

IMMUNOREPERTOIRE NORMALITY ASSESSMENT METHOD AND ITS USE**CROSS REFERENCE TO RELATED APPLICATION**

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/234,424, entitled "Immunorepertoire Normality Assessment Method and Its Use" and filed on September 29, 2015, which is incorporated herein by reference.

FIELD OF INVENTION

[0002] The present disclosure relates to methods for performing diagnostic tests. More specifically, the present disclosure relates to diagnostic tests for the assessment of immunorepertoire diversity relative to a standard index.

BACKGROUND OF INVENTION

[0003] Diagnostic tests may be used to detect the presence or absence of the normal state in a patient, and the results of these tests may be used to screen patients and collectively aid in diagnosis. For example, a normal white blood cell count is between 4,500 and 10,000 cells per microliter. An elevated white blood cell count is not determinative for a specific disease, but it may indicate an underlying problem that requires medical evaluation. Normal ranges of red blood cell counts for women and men are generally different, with a count of 5 to 6 million per microliter being normal for males and 3.6 to 5.6 million being normal for females. Platelet counts are normal if they are within the range of 150,000 to 400,000. In the presence of inflammation, for example, red cell count may go down, white cell count may go up, and platelet count may also be elevated.

[0004] Similarly, heart disease, the tendency to have heart disease, signs of certain cancers, and a variety of genetic diseases may have as their early signs one or more abnormal results for a variety of diagnostic tests. Such diagnostic tests include, for example, measures of albumin, alkaline phosphatase, alanine transaminase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), serum calcium, serum chloride, carbon dioxide, creatinine, direct bilirubin, gamma-glutamyl-transpeptidase (gamma-GT), glucose, lactate dehydrogenase (LDH), serum phosphorus, potassium, serum sodium, total bilirubin, total cholesterol, total protein, and uric acid.

[0005] In addition, blood glucose levels may also be used as early indicators associated with diseases as varied as Cushing syndrome, hyperthyroidism, pancreatic cancer, pancreatitis, pre-diabetes, and diabetes.

[0006] As described above, diagnostic tests are currently available and performed on a regular basis to detect the presence or absence of a normal state in an individual. These tests, however, do not address the diversity of the immunorepertoire in a patient. The ability to detect the presence or absence of a diverse immunorepertoire, relative to a standard, in an individual may assist with an assessment of the health of such individual.

SUMMARY OF INVENTION

[0007] The present disclosure relates to a method for determining the immunorepertoire diversity in a patient. The method comprises: quantifying the expression of third complementarity-determining region ("CDR3") in a population or sub-population of immune system cells in a patient sample; and identifying the percentage of pCDR3 represented by such CDR3s within that population or sub-population, wherein normal immunorepertoire diversity is characterized by the presence of a minimum percentage of pCDR3 and an abnormal immunorepertoire diversity status is characterized by the absence of a minimum percentage of pCDR3.

[0007a] In one aspect, the present invention provides for a method for using immunorepertoire diversity to assess a normal or abnormal status of an individual patient's immunorepertoire, the method comprising the steps of:

(a) determining the pCDR3 sequences, the commonly-shared CDR3 sequences identified from a reference group of individuals, comprising comparing each sample from the reference group and identifying those CDR3 sequences that are most-frequently shared within said reference group;

(b) using primers directed to pCDR3 sequences to detect the presence in the patient's immunorepertoire of individual CDR3s within that shared group of pCDR3 sequences; and

(c) calculating the normality index, comprising dividing the number of pCDR3 sequences from the reference group by the number of pCDR3 sequences present in the patient's immunorepertoire; and

(d) assessing the status of the patient's immunorepertoire, wherein the normality index is compared with a predetermined minimum percentage of

shared pCDR3 sequences, such that the immunorepertoire of the patient is considered normal when the normality index meets or exceeds the minimum percentage of shared pCDR3 sequences and the immunorepertoire of the patient is considered abnormal when the normality index is below the minimum percentage of shared pCDR3 sequences.

[0007b] In another aspect, the present invention provides a method for using immunorepertoire diversity to assess a normal or abnormal status of an individual patient's immunorepertoire, the method comprising:

- (a) amplifying polynucleotides from a population of white blood cells from a human or animal subject in a reaction mix comprising target-specific nested primers to produce a set of first amplicons, at least a portion of the target-specific nested primers comprising additional nucleotides which, during amplification, serve as a template for incorporating into the first amplicons a binding site for at least one common primer;

- (b) transferring a portion of the first reaction mix containing the first amplicons to a second reaction mix comprising at least one common primer;

- (c) amplifying, using the at least one common primer, the first amplicons to produce a set of second amplicons;

- (d) sequencing the second amplicons to identify CDR3 sequences in the subpopulation of white blood cells;

- (e) determining the pCDR3 sequences, the commonly-shared CDR3 sequences identified from a reference group of individuals, comprising comparing each sample from the reference group and identifying those CDR3 sequences that are most-frequently shared within said reference group;

- (f) using the identified CDR3 sequences to calculate the normality index, comprising dividing the number of pCDR3 sequences from the reference group by the number of pCDR3 sequences present in the patient's identified CDR3 sequences; and

- (g) assessing the status of the patient's immunorepertoire, wherein the normality index is compared with a predetermined minimum percentage of shared pCDR3 sequences, such that the immunorepertoire of the patient is considered normal when the normality index meets or exceeds the minimum percentage of shared pCDR3 sequences and the immunorepertoire of the patient

is considered abnormal when the normality index is below the minimum percentage of shared pCDR3 sequences.

[0008] In certain examples, the minimum percentage of pCDR3 in such method is a percentage number selected from about 25 percent to about 75 percent. In other examples, the minimum percentage of pCDR3 is about 25 percent. In other examples, the pCDR3 is composed of about the 1000 most-frequently-shared CDR3. In other examples, the pCDR3 is composed of more than 1000 of the most-frequently-shared CDR3. In yet other examples, the pCDR3 is composed of fewer than 1000 of the most-frequently-shared CDR3. In other examples, the pCDR3 is composed of CDR3 shared by at least 25 percent of a pool of individuals.

[0009] In certain examples, the pCDR3 is composed of CDR3 shared by at least 50 percent of a pool of individuals. In certain examples, such pool contains at least 100 individuals. In other examples, such pool contains about 1000 individuals.

[0010] The present disclosure also relates to a method for determining the immunorepertoire diversity in a patient. The method comprises: amplifying polynucleotides from a population of white blood cells from a human or animal subject in a reaction mix comprising target-specific nested primers to produce a set of first

amplicons, at least a portion of the target-specific nested primers comprising additional nucleotides which, during amplification, serve as a template for incorporating into the first amplicons a binding site for at least one common primer; transferring a portion of the first reaction mix containing the first amplicons to a second reaction mix comprising at least one common primer; amplifying, using the at least one common primer, the first amplicons to produce a set of second amplicons; sequencing the second amplicons to identify CDR3 sequences in the subpopulation of white blood cells; using the identified CDR3 sequences to quantify the percentage of pCDR3 represented by the sample to provide a normality index; and identifying whether the normality index is normal or abnormal, wherein a normal state is characterized by the presence of a minimum percentage of pCDR3 and an abnormal state is characterized by the absence of a minimum percentage of pCDR3.

[0011] In certain examples, such second method measures a population of white blood cells, wherein the population includes about 100,000 randomly-selected cells. In certain examples, the method is repeated about 10 to 100 times, each time with a random selection of cells, to determine the average percentage of pCDR3 expressed by the patient. In certain examples, the minimum percentage of pCDR3 is a percentage number from about 25 to about 75. In certain examples, the pCDR3 is composed of at least the 1000 most-frequently-shared CDR3. In certain examples, the pCDR3 includes SEQ ID NO:1 through SEQ ID NO: 4014 as described herein. For example, the normality index of a patient may be determined based on the percentage of pCDR3 sequences, represented by SEQ ID NO: 1 through SEQ ID NO: 4014, present in a predetermined number of reads of a patient sample. In certain examples, the pCDR3 is composed of CDR3 shared by at least 50 percent of a pool of individuals.

[0012] The reference pool of individuals may be different sizes. In certain examples, the reference pool includes at least 100 individuals. In certain embodiments, the pool contains about 1000 individuals. In certain examples, the pool is composed of healthy control individuals, whereas in other examples, the pool is composed of individuals with one or more specific disease states. The reference pool may further be controlled to account for certain variables of a given patient. In certain examples, the pool of individuals is approximately age-matched to the patient. In other examples, the pool of individuals is gender-matched to the patient.

