~~WHAT IS CLAIMED IS:~~CLAIMS

1. A method to recover a repertoire of T cell receptors (TCRs) from diverse T cell populations, the method comprising:

determining TCR-α and β nucleotide or amino acid sequences within a subject's sample;

selecting one or more subsets of TCRα- and β-chain sequences from the total repertoire;

creating a TCR repertoire by combinatorial pairing of selected TCRα- and β-chain sequences creating a library of TCRαβ pairs; and

identifying at least one TCRαβ pair with desired features from the created TCR repertoire.

2. The method of claim 1, wherein the one or more subsets of TCRα- and β-chain sequences from the total repertoire is selected based on at least one criterion:

on frequency within the T cell population,

on relative enrichment compared to a second T cell population,

on relative difference of DNA and RNA copy numbers of a given TCR chain

on biological properties of the TCR chain, wherein the properties are selected from at least one of: (predicted) antigen-specificity, (predicted) HLA-restriction, antigen-affinity, co-receptor dependency, parental T cell lineage (e.g. CD4 or CD8 T cell) or TCR sequence motifs,

on spatial patterns of gene expression, wherein spatial gene expression patterns are derived from at least one of: originating region in the tissue or co-expression patterns of other genes,

on co-occurrence or occurrence at a similar frequency in multiple samples, for example occurrence in multiple tumor lesions,

assignment to multiple groups to separately recover specific parts of the TCR repertoire,

on a combination of multiple criteria as defined in the different embodiments.

3. The method of claim 2, wherein selection based on frequency within the T cell population is based upon data of the frequency of TCR sequences, which is used to create a separate rank order for TCRα- and β-chains or a combined rank order for TCRα- and β-chains.

~~4. The method of any one of claims 1-3, further comprising determining a frequency threshold that is defined based on the desired depth for TCR repertoire recovery and used to select collections of TCRα- and β-chains based on frequency.~~

~~5. The method of any one of claims 1-4, wherein determining TCR-α and β sequences is achieved by at least one of:~~

~~multiplex PCR;~~

~~TCR-sequence recovery by target enrichment;~~

~~TCR-sequence recovery by 5'RACE and PCR;~~

~~TCR-sequence recovery by spatial sequencing;~~

~~TCR-sequence recovery by RNA-seq;~~

~~and the use of a Unique Molecular Identifier (UMI)~~

~~6. The method of any one of claims 1-5, wherein a recovered TCR-chain sequence is defined as the CDR3 nucleotide sequence together with sufficient 5'- and 3'-nucleotide sequence information to select at least one TCR V- and one TCR J-segment family based on nucleotide sequence alignment to assemble a complete TCR chain sequence.~~

~~7.~~4. The method of any one of claims 1-~~6~~3, wherein creating a TCR repertoire by combinatorial pairing of selected TCRα- and β-chain sequences creating a library of TCRαβ pairs is achieved by at least one of the following:

TCR chain sequences are used to synthesize separate libraries of TCRα- and β-chain DNA or RNA fragments which are subsequently linked into one DNA or RNA fragment in which exactly one TCRα- and one β-chain are linked,

combinations of TCRα- and β-chains are generated by directly synthesizing DNA or RNA fragments in which exactly one TCRα- and one β-chain are linked,

combinations of TCRα- and β-chains are created intracellularly by modification of a pool of cells with separate collections of TCRα- and β-genes in such a way that cells will express at least one TCRα- and one β-chain, and/or

combinations of TCRα- and β-chains are linked in a single-chain TCR construct in which both TCRα and TCRβ Variable chain fragments are fused and in which the single chain TCR construct may be fused to (i) a transmembrane domain alone or (ii) additionally contain intracellular signaling domains, including but not limited to CD3ε or CD3ζ signaling domains alone or in combination with a CD28 signaling domain.

~~8. The method of any one of claims 1-7, wherein identifying at least one TCRαβ pair with desired features from the created TCR repertoire is achieved by at least one of the following:~~

~~a pool of reporter cells modified with the library of generated TCRαβ pairs is stimulated by antigen presenting cells presenting at least one antigen of interest and antigen-reactive reporter cells are isolated based on at least one activation marker for TCR isolation;~~

~~a pool of reporter cells modified with the library of generated TCRαβ pairs is labelled with a fluorescent dye suitable to trace cell proliferation, stimulated by antigen presenting cells expressing at least one antigen of interest, and antigen-reactive reporter cells are isolated based on proliferation for TCR isolation;~~

~~a pool of reporter cells modified with the library of generated TCRαβ pairs is divided into at least two samples; samples are stimulated by antigen presenting cells expressing at least one antigen of interest or not; after stimulation, both reporter cell populations are incubated for a period of time and subsequently both reporter cell populations are analyzed by TCR isolation; comparison of TCRαβ pairs obtained from both samples is used to identify TCR genes with higher abundance in the sample exposed to at least one antigen;~~

~~a pool of reporter cells modified with the library of generated TCRαβ pairs is stimulated by antigen presenting cells presenting at least one antigen of interest and antigen-reactive reporter cells are isolated for TCR isolation based on at least one reporter gene, such as NFAT-GFP or NFAT-YFP that reports on TCR triggering;~~

