGACCAGCTCGTAG	SEQ ID NO: 295
DPB Ro	GGACTCGGCGCTGCAGGGTC	SEQ ID NO: 296
DPA Fo	AGGAGCTGGGGCCATCAAGG	SEQ ID NO: 297
DPA Fi	GACCATGTGTCAACTTATGC	SEQ ID NO: 298
DPA Ri1	CTCAGGGGGATCGTTGGTGG	SEQ ID NO: 299
DPA Ri2	CTCAGGGGGATCATTGGCGG	SEQ ID NO: 300
DPA Ro	CAGCTCCACAGGCTCCTTGG	SEQ ID NO: 301
DQB Fo	ACTCTCCCGAGGATTTCTGTG	SEQ ID NO: 302
DQB Fi	TGTGCTACTTCACCAACGGG	SEQ ID NO: 303
DQB Ri1	ACCTCGTAGTTGTGTCTGCA	SEQ ID NO: 304
DQB Ri2	AACTGGTAGTTGTGTCTGCA	SEQ ID NO: 305
DQB Ro1	ACTCTCCTCTGCAGGATCCC	SEQ ID NO: 306
DQB Ro2	ACTCGCCGCTGCAAGGTCGT	SEQ ID NO: 307
DQB Ro3	ACTCTCCTCTGCAAGATCCC	SEQ ID NO: 308
DQA Fo	TGGTGTAAACTTGTACCAGT	SEQ ID NO: 309
DQA Fi	ACCCATGAATTTGATGGAGA	SEQ ID NO: 310
DQA Ri	GGAACCTCATTTGGTAGCAGC	SEQ ID NO: 311
DQA Ro	ACTTGGAAAACACTGTGACC	SEQ ID NO: 312

What is claimed is:

1. A method for producing an immune status profile for a human and/or animal, the method comprising
 - (a) amplifying, in a first amplification using target-specific primers, at
5 least one DNA and/or RNA from a sample of white blood cells from a human or animal subject to produce at least one first amplicon, at least a portion of the target-specific primers comprising additional nucleotides to incorporate into the at least one first amplicon a binding site for a common primer;
 - 10 (b) rescuing the at least one first amplicon from the first amplification;
 - (c) amplifying, in a second amplification using at least one common primer, the at least one first amplicon to produce at least one second amplicon; and
 - (d) sequencing the at least one second amplicon to identify and
15 quantify one or more DNA sequences representing rearrangements to create an immune status profile.
2. The method of claim 1 further comprising the steps of (e) inputting the one or more DNA sequences into a database to provide data which may be stored on a computer, server, or other electronic storage device, (f) inputting a data set of
20 identifying information and characteristics for an individual corresponding to the sequences as data which may be stored on a computer, server, or other electronic storage device, and (g) evaluating the data of step (e) and step (f) for one or more individuals to determine whether a correlation exists between the one or more sequences and one or more characteristics of the individual corresponding to the
25 one or more sequences.

3. The method of claim 1 wherein the target-specific primers are chosen from among the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 151, SEQ ID NO:

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 304, SEQ ID NO: 305, SEQ ID NO: 306, SEQ ID NO: 307, SEQ ID NO: 308, SEQ ID
 NO: 309, SEQ ID NO: 310, SEQ ID NO: 311, SEQ ID NO: 312, and combinations
 thereof.
- 10 4. A method for producing an immune response profile, the method
 comprising:
- amplifying RNA and/or DNA from a human or animal subject using target-
 specific primers chosen from among the group consisting of SEQ ID NO: 1,
 SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6,
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SEQ ID NO: 307, SEQ ID NO: 308, SEQ ID NO: 309, SEQ ID NO: 310, SEQ
25 ID NO: 311, SEQ ID NO: 312, and combinations thereof to perform a first
amplification producing at least one first amplicon, at least one of the

- target-specific primers containing additional base pairs so that the amplification results in the addition of a binding sequence for at least one common primer into the at least one first amplicon;
- amplifying the at least one first amplicon in a second amplification using at
- 5 least one common primer to produce at least one second amplicon; and
- sequencing the at least one second amplicon to identify and quantify the sequences produced by the first and second amplifications.

Fig. 1

1 2 3 4 5 6 7 8 9 10 11



Fig. 1a

Fig. 1b

1 2 3 4 5 6 7 8 9 10 11 12



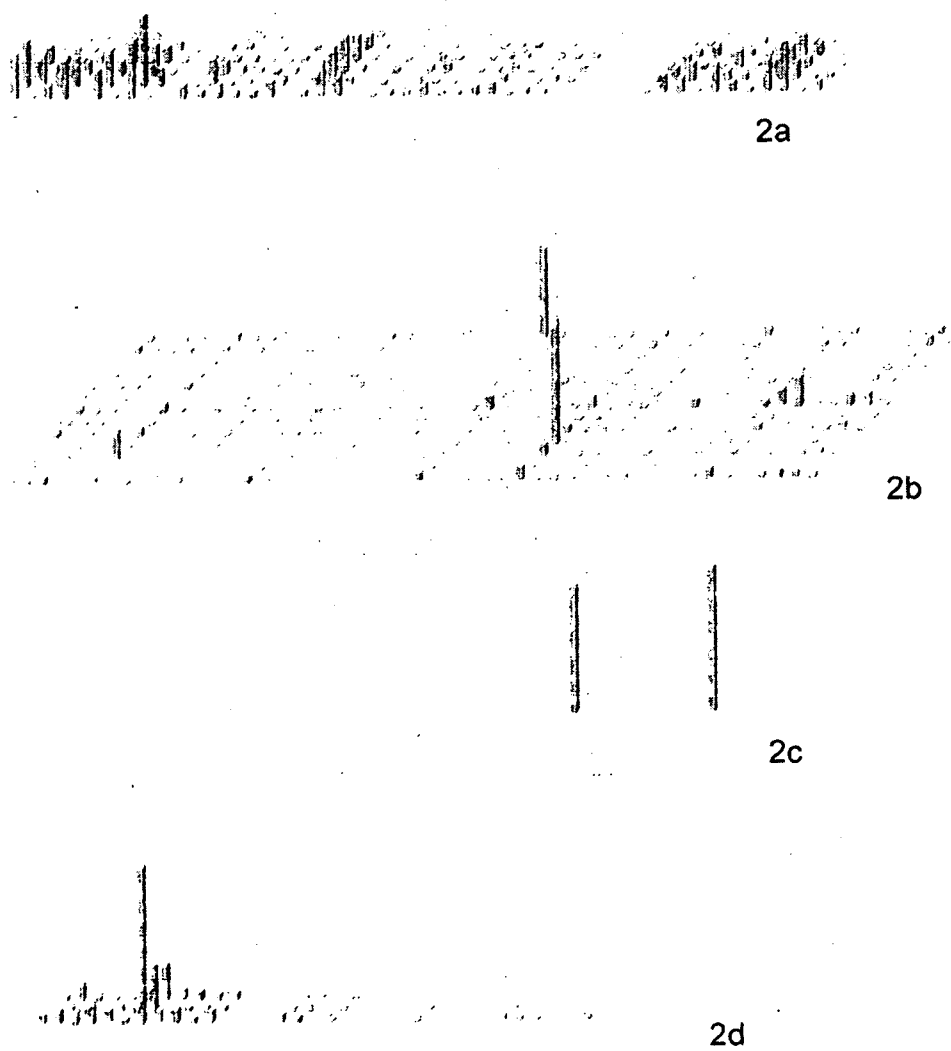


Fig. 2