[0013] In various aspects of the present disclosure, the immune system cells that are analyzed, under any method described herein, may include, for example, all T cells

[panT], functional subsets of T cells such as CD8+ T cells [cytotoxic T (Ta)], CD4+ T cells and their subsets [TH1, TH2, TH17, regulatory T (reg) and follicular T (TFH)], or developmental subsets of T cells such as naive T (Tn), activated T (Ta), memory T (Tm), all B cells (panB) and their subsets such as naive B (Br), activated B (Ba), memory B (Bm), plasma and plasmablast B cells. Such cells may be obtained from peripheral blood or other sources known in the art.

[0014] It will be apparent to those skilled in the art that only the preferred embodiments have been described by way of exemplification and that there are various modifications which fall within the scope of the present disclosure. These and other aspects of the present disclosure will be discussed in greater detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Fig. 1 shows four plots overlaid into a single graph, representing (from top to bottom) the number of total reads, the number of unique CDR3s, the number of pCDR3s, and the normality index.

DETAILED DESCRIPTION

[0016] The inventor has developed a method for determining the diversity of a patient's immunorepertoire by comparing the CDR3 sequences in a patient sample to the most commonly-shared CDR3 sequences (i.e., the pCDR3) of an index group of individuals. This percentage of pCDR3 in a patient's sample is referred to as the "normality index" and may serve as a diagnostic indicator of the immunorepertoire diversity in such patient. The present disclosure further includes a method for determining such subset of highly-shared pCDR3. In one aspect of the present disclosure, the immunorepertoire of a patient is considered normal if the patient's normality index meets or exceeds a minimum percentage, whereas the immunorepertoire of the patient is considered abnormal if the patient's normality index is below such minimum percentage. As used herein, "patient" may refer to either a human or an animal.

[0017] The CDR3 expressed by individuals exhibits tremendous diversity, with up to 10^{15} unique CDR3 possible. As such, the present disclosure uses CDR3 as a basis for immune system diversity. The inventor has determined, based on a sampling of 75 million CDR3, that approximately 81% of randomly-selected CDR3 are unique to a given individual and are not shared among multiple individuals. The present disclosure, however, provides

a method for determining a pool of highly-shared CDR3, thereby enabling a standard index of shared CDR3 by which the diversity of an individual's immunorepertoire may be compared.

[0018] As used herein, "abnormal" means the absence of a minimum percentage of pCDR3 sequences measured in a patient. As such, abnormal indicates a difference from the reference group of individuals used to generate the pCDR3. An abnormal immunorepertoire may, but does not necessarily, indicate the presence or absence of disease in the patient.

[0019] As used herein, "immunorepertoire" means the set of distinct CDR3 sequences detected in the lymphocytes of an individual or individuals, as applicable.

[0020] The method of the present disclosure may be performed using the following steps to identify a normal immune status or an abnormal immune status in a patient, the method comprising the steps of: (a) amplifying polynucleotides from a population of white blood cells from a patient in a reaction mix comprising target-specific nested primers to produce a set of first amplicons, at least a portion of the target-specific nested primers comprising additional nucleotides which, during amplification, serve as a template for incorporating into the first amplicons a binding site for at least one common primer; (b) transferring a portion of the first reaction mix containing the first amplicons to a second reaction mix comprising at least one common primer; (c) amplifying, using the at least one common primer, the first amplicons to produce a set of second amplicons; (d) sequencing the second amplicons to identify CDR3 sequences in the subpopulation of white blood cells, and (e) using the identified CDR3 sequences to quantify the percentage of pCDR3 represented by the sample to provide a normality index; and (f) identifying whether the normality index is normal or abnormal, wherein a normal state is characterized by the presence of a minimum percentage of pCDR3 and an abnormal state is characterized by the absence of a minimum percentage of pCDR3. As shown in FIG. 1, a patient's normality index, pCDR3s and unique CDR3s are correlated.

[0021] In certain embodiments, the sequencing includes about 100,000 reads taken per sample. In certain embodiments, the reads are performed multiple times, for example about 10 to 100 times, using random selection, to obtain an average percentage of pCDR3 sequences present in the sample. The resulting number is divided by the number of pCDR3 in the reference pool to obtain a percentage, referred to as the "normality index," which is a number between 0% and 100%. For example, if a patient sample contains 400 of 1000 pCDR3 sequences, then the patient's normality index is 0.40 or 40%. In other

embodiments at least 10,000 reads are taken. In other embodiments, more than 100,000 reads are taken. In other embodiments, the reads are performed less than 10 times. In other embodiments, the reads are performed more than 100 times.

[0022] In certain embodiments, the index pool is composed of about 1000 individuals. In other embodiments, the index pool contains between 100 and 1000 individuals. In other embodiments, the index pool contains fewer than 100 individuals. In other embodiments, the index pool contains more than 1000 individuals. Relative to the patient, the individuals may be age-matched, gender-matched, healthy, disease-matched, and/or other criteria commonly known in the art when controlling for variables. In certain embodiments, the index pool is composed of healthy controls. In other embodiments, the index pool is composed of a mix of healthy controls and individuals with one or more disease states. In other embodiments, the index pool is composed of individuals with one or more particular disease states.

[0023] In certain embodiments, the CDR3 sequences shared by the index pool (i.e., the pCDR3) are determined by comparing each sample from the index pool and identifying those CDR3s that are shared by at least 50% of the individuals tested in such reference pool. In certain embodiments, the pCDR3 includes about the top 1000 shared CDR3 sequences. In other embodiments, the pCDR3 include at least 100 CDR3 sequences. In other embodiments, the pCDR3 includes more than 1000 CDR3 sequences.

[0024] In certain embodiments, the minimum percentage of pCDR3, used to determine the normal or abnormal state of the immunorepertoire, is about 25%. In other embodiments, the minimum percentage of pCDR3 is between 25% and 75%.

[0025] It has previously been difficult to assess the immune system in a broad manner, because the number and variety of cells in a human or animal immune system is so large that sequencing of more than a small subset of cells has been almost impossible. The inventor developed a semi-quantitative PCR method (arm-PCR, described in more detail in U.S. Patent Application Publication Number 20090253183), which provides increased sensitivity and specificity over previously-available methods, while producing semi-quantitative results. It is this ability to increase specificity and sensitivity, and thereby increase the number of targets detectable within a single sample that makes the method ideal for detecting relative numbers of clonotypes of the immunorepertoire. The inventor has more recently discovered that using this sequencing method allows comparison of a patient's CDR3 sequences to those commonly shared by an index group, which has led to the development of the present method. The method may be used to evaluate the diversity

of the immunorepertoire of subjects relative to an index pool of individuals. For example, the inventor has demonstrated that the presence of disease correlates with decreased immunorepertoire diversity, for example a decrease in the diversity of CDR3 sequences, which can be readily detected using the method of the present disclosure. This method may therefore be useful as an initial diagnostic indicator, much as cell counts and biochemical tests are currently used in clinical practice, of normal versus abnormal immunorepertoire diversity.

[0026] Clonotypes (i.e., clonal types) of an immunorepertoire are determined by the rearrangement of Variable(V), Diverse(D) and Joining(J) gene segments through somatic recombination in the early stages of immunoglobulin (Ig) and T cell receptor (TCR) production of the immune system. The V(D)J rearrangement can be amplified and detected from T cell receptor alpha, beta, gamma, and delta chains, as well as from immunoglobulin heavy chain (IgH) and light chains (IgK, IgL). Cells may be obtained from a patient by obtaining peripheral blood, lymphoid tissue, cancer tissue, or tissue or fluids from other organs and/or organ systems, for example. Techniques for obtaining these samples, such as blood samples, are known to those of skill in the art. Cell counts may be extrapolated from the number of sequences detected by PCR amplification and sequencing.

[0027] The CDR3 region, comprising about 30-90 nucleotides, encompasses the junction of the recombined variable (V), diversity (D) and joining (J) segments of the gene. It encodes the binding specificity of the receptor and is useful as a sequence tag to identify unique V(D)J rearrangements.

[0028] Wang et al. disclosed that PCR may be used to obtain quantitative or semi-quantitative assessments of the numbers of target molecules in a specimen (Wang, M. et al, "Quantitation of mRNA by the polymerase chain reaction," (1989) Proc. Nat'l. Acad. Sci. 86: 9717-9721). Particularly effective methods for achieving quantitative amplification have been described previously by the inventor. One such method is known as arm-PCR, which is described in United States Patent Application Publication Number 20090253183A1.

[0029] Aspects of the present disclosure include arm-PCR amplification of CDR3 from T cells, B cells, and/or subsets of T or B cells. Such cell types may be sorted and isolated using techniques known in the art including, but not limited to, FACS sorting and magnetic bead sorting. The term "population" of cells, as used herein, therefore encompasses what are generally referred to as either "populations" or "sub-populations" of cells. Large numbers of amplified products may then be efficiently sequenced using next-generation sequencing using platforms such as 454 or Illumina, for example.