~~a pool of reporter cells modified with the library of generated TCRαβ pairs is stimulated by antigen presenting cells presenting at least one antigen of interest, and antigen-reactive reporter cells are isolated for TCR isolation based on selection of antigen-specific reporter cells based on selective survival, including but not limited to acquired antibiotic resistance, upon TCR signaling, for example by use of an NFAT-puromycin transgene;~~

~~a pool of reporter cells modified with the library of generated TCRαβ pairs is exposed to one or multiple MHC complexes that carry an antigen of interest; reporter cells binding to an MHC complex are isolated for TCR isolation;~~

~~a pool of reporter cells modified with the library of generated TCRαβ pairs is stimulated by antigen presenting cells expressing at least one antigen of interest; subsequently, TCRαβ pairs of interest are identified using single-cell based droplet PCR or microfluidic approaches to combine TCR isolation with the detection of transcript levels for at least one activation marker; thereby, single reporter cells within the pool of T cells in which TCRαβ transcripts are co-expressed with increased levels of activation marker are detected.~~

~~9.~~5. The method of any one of claims 1-~~8~~4, wherein the subject's sample comprises non-viable starting material.

~~10. The method of any one of claims 1-9, wherein a defined part of the identified TCR repertoire is recovered.~~

~~11.~~6. The method of any one of claims 1-~~10~~5, wherein antigen-specific TCR sequences are recovered.

~~12.~~7. The method of any one of claims 1-~~11~~6, wherein Class I and/or Class II restricted TCR sequences are recovered.

~~13. The method of any one of claims 1-12, wherein at least one of:~~

~~neo-antigen specific TCR sequences~~

~~virus-specific TCR sequences~~

~~shared tumor-antigen specific TCR sequences~~

~~self-antigen specific TCR sequences~~
~~are recovered.~~

~~14.~~8. The method of claim ~~12~~7, further comprising the step of ~~administering~~ providing T cells expressing the neo-antigen specific TCR sequences to be administered as a cancer therapy.

~~15. The method of any one of claims 1-13, wherein the method is for a diagnostic.~~

~~16. The method of claim 15, wherein the diagnostic is to recover TCR repertoires from pathological sites of infection or autoimmunity.~~

~~17. The method of any one of claims 1-15, wherein the method is for the recovery of BCR/antibody repertoires.~~

~~18. The method of any one of claims 1-16, further comprising isolating nucleic acids from a subject that comprise the TCR-α and β nucleotide sequences.~~

~~19. The method of claim 8, wherein the activation marker is selected from the group consisting of: CD25, CD69, CD62L, CD137, IFN-γ, IL-2, TNF-α, GM-CSF, OX40.~~

~~20.~~9. The method of any one of claims 1-~~18~~8, wherein DNA and RNA isolation is from a T cell population that is a mixture of different cell types or part of a tissue sample (such as blood or tumor tissue).

~~21. The method of any one of claims 1-19, wherein the subject's sample comprises cells isolated from a body fluid.~~

~~22.~~10. The method of claim ~~20~~9, wherein the cells are tumor-specific T cells or tumor-infiltrating lymphocytes.

~~23. The method of claims 20-21, wherein the body fluid is selected from the group consisting of blood, urine, serum, serosal fluid, plasma, lymph, cerebrospinal fluid, saliva, sputum, mucosal secretion, vaginal fluid, ascites fluid, pleural fluid, pericardial fluid, peritoneal fluid, and abdominal fluid.~~

~~24.~~11. The method of claim 1, further comprising using the TCRαβ chain sequences for use in ~~to~~ treating a subject suffering from cancer, an immunological disorder, an autoimmune disease, or an infectious disease.

~~25.~~12. The method of any one of claims 1-~~7~~4, wherein identifying at least one TCRαβ pair with desired features from the created TCR repertoire is achieved by at least one of the following:

identification or selection based on at least one activation marker;

identification or selection based on proliferation in response to antigen;

identification or selection based on identification of TCR genes of higher abundance in antigen-stimulated cells as compared to unstimulated cells;

identification or selection based on reporter gene activation by TCR triggering;

identification or selection based on selective survival, including but not limited to acquired antibiotic-resistance upon TCR signaling;

identification or selection based on binding to one or more MHC complexes;

identification or selection using single-cell based droplet PCR or microfluidics;

or any combination thereof.

~~26. The method of claim 8, wherein TCR isolation is achieved by DNA or RNA isolation from bulk antigen-reactive T cells to generate TCRαβ specific PCR product which is analyzed by DNA sequencing or RNA sequencing to determine TCRαβ gene sequences of antigen-reactive T cells or single-cell based droplet PCR or microfluidic approaches to analyze the TCRαβ gene sequences expressed in analyzed single T cells.~~

~~27. The method of claim 8, wherein the reporter cells are T cells.~~

~~28. The method of claim 24, wherein identification or selection using single-cell based droplet PCR or microfluidics further comprises determination of co-expression of activation-associated genes.~~

~~29.~~13. A method of creating multiple T cell libraries, the method comprising: recovering a repertoire of T cell receptors (TCRs) according to the method of claim 1; selection of TCRα- and β-chain sequences from the total repertoire into multiple groups to separately recover

specific parts of the TCR repertoire, wherein multiple T cell libraries are created that are of smaller complexity or that recover specific parts of the TCR repertoire.