[0030]

The arm-PCR method provides highly sensitive, semi-quantitative amplification of multiple polynucleotides in one reaction. The arm-PCR method may also be performed by automated methods in a closed cassette system (iCubate®, Huntsville, Alabama), which is beneficial in the present method because the repertoires of various T and B cells, for example, are so large. In the arm-PCR method, target numbers are increased in a reaction driven by DNA polymerase, which is the result of target-specific primers being introduced into the reaction. An additional result of this amplification reaction is the introduction of binding sites for common primers which will be used in a subsequent amplification by transferring a portion of the first reaction mix containing the first set of amplicons to a second reaction mix comprising common primers. "At least one common primer," as used herein, refers to at least one primer that will bind to such a binding site, and includes pairs of primers, such as forward and reverse primers. This transfer may be performed either by recovering a portion of the reaction mix from the first amplification reaction and introducing that sample into a second reaction tube or chamber, or by removing a portion of the liquid from the completed first amplification, leaving behind a portion, and adding fresh reagents into the tube in which the first amplification was performed. In either case, additional buffers, polymerase, etc., may then be added in conjunction with the common primers to produce amplified products for detection. The amplification of target molecules using common primers gives a semi-quantitative result wherein the quantitative numbers of targets amplified in the first amplification are amplified using common, rather than target-specific primers – making it possible to produce significantly higher numbers of targets for detection and to determine the relative amounts of the cells comprising various rearrangements within a patient blood sample. Also, combining the second reaction mix with a portion of the first reaction mix allows for higher concentrations of target-specific primers to be added to the first reaction mix, resulting in greater sensitivity in the first amplification reaction. It is the combination of specificity and sensitivity, along with the ability to achieve quantitative results by use of a method such as the arm-PCR method, which allows a sufficiently sensitive and quantitative assessment of the CDR3 expressed in a population of cells to produce a normality index that is of diagnostic use.

[0031]

Clonal expansion due to recognition of antigen results in a larger population of cells that recognize that antigen, potentially including antibody-producing B cells or receptor-bearing T cells. This may cause the reads taken pursuant to the method disclosed herein to be biased in favor of the antigen-specific expansion, thereby reducing the percentage of pCDR3 sequences detected. Therefore, a relatively low normality index, for example one

below the minimum percentage, may be indicative of the expansion of a particular population of cells that is prevalent in individuals who have been diagnosed with a particular disease or in individuals recently-vaccinated against a particular antigen.

[0032] Primers for amplifying and sequencing variable regions of immune system cells are available commercially, and have been described in publication such as the inventor's published patent applications W02009137255 and US201000021896A1.

[0033] There are several commercially available high-throughput sequencing technologies, such as Hoffman-LaRoche, Inc.'s 454® sequencing system. In the 454® sequencing method, for example, the A and B adaptor are linked onto PCR products either during PCR or ligated on after the PCR reaction. The adaptors are used for amplification and sequencing steps. When done in conjunction with the arm-PCR technique, A and B adaptors may be used as common primers (which are sometimes referred to as "communal primers" or "superprimers") in the amplification reactions. After A and B adaptors have been physically attached to a sample library (such as PCR amplicons), a single-stranded DNA library is prepared using techniques known to those of skill in the art. The single-stranded DNA library is immobilized onto specifically-designed DNA capture beads. Each bead carries a unique singled-stranded DNA library fragment. The bead-bound library is emulsified with amplification reagents in a water-in-oil mixture, producing microreactors, each containing just one bead with one unique sample-library fragment. Each unique sample library fragment is amplified within its own microreactor, excluding competing or contaminating sequences. Amplification of the entire fragment collection is done in parallel. For each fragment, this results in copy numbers of several million per bead. Subsequently, the emulsion PCR is broken while the amplified fragments remain bound to their specific beads. The clonally amplified fragments are enriched and loaded onto a PicoTiterPlate® device for sequencing. The diameter of the PicoTiterPlate® wells allows for only one bead per well. After addition of sequencing enzymes, the fluidics subsystem of the sequencing instrument flows individual nucleotides in a fixed order across the hundreds of thousands of wells each containing a single bead. Addition of one (or more) nucleotide(s) complementary to the template strand results in a chemilluminescent signal recorded by a CCD camera within the instrument. The combination of signal intensity and positional information generated across the PicoTiterPlate® device allows the software to determine the sequence of more than 1,000,000 individual reads, each is up to about 450 base pairs, with the GS FLX system.

[0034] Having obtained the sequences using a quantitative and/or semi-quantitative method, it is then possible to calculate the normality index, for example, by determining the percentage of pCDR3 represented by a predetermined number of reads of a patient sample. Each patient's normality index may be compared to a predetermined threshold to determine whether the patient's normality index falls within the normal range, and therefore is normal, or below the threshold, and thereby is abnormal.

[0035] The method of the present disclosure provides a physician with an additional clinical test for diagnostic purposes to determine whether a patient's immunorepertoire is abnormal. Further, the method of the present disclosure, particularly if used in an automated system such as that described by the inventor in U.S. Patent Application Publication Number 201000291668A1, may be used to analyze samples from multiple patients, with detection of the amplified targets sequences being accomplished by the use of one or more microarrays.

Examples

Patient Samples

[0036] Whole blood samples (40 ml) collected in sodium heparin or peripheral blood mononuclear cells (PBMCs) were obtained from 1100 individuals, representing a mixed population of both healthy individuals and those with disease. The 1100 individuals were placed randomly into 11 different groups with 100 samples per group.

RNA extraction and repertoire amplification

[0037] RNA extraction was performed using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. For each target, a set of nested sequence-specific primers (Forward-out, Fo; Forward-in, Fi; Reverse-out, Ro; and Reverse-in, Ri) was designed using primer software available at www.irepertoire.com. A pair of common sequence tags was linked to all internal primers (Fi and Ri). Once these tag sequences were incorporated into the PCR products in the first few amplification cycles, the exponential phase of the amplification was carried out with a pair of communal primers. In the first round of amplification, only sequence-specific nested primers were used. The nested primers were then removed by exonuclease digestion and the first-round PCR products were used as templates for a second round of amplification by adding communal primers and a mixture of fresh enzyme and dNTP. Each distinct barcode tag was introduced into amplicon from the same sample through PCR primer.

Sequencing

[0038] Barcode tagged amplicon products from different samples were pooled together and loaded into a 2% agarose gel. Following electrophoresis, DNA fragments were purified from DNA band corresponding to 250-500bp fragments extracted from agarose gel. DNA was sequenced using the 454 GS FLX system with titanium kits (SeqWright, Inc.).

Sequencing data analysis

[0039] Sequences for each sample were sorted out according to barcode tag. Following sequence separation, sequence analysis was performed in a manner similar to the approach reported by Wang et al. (Wang C, et al. High throughput sequencing reveals a complex pattern of dynamic interrelationships among human T cell subsets. Proc Natl Acad Sci USA 107(4): 1518-1523). Briefly, germline V and J reference sequences, which were downloaded from the IMGT server (<http://www.imgt.org>), were mapped onto sequence reads using the program IRmap. The boundaries defining CDR3 region in reference sequences were mirrored onto sequencing reads through mapping information. The enclosed CDR3 regions in sequencing reads were extracted and translated into amino acid sequence.

Determination of pCDR3

[0040] Sequences obtained from each of the 11 groups were compared to determine which CDR3 sequences were detected in at least 50% of the samples in each group. The resulting CDR3 sequences were sorted such that the top 4014 most commonly shared CDR3 sequences were selected as the pCDR3 for purposes of calculating a normality index for patient samples. The CDR3 sequences selected for inclusion in the pCDR3 – SEQ ID NO:1 through SEQ ID NO: 4014 – are shown in Table I.

TABLE I

Sequence	Sequence ID No	Sequence	Sequence ID No
ASSLGETQY	SEQ ID NO: 1	ASSLGYEQY	SEQ ID NO: 7
ASSLSTDQY	SEQ ID NO: 2	ASSLGDTQY	SEQ ID NO: 8
ASSLGTDTQY	SEQ ID NO: 3	ASSLGGNTEAF	SEQ ID NO: 9
ASSPSTDQY	SEQ ID NO: 4	ASSLGGNQPQH	SEQ ID NO: 10
ASSLQETQY	SEQ ID NO: 5	ASSLEETQY	SEQ ID NO: 11
ASSLTDQY	SEQ ID NO: 6	ASSLDSNQPQH	SEQ ID NO: 12
ASSLGQNTEAF	SEQ ID NO: 13	ASSLSDQY	SEQ ID NO: 54
ASSLRETQY	SEQ ID NO: 14	ASSLTVNTEAF	SEQ ID NO: 55
ASSLADQY	SEQ ID NO: 15	ASSPPSTDQY	SEQ ID NO: 56
ASSLSYEQY	SEQ ID NO: 16	ASSLAGNTEAF	SEQ ID NO: 57
ASSLGSNQPQH	SEQ ID NO: 17	ASSLGGSYEQY	SEQ ID NO: 58
ASSSSYEQY	SEQ ID NO: 18	ASSPGQGYEQY	SEQ ID NO: 59

ASSSQETQY	SEQ ID NO: 19	ASSLGQGNTAF	SEQ ID NO: 60
ASSPQETQY	SEQ ID NO: 20	ASSLGGSNQPQH	SEQ ID NO: 61
ASSLGGTEAF	SEQ ID NO: 21	ASSFQETQY	SEQ ID NO: 62
ASSSTDTQY	SEQ ID NO: 22	ASSLQGYEQY	SEQ ID NO: 63
ASSLGGTDTQY	SEQ ID NO: 23	ASSLGADTQY	SEQ ID NO: 64
ASSLQGNTEAF	SEQ ID NO: 24	ASSLTGGTEAF	SEQ ID NO: 65
ASSLAGGDTQY	SEQ ID NO: 25	ASSLGQGNQPQH	SEQ ID NO: 66
ASSLGNTEAF	SEQ ID NO: 26	ASSLGSSYEQY	SEQ ID NO: 67
ASSFGETQY	SEQ ID NO: 27	ASSLGSYNEQF	SEQ ID NO: 68
ASSLGGYEQY	SEQ ID NO: 28	ASSLGGSSYEQY	SEQ ID NO: 69
ASSLGLNTEAF	SEQ ID NO: 29	ASSLAGYEQY	SEQ ID NO: 70
ASSLGSTDTQY	SEQ ID NO: 30	ASSLAGSTDTQY	SEQ ID NO: 71
ASSLTGNTEAF	SEQ ID NO: 31	ASSLKETQY	SEQ ID NO: 72
ASSLGRNTEAF	SEQ ID NO: 32	ASSLGGGTEAF	SEQ ID NO: 73
ASSPSYEQY	SEQ ID NO: 33	ASSRDSNQPQH	SEQ ID NO: 74
ASSPSSYEQY	SEQ ID NO: 34	ASSLGPNTAF	SEQ ID NO: 75
ASSLYNEQF	SEQ ID NO: 35	ASSLGGDTQY	SEQ ID NO: 76
ASSLGVNTEAF	SEQ ID NO: 36	ASSSSSYEQY	SEQ ID NO: 77
ASSSSTDTQY	SEQ ID NO: 37	ASSLGNQPQH	SEQ ID NO: 78
ASSLAGTDTQY	SEQ ID NO: 38	ASSSTYEQY	SEQ ID NO: 79
ASSFTDTQY	SEQ ID NO: 39	ASSLGGGDTQY	SEQ ID NO: 80
ASSLGQGYEQY	SEQ ID NO: 40	ASSLSSYNEQF	SEQ ID NO: 81
ASSLGNEQF	SEQ ID NO: 41	ASSFSTDTQY	SEQ ID NO: 82
ASSLGTEAF	SEQ ID NO: 42	ASSLGMNTEAF	SEQ ID NO: 83
ASSPGTDTQY	SEQ ID NO: 43	ASSPDSYEQY	SEQ ID NO: 84
ASSLSSYEQY	SEQ ID NO: 44	ASSPGYEQY	SEQ ID NO: 85
ASSLDSYEQY	SEQ ID NO: 45	ASSPRTDTQY	SEQ ID NO: 86
ASSLGSYEQY	SEQ ID NO: 46	ASSLDTDTQY	SEQ ID NO: 87
ASSLDRNTEAF	SEQ ID NO: 47	ASSLGGETQY	SEQ ID NO: 88
ASSLNTEAF	SEQ ID NO: 48	ASSRQETQY	SEQ ID NO: 89
ASSSYNEQF	SEQ ID NO: 49	ASSLAETQY	SEQ ID NO: 90
ASSPGETQY	SEQ ID NO: 50	ASSLGQGAYEQY	SEQ ID NO: 91
ASSLGGSTDTQY	SEQ ID NO: 51	ASSLNSNQPQH	SEQ ID NO: 92
ASSLGGNEQF	SEQ ID NO: 52	ASSLQGNQPQH	SEQ ID NO: 93
ASSQGYEQY	SEQ ID NO: 53	ASSRTDTQY	SEQ ID NO: 94
ASSLNTGELF	SEQ ID NO: 95	ASSSQGYEQY	SEQ ID NO: 136
ASSLGQGTDTQY	SEQ ID NO: 96	ASSLVETQY	SEQ ID NO: 137
ASSLGQNYGYT	SEQ ID NO: 97	ASSLGENTEAF	SEQ ID NO: 138
ASSLSGNTEAF	SEQ ID NO: 98	ASSPGGYEQY	SEQ ID NO: 139
ASSLSSNQPQH	SEQ ID NO: 99	ASSLAGSYEQY	SEQ ID NO: 140
ASSLTGNQPQH	SEQ ID NO: 100	ASSLSGANVLT	SEQ ID NO: 141

ASSPQGYEQY	SEQ ID NO: 101	ASSPQGNTEAF	SEQ ID NO: 142
ASSLSETQY	SEQ ID NO: 102	ASSLLETQY	SEQ ID NO: 143
ASSLGGEQY	SEQ ID NO: 103	ASSRGYEQY	SEQ ID NO: 144
ASSLGYNEQF	SEQ ID NO: 104	ASSLLNTEAF	SEQ ID NO: 145
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ASSLDSYNEQF	SEQ ID NO: 117	ASSLNNEQF	SEQ ID NO: 158
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ASSYTDQY	SEQ ID NO: 120	ASSLTENTEAF	SEQ ID NO: 161
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ASSPLNTEAF	SEQ ID NO: 673	ASSLGLAGYNEQF	SEQ ID NO: 714
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ASSLTNTEAF	SEQ ID NO: 687	ASSLQGYGYT	SEQ ID NO: 728
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ASSLEQNTEAF	SEQ ID NO: 1250	ASSGAYEQY	SEQ ID NO: 1291
ASSLAGPYEQY	SEQ ID NO: 1251	ASSRLNTEAF	SEQ ID NO: 1292
ASSLGLNNEQF	SEQ ID NO: 1252	ASSLGDNEQF	SEQ ID NO: 1293
ASSLVNQPPH	SEQ ID NO: 1253	ASSRDSNYGYT	SEQ ID NO: 1294
ASSVGETQY	SEQ ID NO: 1254	ASSLGTSGTDTQY	SEQ ID NO: 1295
ASSLDRLNTEAF	SEQ ID NO: 1255	ASSPLNEQF	SEQ ID NO: 1296
ASSQGGETQY	SEQ ID NO: 1256	ASSRDRDQPPH	SEQ ID NO: 1297
ASSRDRGETQY	SEQ ID NO: 1257	ASSPTANTEAF	SEQ ID NO: 1298
ASSFGGQETQY	SEQ ID NO: 1258	ASSQDLNQPPH	SEQ ID NO: 1299
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ASSFSGGTDQY	SEQ ID NO: 1262	ASSPSGGTDQY	SEQ ID NO: 1303
ASRGYNEQF	SEQ ID NO: 1263	ASSLRDEQF	SEQ ID NO: 1304
ASSQDLGQPPH	SEQ ID NO: 1264	ASSLQGQETQY	SEQ ID NO: 1305
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ASSLDGSYEQY	SEQ ID NO: 1268	ASSRSNQPPH	SEQ ID NO: 1309
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ASSLVQNTEAF	SEQ ID NO: 1281	ASSLGLADTQY	SEQ ID NO: 1322
ASSQGPNTAF	SEQ ID NO: 1282	ASSPLAGGYNEQF	SEQ ID NO: 1323
ASSPGGYGYT	SEQ ID NO: 1283	ASSLRGYGYT	SEQ ID NO: 1324
ASSLGVYNEQF	SEQ ID NO: 1325	ASSLRDSNQPPH	SEQ ID NO: 1366
ASSWGNTEAF	SEQ ID NO: 1326	ASSFGGGTDQY	SEQ ID NO: 1367
ASSRDSYGYT	SEQ ID NO: 1327	ASSYLETQY	SEQ ID NO: 1368
ASSLPNTEAF	SEQ ID NO: 1328	ASSSGTSTDQY	SEQ ID NO: 1369
ASSSGQLNTEAF	SEQ ID NO: 1329	ASSRTSGSTDQY	SEQ ID NO: 1370
ASSRGMNTEAF	SEQ ID NO: 1330	ASSPGQSNQPPH	SEQ ID NO: 1371