~~30. The method of claim 29, wherein selection of TCRα- and β-chain sequences is based on frequency range.~~

~~31.~~14. A method of identifying a nucleotide sequence from a combinatorial library of nucleic acids, comprising:

providing a combinatorial library comprising a plurality of variant nucleic acids, each of the plurality of variant nucleic acids comprising a contiguous portion of at least 600 bp, wherein the contiguous portion comprises a combination of two or more variant nucleotide subsequences, wherein a first variant nucleotide subsequence of the two or more variant nucleotide subsequences defines a first end of the contiguous portion and a second variant nucleotide subsequence of the two or more variant nucleotide subsequences defines a second end of the contiguous portion opposite the first end;

introducing the library into a population of cells configured to express one or more polypeptides encoded by a member of the plurality of variant nucleic acids;

selecting a subpopulation of the population of cells based on at least one functional property dependent on the combination of the two or more variant nucleotide subsequences, wherein the subpopulation comprises a plurality of cells;

isolating a subset of the plurality of variant nucleic acids from the subpopulation;

determining nucleotide sequences of the contiguous portion of individual members of the subset; and

identifying at least one combination of the two or more variant nucleotide subsequences based on the nucleotide sequences.

~~32.~~15. The method of claim ~~1~~14, wherein the one or more polypeptides comprises:

T cell receptor α (TCRα)- and TCRβ-chains;

a chimeric antigen receptor (CAR);

a switch receptor; or

one or more chains of an antibody or antigen binding fragment thereof.

~~33.~~16. The method of claim ~~31~~14 or ~~32~~15, wherein the first variant nucleotide subsequence encodes a TCRα variant amino acid sequence and the second variant nucleotide subsequence encodes a TCRβ variant amino acid sequence.

~~34.~~17. The method of any one of claims ~~31~~ 14 to ~~33~~16, wherein the two or more variant nucleotide subsequences encode one or more of: a TCR V region, a TCR complementarity determining region 3 (CDR3), a TCR J-segment, and a TCR constant region.

~~35. The method of claim 31 or 32, wherein each of the two or more variant nucleotide subsequences encodes an antigen binding domain, a hinge domain, a transmembrane domain, or one or more intracellular signaling domains of a CAR.~~

~~36. The method of any one of claims 31-35, wherein the contiguous portions is from 600 bp to 15,000 bp long.~~

~~37. The method of any one of claims 31-36 wherein providing comprises generating the library.~~

~~38. The method of claim 37, wherein generating the library comprises:~~

~~identifying two or more sets of variant nucleotide subsequences encoding two or more sets of variant amino acid sequences of the one or more polypeptides, wherein the at least one functional property depends on a combination of variant amino acid sequences from each of the two or more sets of variant amino acid sequences; and~~

~~assembling the contiguous portion by combining a variant nucleotide subsequence from each of the two or more sets of variant nucleotide subsequences to thereby generate the member of the plurality of variant nucleic acids.~~

~~39. The method of any one of claims 31-38, wherein an edit distance among contiguous portions of the plurality of variant nucleic acids in the library is maximized.~~

~~40. The method of any one of claims 31-39, wherein the library comprises at least 100 different combinations of the two or more variant nucleotide subsequences.~~

~~41. The method of any one of claims 31-40, wherein introducing comprises introducing the library via viral transduction, transposon-based gene delivery, or nuclease-mediated site-specific integration.~~

~~42. The method of claim 41, wherein introducing comprises virally transducing the population of cells at a multiplicity of infection (MOI) of 5 or less.~~

~~43.~~18. The method of any one of claims ~~31~~14-~~42~~17, comprising adjusting a size of the population of cells based on a number of different combinations of the two or more variant nucleotide subsequences in the library.

~~44.~~19. The method of any one of claims ~~31~~14-~~43~~18, wherein the population of cells comprises immortalized T cells or primary T cells.

~~45. The method of claim 44, wherein the immortalized T cells or primary T cells are human T cells.~~

~~46. The method of any one of claims 31-45, further comprising using a marker to select or screen for cells in the population of cells expressing at least one of the plurality of variant nucleic acids.~~

~~47. The method of claim 46, wherein the marker is a cytotoxin resistance marker and/or a cell surface marker.~~

~~48. The method of any one of claims 31-47, wherein cells of the population of cells are genetically modified.~~

~~49. The method of any one of claims 31-47, wherein cells of the population do not comprise an endogenous polypeptide conferring the at least one functional property to the cells.~~

~~50. The method of any one of claims 1 to 49, wherein the cells are genetically modified to eliminate or reduce expression of one or more of CD4, CD8 and CD28.~~