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ASSPLGDTQY	SEQ ID NO: 1334	ASSPDYNEQF	SEQ ID NO: 1375
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ASSLEYNEQF	SEQ ID NO: 1740	ASSLVPNTEAF	SEQ ID NO: 1781

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ASSLGGNNEQF	SEQ ID NO: 1769	ASSEGETQY	SEQ ID NO: 1810
ASSPPSYNEQF	SEQ ID NO: 1770	ASSFGGDTQY	SEQ ID NO: 1811
ASSFSGSYEYQ	SEQ ID NO: 1771	ASSSIYEYQ	SEQ ID NO: 1812
ASSLVGGNEQF	SEQ ID NO: 1772	ASSPRDSYEYQ	SEQ ID NO: 1813
ASSFLTDTQY	SEQ ID NO: 1773	ASSYSLNTEAF	SEQ ID NO: 1814
ASSWGTEAF	SEQ ID NO: 1774	ASSFPGNTEAF	SEQ ID NO: 1815
ASSFGGNYGYT	SEQ ID NO: 1775	ASSQVGYEYQ	SEQ ID NO: 1816
ASSLTQNTEAF	SEQ ID NO: 1817	ASSRTGTDQY	SEQ ID NO: 1858
ASSLQDTQY	SEQ ID NO: 1818	ASSYNTEAF	SEQ ID NO: 1859
ASSPGQGNEKLF	SEQ ID NO: 1819	ASSLGGGEQF	SEQ ID NO: 1860
ASSLGGANVLT	SEQ ID NO: 1820	ASSDYEYQ	SEQ ID NO: 1861
ASSRQMNTAF	SEQ ID NO: 1821	ASSPRGNQPQH	SEQ ID NO: 1862
ASSLRDQPQH	SEQ ID NO: 1822	ASSPRMNTAF	SEQ ID NO: 1863

ASSLQGSYNEQF	SEQ ID NO: 1823	ASSPLGTEAF	SEQ ID NO: 1864
ASSPGGSSYNEQF	SEQ ID NO: 1824	ASSPRTGQETQY	SEQ ID NO: 1865
ASSLWGTEAF	SEQ ID NO: 1825	ASSPTGNEQF	SEQ ID NO: 1866
ASSTGLNTEAF	SEQ ID NO: 1826	ASSPRNEQF	SEQ ID NO: 1867
ASSPDRGYEQY	SEQ ID NO: 1827	ASSYSGDTQY	SEQ ID NO: 1868
ASSRQLNTEAF	SEQ ID NO: 1828	ASSPQGDTQY	SEQ ID NO: 1869
ASSFGGSSYNEQF	SEQ ID NO: 1829	ASSPATDTQY	SEQ ID NO: 1870
ASSLGNNEQF	SEQ ID NO: 1830	ASSLGTSSYNEQF	SEQ ID NO: 1871
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ASSSGGQPQH	SEQ ID NO: 1832	ASSFQGGTEAF	SEQ ID NO: 1873
ASSLNEKLF	SEQ ID NO: 1833	ASSPGGSSYEQY	SEQ ID NO: 1874
ASSSGLSTDYQY	SEQ ID NO: 1834	ASSLAGGYGYT	SEQ ID NO: 1875
ASSLPYNEQF	SEQ ID NO: 1835	ASSLSRNQPQH	SEQ ID NO: 1876
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ASSPGRMNTAF	SEQ ID NO: 1837	ASSFTENTAF	SEQ ID NO: 1878
ASSRDRNYGYT	SEQ ID NO: 1838	ASSFGANTEAF	SEQ ID NO: 1879
ASSFRVNTAF	SEQ ID NO: 1839	ASSYGTEAF	SEQ ID NO: 1880
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ASSLGQSSYEQY	SEQ ID NO: 1841	ASSLGATNEKLF	SEQ ID NO: 1882
ASSSMNTAF	SEQ ID NO: 1842	ASSLTGRTEAF	SEQ ID NO: 1883
ASSSSSTDYQY	SEQ ID NO: 1843	ASSLNMNTAF	SEQ ID NO: 1884
ASSRQGDTEAF	SEQ ID NO: 1844	ASSDSSYEQY	SEQ ID NO: 1885
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ASSPTGQETQY	SEQ ID NO: 1847	ASSQGGYGYT	SEQ ID NO: 1888
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ASSLRGSSYEQY	SEQ ID NO: 1851	ASSLQGSYEQY	SEQ ID NO: 1892
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ASSQDGYEQY	SEQ ID NO: 1853	ASSYSGEQY	SEQ ID NO: 1894
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ASSLNNQPQH	SEQ ID NO: 1856	ASSLLAGSYNEQF	SEQ ID NO: 1897
ASSSVYEQY	SEQ ID NO: 1857	ASSQGANTEAF	SEQ ID NO: 1898
ASSVGLNTEAF	SEQ ID NO: 1899	ASSRDRSTDYQY	SEQ ID NO: 1940
ASSLYEQY	SEQ ID NO: 1900	ASSPQGDTEAF	SEQ ID NO: 1941
ASSLGVSNQPQH	SEQ ID NO: 1901	ASSTNTEAF	SEQ ID NO: 1942
ASSWGQNTAF	SEQ ID NO: 1902	ASSPGQNQPQH	SEQ ID NO: 1943
ASRTGGTDYQY	SEQ ID NO: 1903	ASSQVGNTAF	SEQ ID NO: 1944
ASSVADTQY	SEQ ID NO: 1904	ASSPLETQY	SEQ ID NO: 1945

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ASSRGTGELF	SEQ ID NO: 1906	ASSPTGGYEQY	SEQ ID NO: 1947
ASRDSSYEQY	SEQ ID NO: 1907	ASSPTGYGYT	SEQ ID NO: 1948
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ASSSTANTEAF	SEQ ID NO: 1909	ASSFRGETQY	SEQ ID NO: 1950
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ASSRGLQETQY	SEQ ID NO: 1911	ASSLDGETQY	SEQ ID NO: 1952
ASSLLGSTDTQY	SEQ ID NO: 1912	ASSDGYEQY	SEQ ID NO: 1953
ASSPDGYEQY	SEQ ID NO: 1913	ASSPGLAYNEQF	SEQ ID NO: 1954
ASSLARDTQY	SEQ ID NO: 1914	ASSLIGETQY	SEQ ID NO: 1955
ASSLGGGADTQY	SEQ ID NO: 1915	ASSRGDTEAF	SEQ ID NO: 1956
ASSQGSYNEQF	SEQ ID NO: 1916	ASSSQMNTEAF	SEQ ID NO: 1957
ASSLTGGETQY	SEQ ID NO: 1917	ASSQDRGTDQY	SEQ ID NO: 1958
ASSPGTTYEQY	SEQ ID NO: 1918	ASSLTSGGTDQY	SEQ ID NO: 1959
ASSPGTANYGYT	SEQ ID NO: 1919	ASSFGGSYNEQF	SEQ ID NO: 1960
ASSFSSGNTIY	SEQ ID NO: 1920	ASSLGPGLF	SEQ ID NO: 1961
ASSDRGNTEAF	SEQ ID NO: 1921	ASSQDGETQY	SEQ ID NO: 1962
ASSLAGGEQY	SEQ ID NO: 1922	ASSLEGGQPQH	SEQ ID NO: 1963
ASSPDRDTEAF	SEQ ID NO: 1923	ASSPVNTEAF	SEQ ID NO: 1964
ASSPLNQPH	SEQ ID NO: 1924	ASSFGGQPQH	SEQ ID NO: 1965
ASSESSYEQY	SEQ ID NO: 1925	ASSYGLNTEAF	SEQ ID NO: 1966
ASSPGGGYEQY	SEQ ID NO: 1926	ASSPGTASYEQY	SEQ ID NO: 1967
ASRGQLNTEAF	SEQ ID NO: 1927	ASSLGRGNEQF	SEQ ID NO: 1968
ASSRTGNYGYT	SEQ ID NO: 1928	ASSLVGYGYT	SEQ ID NO: 1969
ASSSNSYEQY	SEQ ID NO: 1929	ASSPTYEQY	SEQ ID NO: 1970
ASSLGTEETQY	SEQ ID NO: 1930	ASSFRGYEQY	SEQ ID NO: 1971
ASSLVDTEAF	SEQ ID NO: 1931	ASSLDGNTIY	SEQ ID NO: 1972
SARDSYEQY	SEQ ID NO: 1932	ASSTGNQPQH	SEQ ID NO: 1973
ASSYSGTEAF	SEQ ID NO: 1933	ASSAGETQY	SEQ ID NO: 1974
ASSLGTENTEAF	SEQ ID NO: 1934	ASSQGSSYEQY	SEQ ID NO: 1975
ASSYSSYNEQF	SEQ ID NO: 1935	ASSLRDSSYEQY	SEQ ID NO: 1976
ASSWTENTEAF	SEQ ID NO: 1936	ASSLRQGTDTQY	SEQ ID NO: 1977
ASSLGAGTDQY	SEQ ID NO: 1937	ASSPGSGNTIY	SEQ ID NO: 1978
ASSPTSGSTDQY	SEQ ID NO: 1938	ASSLQGGTDQY	SEQ ID NO: 1979
ASSPNYGYT	SEQ ID NO: 1939	ASSLTGGQPQH	SEQ ID NO: 1980
ASSLGVGTEAF	SEQ ID NO: 1981	ASSPGTAYNEQF	SEQ ID NO: 2022
ASSPGTSGSYEQY	SEQ ID NO: 1982	ASSDSSTDQY	SEQ ID NO: 2023
ASSRGYNEQF	SEQ ID NO: 1983	ASSQVNEQF	SEQ ID NO: 2024
ASSPPNTEAF	SEQ ID NO: 1984	ASSLAGGAYEQY	SEQ ID NO: 2025
ASSLLAGAYNEQF	SEQ ID NO: 1985	ASSLGRDYEQY	SEQ ID NO: 2026
ASSPDRLNTEAF	SEQ ID NO: 1986	ASSLGFTDTQY	SEQ ID NO: 2027

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ASSQDRGNQPQH	SEQ ID NO: 1990	ASSPGQANTEAF	SEQ ID NO: 2031
ASSRTSGSYNEQF	SEQ ID NO: 1991	ASSGGNTEAF	SEQ ID NO: 2032
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ASSLVTEAF	SEQ ID NO: 1993	ASSFGQETQY	SEQ ID NO: 2034
ASSLLENTEAF	SEQ ID NO: 1994	ASSLAGGPTDTQY	SEQ ID NO: 2035
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ASSGYEQY	SEQ ID NO: 1996	SARQGNQPQH	SEQ ID NO: 2037
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ASSLAPYEQY	SEQ ID NO: 1998	ASSLEGYNEQF	SEQ ID NO: 2039
ASSFRGSYEQY	SEQ ID NO: 1999	ASSPGDYGYT	SEQ ID NO: 2040
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ASSFPGTDQY	SEQ ID NO: 2004	ASSLEGDQPQH	SEQ ID NO: 2045
ASSFRGGTEAF	SEQ ID NO: 2005	SARLAGGQETQY	SEQ ID NO: 2046
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ASSLLYNEQF	SEQ ID NO: 2007	ASSSSGTDQY	SEQ ID NO: 2048
ASSEAYEQY	SEQ ID NO: 2008	ASSQTVNTEAF	SEQ ID NO: 2049
ASSLRGEQF	SEQ ID NO: 2009	ASSEGNTEAF	SEQ ID NO: 2050
ASSFTVNTEAF	SEQ ID NO: 2010	ASSSLDTQY	SEQ ID NO: 2051
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ASSPLSTDQY	SEQ ID NO: 2013	ASSFSENTEAF	SEQ ID NO: 2054
ASSGTYEQY	SEQ ID NO: 2014	ASSSGGDTQY	SEQ ID NO: 2055
ASSSGGSTDTQY	SEQ ID NO: 2015	ASSLDSMNTEAF	SEQ ID NO: 2056
ASSLSGMNTEAF	SEQ ID NO: 2016	SARQGNTEAF	SEQ ID NO: 2057
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ASSQGAYEQY	SEQ ID NO: 2019	ASSLGRSNQPQH	SEQ ID NO: 2060
ASSLVGGYEQY	SEQ ID NO: 2020	ASSLGPNEQF	SEQ ID NO: 2061
ASSGQGYGYT	SEQ ID NO: 2021	ASSSPGTDQY	SEQ ID NO: 2062
ASSFSRNTEAF	SEQ ID NO: 2063	ASSSSGYEQY	SEQ ID NO: 2104
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ASSPLGYEQY	SEQ ID NO: 2066	ASSLENQPQH	SEQ ID NO: 2107
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ASSLSGTYEQY	SEQ ID NO: 2068	ASSPGQGTEAF	SEQ ID NO: 2109

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ASSRQVNTEAF	SEQ ID NO: 2070	ASSQSGNTIY	SEQ ID NO: 2111
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ASSFDNEQF	SEQ ID NO: 2101	ASSLGTGNYGYT	SEQ ID NO: 2142
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ASSYGNEQF	SEQ ID NO: 2145	ASSLGGNEQY	SEQ ID NO: 2186
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ASSDRGYEQY	SEQ ID NO: 2147	ASSQVNTEAF	SEQ ID NO: 2188
ASSQTGGTEAF	SEQ ID NO: 2148	ASSPPGQGYEQY	SEQ ID NO: 2189
ASSPGLAGGNEQF	SEQ ID NO: 2149	ASSYSENTEAF	SEQ ID NO: 2190
ASSQGGEQF	SEQ ID NO: 2150	SARGGYEQY	SEQ ID NO: 2191

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ASSRGGYEQY	SEQ ID NO: 2152	ASSPGQVNTEAF	SEQ ID NO: 2193
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ASSVGNTEAF	SEQ ID NO: 2154	ASSSRNTEAF	SEQ ID NO: 2195
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ASSLGGGSTDQY	SEQ ID NO: 2156	ASSFTGELF	SEQ ID NO: 2197
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ASSPDRAYEQY	SEQ ID NO: 2161	ASSLQGNTGELF	SEQ ID NO: 2202
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ASRGGTDTQY	SEQ ID NO: 2165	ASSLGNTDTQY	SEQ ID NO: 2206
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ASSSTGSYEQY	SEQ ID NO: 2175	ASSLTNQPQH	SEQ ID NO: 2216
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ASSPPGNEQF	SEQ ID NO: 2178	ASSLVGGETQY	SEQ ID NO: 2219
ASSQGPDTQY	SEQ ID NO: 2179	ASSRQGQETQY	SEQ ID NO: 2220
ASSGGSYEQY	SEQ ID NO: 2180	ASSLSGAYEQY	SEQ ID NO: 2221
ASSLGLAYNEQF	SEQ ID NO: 2181	ASSSGLYNEQF	SEQ ID NO: 2222
ASSNSNQPQH	SEQ ID NO: 2182	ASSLMGNTEAF	SEQ ID NO: 2223
ASRNTGELF	SEQ ID NO: 2183	ASRNTDTQY	SEQ ID NO: 2224
ASSRSYNEQF	SEQ ID NO: 2184	ASSLGGSDTQY	SEQ ID NO: 2225
ASSQVTDQY	SEQ ID NO: 2185	ASSPPQETQY	SEQ ID NO: 2226
ASSLTGTNTEAF	SEQ ID NO: 2227	ASSRDYNEQF	SEQ ID NO: 2268
ASSLASGSYEQY	SEQ ID NO: 2228	ASSLDQNTEAF	SEQ ID NO: 2269
ASSLEGDTAF	SEQ ID NO: 2229	ASSPPGYEQY	SEQ ID NO: 2270
ASSQDRGYGYT	SEQ ID NO: 2230	ASSRTGDQPQH	SEQ ID NO: 2271
ASSLASGNTIY	SEQ ID NO: 2231	ASSLLRNTEAF	SEQ ID NO: 2272
ASSPGGMNTEAF	SEQ ID NO: 2232	ASSYGGYEQY	SEQ ID NO: 2273