~~51. The method of claim 48 or 49, wherein the cells are reconstituted with CD4 and/or CD8 and utilized to screen for Class I and/or Class II restricted TCR sequences.~~

~~52. The method of claim 50 or 51, wherein the cells are T cells.~~

~~53. The method of any one of claims 31-52, wherein selecting comprises selecting the subpopulation based on expression of a detectable marker, wherein the expression depends on the at least one functional property of the one or more polypeptides.~~

~~54. The method of claim 53, wherein the detectable marker comprises a cell-surface marker, a cytokine marker, a cell proliferation marker, a transcription reporter, a signal transduction reporter, and/or a cytotoxicity reporter.~~

~~55. The method of claim 54, wherein~~

~~the cell-surface marker comprises one or more of: CD25, CD69, CD62L, CD137, OX40;~~

~~the cytokine marker comprises one or more of: IFN-γ, IL-2, TNF-α, IL-8, GM-CSF;~~

~~the transcription reporter comprises one or more of: NF-κB, NFAT, AP-1;~~

~~the signal transduction reporter comprises one or more of: ZAP70, ERK1/2; and~~

~~the cytotoxicity reporter comprises one or more of: CD107A, CD107B.~~

~~56.~~20. The method of any one of claims ~~31-55~~14-19, wherein selecting comprises contacting the population of cells with one or more of:

a second population of cells;

a ligand for the one or more polypeptides;

an agonist or antagonist of the one or more polypeptides; and

a small molecule,

wherein a change in the subpopulation induced by the contacting depends on the at least one functional property of the one or more polypeptides.

~~57. The method of claim 56, wherein selecting further comprises:~~

~~detecting the presence or absence of the change, and/or a magnitude of the change; and~~

~~selecting the subpopulation based on the detecting.~~

~~58.~~21. The method of claim ~~56~~20 ~~or 57~~, wherein the second population of cells comprises antigen-presenting cells.

~~59. The method of claim 58, wherein the antigen-presenting cells comprise B-cells and/or dendritic cells.~~

~~60. The method of any one of claims 56-59, wherein the second population of cells comprises primary cells or immortalized cells.~~

~~61. The method of any one of claims 56-60, wherein variant nucleotide sequences of the library are derived from cells expressing a variant polypeptide comprising an amino acid encoded by the variant nucleotide subsequences, wherein the cells are obtained from a subject, and wherein the second population of cells is derived from the subject.~~

~~62. The method of any one of claims 31-61, wherein selecting comprises selecting a first subpopulation of the population of cells based on a measure of the at least one functional property above or below a threshold.~~

~~63. The method of claim 62, wherein the threshold is determined based on a measure of the functional property in an unselected subpopulation of the population of cells.~~

~~64. The method of claim 62, wherein selecting further comprises selecting a second subpopulation of the population of cells based on a second measure of the at least one functional property above or below a second threshold, wherein the first and second subpopulations are non-overlapping.~~

~~65. The method of claim 64, wherein identifying the at least one combination comprises comparing an abundance of the at least one combination between the first and second subpopulations.~~

~~66.~~22. The method of any one of claims ~~31-64~~14-21, wherein identifying the at least one combination comprises measuring an enrichment of the at least one combination in the subpopulation relative to a control population of cells.

~~67.~~23. The method of claim ~~66~~22, wherein the population of cells comprises the control population of cells.

~~68.~~24. The method of claim 22~~67~~ or claim 23, wherein the subpopulation and control population of cells are non-overlapping, wherein non-overlapping denotes that the cells in both populations have a different activation status, but can carry a same variant nucleic acid.

~~69. The method of claim 66, wherein the control population of cells are selected based on a second functional property that is different from the at least one functional property.~~

~~70. The method of any one of claims 31-69, wherein the subpopulation comprises a plurality of cells.~~

~~71.~~25. The method of any one of claims ~~31-70~~14-24, wherein the isolating does not comprise isolating single clones of the subpopulation based on the at least one functional property.

~~72. The method of any one of claims 31-71, wherein the subpopulation comprises at least 1,000 cells.~~

~~73. The method of any one of claims 31-72, wherein determining comprises sequencing the individual members by generating sequencing reads of at least 600 bp of the contiguous portion.~~

~~74. The method of claim 73, wherein the sequencing reads are between 600 bp and 15,000 bp long.~~

~~75. The method of any one of claims 31-74, wherein determining comprises amplifying at least the contiguous portion of the individual members.~~

~~76. The method of claim 75, wherein amplifying comprises using an amplification primer that hybridizes to an invariant nucleotide subsequence, wherein each of the plurality of variant nucleic acids comprises the invariant nucleotide subsequence, and~~

~~wherein the invariant nucleotide subsequence encodes an invariant amino acid sequence of the one or more polypeptides.~~

~~77. The method of claim 76, wherein the one or more polypeptides comprises TCRα- and TCRβ-chains, and wherein the invariant amino acid sequence comprises a TCRα constant region.~~

~~78. The method of claim 76, wherein the one or more polypeptides comprises TCRα- and TCRβ-chains, and wherein the invariant amino acid sequence comprises a TCRβ constant region.~~

~~79. The method of any one of claims 31-78, wherein the isolating comprises using CRISPR/Cas9-mediated targeted fragmentation of genomic DNA from the subpopulation.~~

~~80. The method of any one of claims 31-78, wherein the determining comprises obtaining an average coverage of at least 10 for each of the nucleotide sequences of the contiguous portion, before or in the absence of any amplification of the individual members.~~

~~81. The method of any one of claims 31-80, wherein the determining comprises obtaining an average coverage of at least 25, at least 50, at least 100, at least 200, at least 300, at least 400, at least 500 or at least 1,000 for each of the nucleotide sequences of the contiguous portion.~~

~~82.~~26. A method of identifying nucleotide sequences encoding T cell receptor α (TCRα)- and TCRβ-chains from a combinatorial library of nucleic acids, comprising:

providing a library comprising a plurality of variant nucleic acids, each of the plurality of variant nucleic acids comprising a contiguous portion of at least 600 bp, wherein the contiguous portion comprises:

a combination of

a first variant nucleotide subsequence encoding a TCRα variant amino acid sequence and defining a first end of the contiguous portion, and

a second variant nucleotide subsequence encoding a TCRβ variant amino acid sequence and defining a second end of the contiguous portion opposite the first end;

introducing the library into a population of immortalized T cells configured to express TCRα- and TCRβ-chains encoded by a member of the plurality of variant nucleic acids;

selecting a subpopulation of the population of immortalized T cells based on an expression of a T cell activation marker above a threshold level in response to contacting the immortalized T cells with immortalized B cells expressing an antigen, wherein the subpopulation comprises a plurality of T cells;

isolating a subset of the plurality of variant nucleic acids from the subpopulation;

determining nucleotide sequences of the contiguous portion of individual members of the subset; and

identifying at least one combination of the first and second variant nucleotide subsequences based on an enrichment of the at least one combination in the nucleotide sequences of the subset relative to a control.

~~83.~~27. The method of claim ~~82~~26, further comprising:

selecting a second subpopulation of the population of immortalized T cells based on the expression of the T cell activation marker below a second threshold level in response to contacting the immortalized T cells with the immortalized B cells, wherein the second subpopulation comprises a second plurality of T cells, and wherein the subpopulation and second subpopulation are non-overlapping;

isolating a second subset of the plurality of variant nucleic acids from the second subpopulation; and

determining second nucleotide sequences of the contiguous portion of individual members of the second subset,

wherein the at least one combination is identified based on an enrichment of the at least one combination in the subset relative to the at least one combination in the second nucleotide sequences of the second subset.

~~84.~~28. A method of identifying a nucleotide sequence encoding a chimeric antigen receptor (CAR) hinge domain, transmembrane domain, and/or an intracellular signaling domain from a combinatorial library of nucleic acids, comprising:

providing a library comprising a plurality of variant nucleic acids, each of the plurality of variant nucleic acids comprising a contiguous portion of at least 600 bp, wherein the contiguous portion comprises a combination of two or more of:

a first variant nucleotide subsequence encoding a CAR hinge domain;

a second variant nucleotide subsequence encoding a CAR transmembrane domain; and

a third variant nucleotide subsequence encoding a CAR intracellular signaling domain,

wherein one of the first, second or third variant nucleotide subsequences define a first end of the contiguous portion, and wherein another one of the first, second or third variant nucleotide subsequences defines a second end of the contiguous portion opposite the first end;

introducing the library into a population of cells configured to express a CAR encoded by a member of the plurality of variant nucleic acids, wherein the population of cells comprises a population of immortalized T cells or primary human T cells;

selecting a subpopulation of the population of cells based on cell proliferation above a threshold level in response to contacting the cells with antigen-presenting cells expressing an antigen specific to an antigen-binding domain of the CAR, wherein the subpopulation comprises a plurality of cells;

isolating a subset of the plurality of variant nucleic acids from the subpopulation;

determining nucleotide sequences of the contiguous portion of individual members of the subset; and

identifying at least one combination of the first, second, and third variant nucleotide subsequences based on an enrichment of the at least one combination in the nucleotide sequences of the subset relative to a control.

~~85.~~29. The method of claim ~~84~~28, wherein there is more than one CAR intracellular signaling domain.

~~86. The method of claim 85, wherein there are at least two CAR intracellular signaling domains.~~

~~87. The method of claim 84, wherein at least two of the following are the at least two CAR intracellular signaling domains: CD3ε, CD3ζ ITAM1, CD3ζ ITAM12, CD3ζ ITAM123, CD3ζ with any ITAM of CD3δ, CD3ε and CD3γ, CD8α, CD28, ICOS, 4-1BB (CD137), OX40 (CD134), CD27, and CD2~~

~~88.~~30. The method of claim ~~84~~28, comprising:

selecting a second subpopulation of the population of cells based on cell proliferation below a second threshold level in response to contacting the cells with the antigen-presenting cells, wherein the second subpopulation comprises a second plurality of cells, and wherein the subpopulation and second subpopulation are non-overlapping;

isolating a second subset of the plurality of variant nucleic acids from the second subpopulation;

determining second nucleotide sequences of the contiguous portion of individual members of the second subset, and

wherein the at least one combination is identified based on an enrichment of the at least one combination in the subset relative to the at least one combination in the second nucleotide sequences of the second subset.