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ASSFRNQPDH	SEQ ID NO: 2234	ASSQGDYGYT	SEQ ID NO: 2275
ASSPGSSGNTIY	SEQ ID NO: 2235	ASSPTGSGNTIY	SEQ ID NO: 2276
ASSLSGGYNEQF	SEQ ID NO: 2236	ASSRDQETQY	SEQ ID NO: 2277
ASSLRQGYEQY	SEQ ID NO: 2237	ASSLGGPTDTQY	SEQ ID NO: 2278
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ASSGSYNEQF	SEQ ID NO: 2241	ASSLEGGNTTEAF	SEQ ID NO: 2282
ASSQGQNYGYT	SEQ ID NO: 2242	ASSPGTDYEQY	SEQ ID NO: 2283
ASSLLGGTDTQY	SEQ ID NO: 2243	ASSLGRNTGELF	SEQ ID NO: 2284
ASSLVTGELF	SEQ ID NO: 2244	ASSYGGSYEQY	SEQ ID NO: 2285
ASSLGQGGYGYT	SEQ ID NO: 2245	ASSLGNYEQY	SEQ ID NO: 2286
ASSPGTSSYEQY	SEQ ID NO: 2246	ASSLTGANVLT	SEQ ID NO: 2287
ASSTNTGELF	SEQ ID NO: 2247	ASSSTGYGYT	SEQ ID NO: 2288
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ASSPGPDQY	SEQ ID NO: 2249	ASGTYEY	SEQ ID NO: 2290
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ASSQGQGNTEAF	SEQ ID NO: 2251	ASSFGQQETQY	SEQ ID NO: 2292
ASSLRPNTEAF	SEQ ID NO: 2252	ASSLAGSQETQY	SEQ ID NO: 2293
ASSLDRNEQF	SEQ ID NO: 2253	ASSPTGGNTTEAF	SEQ ID NO: 2294
ASSPDYEQY	SEQ ID NO: 2254	ASSLVGLNTEAF	SEQ ID NO: 2295
ASSSQVNTEAF	SEQ ID NO: 2255	ASRGQETQY	SEQ ID NO: 2296
ASSLMETQY	SEQ ID NO: 2256	ASSFPSTDQY	SEQ ID NO: 2297
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ASSSDSTDQY	SEQ ID NO: 2258	ASSPTGTYEY	SEQ ID NO: 2299
ASSSQGDTEAF	SEQ ID NO: 2259	ASSAGNTTEAF	SEQ ID NO: 2300
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ASSSGGSNQPDH	SEQ ID NO: 2262	ASSLEGADTQY	SEQ ID NO: 2303
ASSLAVQETQY	SEQ ID NO: 2263	ASSPTSGTDTQY	SEQ ID NO: 2304
ASSRTGGNTTEAF	SEQ ID NO: 2264	ASSFGTANTEAF	SEQ ID NO: 2305
ASSLQGTGELF	SEQ ID NO: 2265	ASSLKGYEQY	SEQ ID NO: 2306
ASSSTGSNQPDH	SEQ ID NO: 2266	ASSLYRNTEAF	SEQ ID NO: 2307
ASSYSGGYEQY	SEQ ID NO: 2267	ASSLAGPDQY	SEQ ID NO: 2308
ASSPRRNTEAF	SEQ ID NO: 2309	ASSDRGTDQY	SEQ ID NO: 2350
ASSLTGGNEQF	SEQ ID NO: 2310	ASSLAGAQETQY	SEQ ID NO: 2351
ASRKNTTEAF	SEQ ID NO: 2311	ASSLSYGYT	SEQ ID NO: 2352
ASSQDSTDQY	SEQ ID NO: 2312	ASSLEGNTIY	SEQ ID NO: 2353
ASSLVGNTIY	SEQ ID NO: 2313	ASSLAGGTGELF	SEQ ID NO: 2354
ASSPGLNQPDH	SEQ ID NO: 2314	ASSPGQGGNQPDH	SEQ ID NO: 2355

ASSSGTSYEQY	SEQ ID NO: 2315	ASSLGLAGVNEQF	SEQ ID NO: 2356
ASSLQGRTEAF	SEQ ID NO: 2316	ASSLLGSYEQY	SEQ ID NO: 2357
ASSLDRADTQY	SEQ ID NO: 2317	ASSSSGGNEQF	SEQ ID NO: 2358
ASSPQGSYEQY	SEQ ID NO: 2318	ASSPSSNQ PQH	SEQ ID NO: 2359
ASSPTGSSYNEQF	SEQ ID NO: 2319	ASSGTSGTDTQY	SEQ ID NO: 2360
ASRDYNEQF	SEQ ID NO: 2320	ASRSSTDYEQY	SEQ ID NO: 2361
ASSSGPYNEQF	SEQ ID NO: 2321	ASSPGRNQ PQH	SEQ ID NO: 2362
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ASSYSGGNTEAF	SEQ ID NO: 2324	ASSVGSNQ PQH	SEQ ID NO: 2365
ASSRNYEQY	SEQ ID NO: 2325	ASSTDRNTEAF	SEQ ID NO: 2366
ASSVDRNTEAF	SEQ ID NO: 2326	SARGSNQ PQH	SEQ ID NO: 2367
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ASSFGTGGYEQY	SEQ ID NO: 2328	ASSPGEETQY	SEQ ID NO: 2369
ASSPGTAGNTIY	SEQ ID NO: 2329	ASSLAPTDYEQY	SEQ ID NO: 2370
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ASSLGGAGNTIY	SEQ ID NO: 2331	ASSRYSNQ PQH	SEQ ID NO: 2372
ASSLARNEQF	SEQ ID NO: 2332	ASSLAGGTYNEQF	SEQ ID NO: 2373
ASSLEGYSNQ PQH	SEQ ID NO: 2333	ASSYSVNTEAF	SEQ ID NO: 2374
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ASSFPDTQY	SEQ ID NO: 2335	ASSYRGTDYEQY	SEQ ID NO: 2376
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ASSPQGSGNTIY	SEQ ID NO: 2337	ASSPRSYEQY	SEQ ID NO: 2378
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ASSQDRGNEQF	SEQ ID NO: 2339	ASSPRQGNQ PQH	SEQ ID NO: 2380
ASSPTGDQ PQH	SEQ ID NO: 2340	ASSSQGYGYT	SEQ ID NO: 2381
ASSYSRGYEQY	SEQ ID NO: 2341	ASSLDLTDYEQY	SEQ ID NO: 2382
ASSLLGDTEAF	SEQ ID NO: 2342	ASSLDRGEQY	SEQ ID NO: 2383
ASRRGTNTEAF	SEQ ID NO: 2343	ASSYSNTEAF	SEQ ID NO: 2384
ASSLLAGYNEQF	SEQ ID NO: 2344	ASSPRGGNQ PQH	SEQ ID NO: 2385
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ASSLTGPNTAF	SEQ ID NO: 2346	ASSSGQAYEQY	SEQ ID NO: 2387
ASSFSGTEAF	SEQ ID NO: 2347	ASSYGGTDYEQY	SEQ ID NO: 2388
ASSLQGAGNTIY	SEQ ID NO: 2348	ASSPTGGSYEQY	SEQ ID NO: 2389
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ASSQTGYEQY	SEQ ID NO: 2395	ASSLAGGRETQY	SEQ ID NO: 2436
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ASSQVSYEQY	SEQ ID NO: 2886	ASSPGTGVEYEQY	SEQ ID NO: 2927
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ASSFRTEAF	SEQ ID NO: 2906	ASSLTVSYEQY	SEQ ID NO: 2947
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SARTGGTEAF	SEQ ID NO: 2911	ASSAGGTEAF	SEQ ID NO: 2952
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ASSLGQENTTEAF	SEQ ID NO: 2913	ASSQGRETQY	SEQ ID NO: 2954
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ASSSNQETQY	SEQ ID NO: 3414	ASSPAGTDTQY	SEQ ID NO: 3455
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ASSQGGTEAF	SEQ ID NO: 3457	ASSYGENTEAF	SEQ ID NO: 3498
ASSLGGRNQPQH	SEQ ID NO: 3458	ASSLGGTTDTQY	SEQ ID NO: 3499
ASSLVRDTEAF	SEQ ID NO: 3459	ASSSDRNYGYT	SEQ ID NO: 3500
ASSLLDTEAF	SEQ ID NO: 3460	ASSSGQGQETQY	SEQ ID NO: 3501
ASSQAGNTEAF	SEQ ID NO: 3461	ASSPGASTDTQY	SEQ ID NO: 3502
ASSLGLNYGYT	SEQ ID NO: 3462	ASSEGDTQY	SEQ ID NO: 3503

ASSWTSGSTDTQY	SEQ ID NO: 3463	ASSPDRSSYEYQY	SEQ ID NO: 3504
ASSLGEQF	SEQ ID NO: 3464	ASSPTGGQETQY	SEQ ID NO: 3505
ASSLLRDTQY	SEQ ID NO: 3465	ASSYPDTQY	SEQ ID NO: 3506
ASSPSGGSYEYQY	SEQ ID NO: 3466	ASSPRLAGGTDTQY	SEQ ID NO: 3507
ASRGGGTDTQY	SEQ ID NO: 3467	ASSLPSSYEYQY	SEQ ID NO: 3508
ASSLGPSSYEYQY	SEQ ID NO: 3468	ASSRTSYEYQY	SEQ ID NO: 3509
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ASSQDTEAF	SEQ ID NO: 3470	ASSRDRNTGELF	SEQ ID NO: 3511
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SARDSSYEYQY	SEQ ID NO: 3472	ASSSGQGNSPLH	SEQ ID NO: 3513
ASSQGSYGYT	SEQ ID NO: 3473	ASSVGNEQF	SEQ ID NO: 3514
ASSLRPDTQY	SEQ ID NO: 3474	ASSGGDTQY	SEQ ID NO: 3515
ASSQGTNTEAF	SEQ ID NO: 3475	ASSFDGNTEAF	SEQ ID NO: 3516
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ASSVGTEAF	SEQ ID NO: 3479	ASSRGPYEYQY	SEQ ID NO: 3520
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ASSAQGYEQY	SEQ ID NO: 3542	ASSLGQIYEYQY	SEQ ID NO: 3583
ASSSSSGANVLT	SEQ ID NO: 3543	ASSLGLAVYNEQF	SEQ ID NO: 3584
ASSFGGADTQY	SEQ ID NO: 3544	ASSFRDTEAF	SEQ ID NO: 3585

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ASSVSNQPH	SEQ ID NO: 3656	ASRG TANTEAF	SEQ ID NO: 3697
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ASSLETEAF	SEQ ID NO: 3713	ASSRGDEQF	SEQ ID NO: 3754
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ASSFYTEAF	SEQ ID NO: 3872	ASSLKGTDTQY	SEQ ID NO: 3913

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ASSLEQETQY	SEQ ID NO: 3874	ASSLRGGQETQY	SEQ ID NO: 3915
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ASSLVSTEAF	SEQ ID NO: 3876	ASSQGRTEAF	SEQ ID NO: 3917
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ASSLGGVTDQY	SEQ ID NO: 3954	ASSLGQTDQY	SEQ ID NO: 3987

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ASRGQPNTEAF	SEQ ID NO: 3971	ASSAYTDTQY	SEQ ID NO: 4004
ASRDRAEYQY	SEQ ID NO: 3972	ASSLGQNTDTQY	SEQ ID NO: 4005
ASSLARNQPQH	SEQ ID NO: 3973	ASSLGAGSYEQY	SEQ ID NO: 4006
ASSSDGYEQY	SEQ ID NO: 3974	ASSQGGGYEQY	SEQ ID NO: 4007
ASSTGQGNTAF	SEQ ID NO: 3975	ASSLRTNTEAF	SEQ ID NO: 4008
AISESYEQY	SEQ ID NO: 3976	ASSLARSTDYQY	SEQ ID NO: 4009
ASSLAPYNEQF	SEQ ID NO: 3977	ASSSRDSSYEYQY	SEQ ID NO: 4010
ASSLSGGADTQY	SEQ ID NO: 3978	ASSSGTGNGNPQH	SEQ ID NO: 4011
ASSWGLNTEAF	SEQ ID NO: 3979	ASSLWGQETQY	SEQ ID NO: 4012
ASSRTAQETQY	SEQ ID NO: 3980	ASSRQGSNTIY	SEQ ID NO: 4013
ASSTSNQPQH	SEQ ID NO: 3981	ASSPYTEAF	SEQ ID NO: 4014

[0041] This application references various publications. The disclosures of these publications, in their entireties, are hereby incorporated by reference into this application to describe more fully the state of the art to which this application pertains. The references disclosed are also individually and specifically incorporated herein by reference for material contained within them that is discussed in the sentence in which the reference is relied on.

[0042] The methodologies and the various embodiments thereof described herein are exemplary. Various other embodiments of the methodologies described herein are possible.

CLAIMS

Now, therefore, the following is claimed:

1. A method for using immunorepertoire diversity to assess a normal or abnormal status of an individual patient's immunorepertoire, the method comprising the steps of:

(a) determining the pCDR3 sequences, the commonly-shared CDR3 sequences identified from a reference group of individuals, comprising comparing each sample from the reference group and identifying those CDR3 sequences that are most-frequently shared within said reference group;

(b) using primers directed to pCDR3 sequences to detect the presence in the patient's immunorepertoire of individual CDR3s within that shared group of pCDR3 sequences; and

(c) calculating the normality index, comprising dividing the number of pCDR3 sequences from the reference group by the number of pCDR3 sequences present in the patient's immunorepertoire; and

(d) assessing the status of the patient's immunorepertoire, wherein the normality index is compared with a predetermined minimum percentage of shared pCDR3 sequences, such that the immunorepertoire of the patient is considered normal when the normality index meets or exceeds the minimum percentage of shared pCDR3 sequences and the immunorepertoire of the patient is considered abnormal when the normality index is below the minimum percentage of shared pCDR3 sequences.

2. The method of claim 1 wherein the CDR3 sequences are from a population of cells selected from the group consisting of all T cells [panT], CD8+ T cells [cytotoxic T (Ta)], CD4+ T cells and their subsets [TH1, TH2, TH17, regulatory T (Treg), follicular T (TFH)], developmental subsets of T cells, such as naive T (Tn), activated T (Ta), memory T (Tm), all B cells (panB), naive B (Br), activated B (Ba), memory B (Bm), plasma and plasmablast B cells.

3. The method of claim 1, wherein the minimum percentage of pCDR3 is a percentage number selected from about 25 percent to about 75 percent.

4. The method of claim 1, wherein the minimum percentage of pCDR3 is about 25 percent.

5. The method of claim 1, where the pCDR3 is composed of about the 1000 most-frequently-shared CDR3.

6. The method of claim 1, where the pCDR3 is composed of more than 1000 of the most-frequently-shared CDR3.

7. The method of claim 1, where the pCDR3 is composed of fewer than 1000 of the most-frequently-shared CDR3.

8. The method of claim 1, wherein the pCDR3 is composed of CDR3 shared by at least 25 percent of a pool of individuals.

9. The method of claim 1, wherein the pCDR3 is composed of CDR3 shared by at least 50 percent of a pool of individuals.

10. The method of claim 9, wherein the pool contains at least 100 individuals.

11. The method of claim 9, wherein the pool contains about 1000 individuals.

12. A method for using immunorepertoire diversity to assess a normal or abnormal status of an individual patient's immunorepertoire, the method comprising:

(a) amplifying polynucleotides from a population of white blood cells from a human or animal subject in a reaction mix comprising target-specific nested primers to produce a set of first amplicons, at least a portion of the target-specific nested primers comprising additional nucleotides which, during amplification, serve as a template for incorporating into the first amplicons a binding site for at least one common primer;

(b) transferring a portion of the first reaction mix containing the first amplicons to a second reaction mix comprising at least one common primer;

(c) amplifying, using the at least one common primer, the first amplicons to produce a set of second amplicons;

(d) sequencing the second amplicons to identify CDR3 sequences in the subpopulation of white blood cells;

(e) determining the pCDR3 sequences, the commonly-shared CDR3 sequences identified from a reference group of individuals, comprising comparing each

sample from the reference group and identifying those CDR3 sequences that are most-frequently shared within said reference group;

(f) using the identified CDR3 sequences to calculate the normality index, comprising dividing the number of pCDR3 sequences from the reference group by the number of pCDR3 sequences present in the patient's identified CDR3 sequences; and

(g) assessing the status of the patient's immunorepertoire, wherein the normality index is compared with a predetermined minimum percentage of shared pCDR3 sequences, such that the immunorepertoire of the patient is considered normal when the normality index meets or exceeds the minimum percentage of shared pCDR3 sequences and the immunorepertoire of the patient is considered abnormal when the normality index is below the minimum percentage of shared pCDR3 sequences.

13. The method of claim 12, wherein the population of cells is selected from the group consisting of all T cells [panT], CD8+ T cells [cytotoxic T (Ta)], CD4+ T cells and their subsets [TH1, TH2, TH17, regulatory T (Treg), follicular T (TFH)], developmental subsets of T cells, such as naive T (Tn), activated T (Ta), memory T (Tm), all B cells (panB), naive B (Br), activated B (Ba), memory B (Bm), plasma and plasmablast B cells.

14. The method of claim 12, wherein the population of white blood cells is about 100,000 randomly-selected cells.

15. The method of claim 12, wherein the method is repeated about 10 to 100 times, each time with a random selection of cells, to determine the average percentage of pCDR3 expressed by the patient.

16. The method of claim 12, wherein the minimum percentage of pCDR3 is a percentage number from about 25 to about 75.

17. The method of claim 12, wherein the minimum percentage of pCDR3 is about 25 percent.

18. The method of claim 12, wherein the pCDR3 is composed of at least the 1000 most-frequently-shared CDR3.

19. The method of claim 12, wherein the pCDR3 includes SEQ ID NO:1 through SEQ ID NO: 4014.

20. The method of claim 12, wherein the pCDR3 is composed of CDR3 shared by at least 50 percent of a pool of individuals.

21. The method of claim 20, wherein the pool contains at least 100 individuals.

22. The method of claim 20, wherein the pool contains about 1000 individuals.

23. The method of claim 20, wherein the pool is composed of healthy control individuals.

24. The method of claim 20, wherein the pool is composed of individuals with one or more specific disease states.

25. The method of claim 20, wherein the pool of individuals is approximately age-matched to the patient.

26. The method of claim 20, wherein the pool of individuals is gender-matched to the patient.