~~89.~~31. A method of identifying a nucleotide sequence from a combinatorial library of nucleic acids, comprising:

providing a combinatorial library comprising a plurality of variant nucleic acids, each of the plurality of variant nucleic acids comprises a contiguous portion of at least 600 bp;

introducing the library into a population of cells configured to express one or more polypeptides encoded by a member of the plurality of variant nucleic acids;

selecting a subpopulation of the population of cells based on at least one functional property dependent on the contiguous portion of at least 600 bp, wherein the subpopulation comprises a plurality of cells;

isolating a subset of the plurality of variant nucleic acids from the subpopulation;

determining nucleotide sequences of the contiguous portion of individual members of the subset; and

identifying the contiguous portion of at least 600 bp based on the nucleotide sequences.

~~90. The method of claim 89, wherein variability in the contiguous portion of at least 600 bp is distributed throughout 600 basepairs.~~

~~91.~~ 32. A method of identifying nucleotide sequences encoding antigen-specific T cell receptor α (TCRα)- and TCRβ-chain pairs from a library of nucleic acids, comprising:

introducing a library into a population of cells able to express TCRα- and TCRβ-chains encoded by a member of a plurality of variant nucleic acids,

selecting a subpopulation of the population of cells based on an expression of a marker above a threshold level in response to antigen, wherein the subpopulation comprises a plurality of cells,

isolating a subset of the plurality of variant nucleic acids from the subpopulation,

determining nucleotide sequences of the variant nucleic acids, and

identifying at least one variant nucleotide sequence based on an enrichment of the nucleotide sequences within the subset relative to a control.

~~92. The method of claim 91 or 90, further comprising providing the library comprising the plurality of variant nucleic acids encoding TCR alpha and TCR beta chains.~~

~~93.~~ 33. A method of identifying nucleotide sequences encoding T cell receptor α (TCRα)- and TCRβ-chains from a sample, comprising:

sequencing TCR-α and β chains in a sample,

selecting and combinatorial pairing TCRα- and β-chain sequences to create a library of TCRαβ pairs,

introducing the library of TCRαβ pairs into a pool of reporter cells,

stimulating the reporter cells that are modified with the library of TCRαβ pairs with antigen presenting cells presenting at least one antigen of interest,

determining TCRαβ pairs specific to the at least one antigen of interest, and

introducing the TCRαβ pairs into cells and selecting cells containing the TCRαβ pairs.

~~94. The method of claim 14, further comprising a step of administering T cells expressing the antigen specific TCR sequences to diagnose or treat an infection or autoimmunity.~~

~~95. The method of claim 14, wherein the T cells can be autologous or allogeneic.~~

~~96.~~ 34. The method of claim ~~8~~26, wherein the activation marker is CD69, and wherein two cell populations are isolated, one cell population with high expression of CD69 and the other cell population with low expression of CD69.

~~97.~~ 35. A nucleotide library comprising the repertoire of T cell receptors recovered according to any one of claims 1-~~30~~ 13 and ~~95, 96~~34.

~~98.~~ 36. A nucleotide construct comprising the nucleotide sequence identified according to any one of claims 1-~~96~~35.

~~99.~~ 37. A cell comprising the nucleotide construct according to claim ~~98~~36.

~~100.~~ 38. A method of identifying a nucleotide sequence encoding an antigen-specific T cell receptor α (TCRα)- and TCRβ-chain pair from a library of nucleic acids, the method comprising:

introducing the nucleic acid library into a population of cells able to express TCRα- and TCRβ-chains to make a library of cells;

selecting a first population of the library of cells based on an expression of a marker above a first threshold level in response to an antigen; and

isolating a first population of variant nucleic acids from the first population of the library.

~~101.~~ 39. The method of claim ~~100~~38, further comprising:

determining at least one nucleotide sequences or nucleic acid identity of the first population of variant nucleic acids; and

identifying at least one variant nucleotide sequence based on an enrichment of the nucleotide sequences within the subset relative to a control.

~~102.~~ 40. The method of claim 37 or 39~~100 or 101~~, wherein the threshold level is based on at least one of:

d) recovery of a percentage of the total pool of cells based on expression of a marker; or

e) recovery of a minimal number of cells from the total pool of cells; or

f) recovery of cells retained by a magnet based on binding of a magnetic probe to at least one marker expressed in response to an antigen

~~103.~~ 41. The method of any one of claims 22-24, 26, 28, 32 and 39 ~~66-69, 82, 84, 91, or 101~~, wherein the control is a second population of cells that is below a second threshold.

~~104.~~ 42. The method of any one of claims 22-24, 26, 28, 32 and 39 ~~66-69, 82, 84, 91, or 101~~, wherein the control is one or more of:

a reference population of cells,

the combinatorial library of nucleic acids that was introduced into the population of cells

a population of cells sorted from a same population of cells as the first population based on an expression marker below a second threshold,

at least one population of cells obtained from cocultures of reporter T cells expressing the relevant TCR library with antigen presenting cells such as B cells that are not presenting any exogenous antigens,

~~105.~~ 43. The method of claims ~~104~~42, wherein the control (or bottom sample) is sorted from a same population of cells as the top sample, but having low activation marker expression or wherein the bottom sample is obtained from cocultures of reporter T cells expressing the relevant TCR library, and B cells that are not engineered to express exogenous antigens.

~~106.~~ 44. The method of any one of claims 37-43~~100-105~~, further comprising adding an antigen to the population of cells.

~~107.~~ 45. The method of any one of claims 37-44~~100-106~~, wherein the isolating a first population and/or the control is achieved by at least one of a) magnetic bead enrichment, b) flow cytometry sorting, or c) both.

~~108.~~ 46. A method of identifying a nucleotide sequence encoding a T cell receptor α (TCRα)- and TCRβ-chain from a library of nucleic acids, the method comprising:

introducing the nucleic acid library into a population of cells able to express TCRα- and TCRβ-chains to make a library of cells; and

determining at least one nucleotide sequence or nucleic acid identity of the first population of variant nucleic acids based on an enrichment of the nucleotide sequence within the subset relative to a control.

~~109. The method of claim 108, wherein the at least one nucleic acid is isolated from a first population of cells.~~

~~110. The method of claim 109, wherein the first population of cells is selected based on an expression of a marker above a first threshold level in response to an antigen.~~

~~111.~~ 47. A method of identifying a nucleotide sequence from a library of nucleic acids, comprising:

introducing the library of nucleic acids into a population of cells to form a library of cells;

contacting the library of cells with a first population of cells;

selecting a sub-population of the library of cells based on expression of at least one marker by magnetic bead enrichment; and

identifying at least one nucleotide sequence based on a statistically significant enrichment or depletion of the nucleotide sequences within the sub-population relative to a control.

~~112. The method of claim 111, wherein at least some of the sub-population of cells are configured to express one or more polypeptides encoded by a member of the library of nucleic acids.~~

~~113. The method of claim 111, wherein marker expression is linked to an introduced nucleic acid from the library, wherein linked denotes that the introduced nucleic acid alters marker expression.~~

~~114. The method of claim 111, wherein selecting is based upon marker expression above a threshold level.~~

~~115. The method of claim 111, wherein selecting is based upon an expression of at least two markers above a threshold level.~~

~~116.~~ 48. The method of any one of claims 26, 32-33 and 38~~8, 82, 91, 93 or 100~~, wherein identifying or stimulating or providing antigen comprises one or more of:

a) selecting a number of antigens;

b) creating antigen-pools in which each antigen is present in exactly two antigen pools

c) evaluating reactivity of reporter cells expressing at least one T cell receptor against each of the antigen pools; and

d) determine whether the at least one T cell receptor is reactive towards any of the selected antigens by evaluating for reactivity against exactly two antigen pools.

~~117.~~ 49. The method of claim ~~116~~48, wherein reactivity against exactly two antigen pools is detected by pairwise enrichment analysis.

~~118. The method of claim 111, wherein the library is a TCR library.~~

~~119. The method of claim 111, wherein one employs an activation marker.~~

~~120. The method of claim 111, wherein one employs a top-bottom comparison to evaluate reactivity.~~

~~121.~~ 50. The method of claim 48, ~~any one of the preceding claims involving an evaluation of reactivity or further comprising an evaluation of reactivity,~~ wherein one employs a top-bottom comparison to evaluate reactivity.

~~122. The method of any one of the preceding claims involving a library, wherein the nucleotide sequence of the plurality of variant nucleic acids in the library is optimized based at least one of the following:~~

~~introduction of preferable codon usage for the host cell,~~

~~optimization of mRNA structural stability,~~

~~avoidance of repetitive sequences,~~

~~avoidance of long stretches of homopolymers, and~~

~~avoidance of large differences in local GC content within a given variant nucleic acid sequence~~

~~123.~~ 51. The method of claim ~~91~~32, wherein the antigen is presented via an antigen-presenting cell.

~~124.~~ 52. The method of claim ~~91~~32, wherein the library is a combinatorial library.

~~125.~~ 53. The method of any one of the preceding claims, wherein the antigen is provided by a cell.

~~126. The method of any one of the preceding claims, wherein the process involves a high degree of antigen diversity and/or complexity.~~

~~127.~~ 54. The method of any of the preceding methods involving a library, wherein the library is a combinatorial library.

~~128. The method above, wherein the combinatorial library is a TCR library.~~

~~129.~~ 55. A collection of cells, the collection comprising:

a set of at least two T cells, wherein each is configured to express at least one TCR alpha and TCR beta pair, wherein the TCR alpha and the TCR beta are each from a subject, wherein the T cells do not express an endogenous TCR, and wherein the set are configured for activation of one or more T cell activation markers; and

a set of at least two B cells, wherein each of the at least two B cells is configured to express at least one exogenous neo-antigen (or antigen), such that there are at least two exogenous neo-antigens (or antigens) capable of being produced, and wherein the at least two exogenous neo-antigens (or antigens) are the same as those in the subject.

~~130. The composition of claim 129, wherein the set of at least two B cells comprises:~~

~~at least a first B cell that produces the exogenous neo-antigen (or antigen); and~~

~~at least a second B cell that produces the a second exogenous neo-antigen (or antigen).~~

~~131.~~ 56. A library of TCR expressing cells, the library of TCR expressing cells comprising: a set of at least three T cells,

wherein at least two of the T cells are configured to express at least two TCR alpha and TCR beta pairs (at least two TCR pairs),

wherein the at least two TCR pairs are from a subject,

wherein the at least three T cells do not express an endogenous TCR,

wherein the at least three T cells are configured for activation of one or more T cell activation markers, upon binding to an antigen (or neo-antigen), presented by a B cell,

wherein an amount of genomic copies of each TCR pair as reflected in a number of TCR cells is such that one gets a read on every TCR in the sample, and

wherein at least one of the TCRs is not distributed equally throughout a composition comprising the library.

~~132. The library of TCR expressing cells of claim 131, wherein a distribution of at least one T cells is altered by binding to an antigen presented by a B cell.~~

~~133. The library of TCR expressing cells of claim 131, wherein the at least two TCR pairs are approximately evenly present in the library of TCR expressing cells.~~

~~134.~~ 57. Use of at least two TCR pairs in the preparation of a medicament for treating tumor in a subject, wherein the TCR pairs are provided according to ~~A method of treating a subject, the~~a method comprising:

identifying a subject having a tumor;

providing a set of at least two T cells, each of which is configured to express at least one different TCR alpha and TCR beta pair, wherein each of the TCR alpha and the TCR beta are from the subject,

providing a set of at least two B cells, wherein the set of B cells is configured to express at least two exogenous neo-antigens, and wherein the at least two exogenous neoantigens are the same as those neo-antigens found in the subject;

combining the set of at least two T cells with the set of at least two B cells and selecting a combination of at least two TCR pairs based upon activation of the at least two T cells via the at least two exogenous neo-antigens; and

~~administering~~ providing the combination of at least two TCR pairs to be administered to the subject~~, thereby treating the tumor~~.

~~135. The method of claim 134, wherein treating reduces a size of the tumor.~~

~~136.~~ 58. Use of at least two TCR pairs in the preparation of a medicament for treating tumor in a subject, therein the TCR pairs are provided according to a~~A method of treating a subject,~~ the method comprising:

identifying a subject having a tumor;

providing a set of at least two T cells, each of which is configured to express at least one different TCR alpha and TCR beta pair, wherein each of the TCR alpha and the TCR beta are from the subject,

providing a set of at least two antigen presenting cells, wherein the set of antigen-presenting cells originates from the subject, is configured to express at least two exogenous neo-antigens, and wherein the at least two exogenous neoantigens are the same as those neo-antigens found in the subject;

combining the set of at least two T cells with the set of at least two antigen present cells and selecting a combination of at least two TCR pairs based upon activation of the at least two T cells via the at least two exogenous neo-antigens; and

providing ~~administering~~ the combination of at least two TCR pairs to be administered to the subject~~, thereby treating the tumor~~.

~~137.~~ 59. A pharmaceutical composition comprising:

a first TCR pair, capable of binding ~~that binds~~ to a first antigen (or neo-antigen) in a subject's tumor; and

a second TCR pair, ~~that binds~~capable of binding to a second antigen (or neo-antigen) in the subject's tumor.

~~138.~~ 60. The pharmaceutical composition of claim ~~137~~59, wherein the first TCR pair is MHC-class I restricted and wherein the second TCR pair is MHC-class II restricted.

~~139.~~ 61. A pharmaceutical composition comprising:

a first TCR pair, that binds to a first antigen and is MHC-class I restricted; and

a second TCR pair, that binds to a second antigen and is MHC-class II restricted.

~~140.~~ 62. The pharmaceutical composition of any one of claims 58-61~~137-139~~, further comprising a third TCR pair.

~~141. The pharmaceutical composition of any one of claims 137-140, wherein the first TCR pair binds to a neo-antigen from a tumor, wherein the second TCR pair binds to a neo-antigen from the tumor, and wherein both the first and second TCR pairs are present in a host of the tumor.~~

~~142.~~ 63. A collection of cells, the collection comprising:

a set of at least two T cells, wherein each is configured to express at least one TCR alpha and TCR beta pair, wherein the pair is from a subject, wherein the T cells do not express an endogenous TCR, and wherein the set are configured for activation of one or more T cell activation markers; and

a set of at least two antigen present cells (APCs), wherein each of the at least two APCs is configured to express at least one exogenous neo-antigen (or antigen), such that there are at least two exogenous neo-antigens (or antigens) capable of being produced, and wherein the at least two exogenous neo-antigens (or antigens) are the same as those in the subject.

~~143. The method of any one of the methods above, wherein, the TCR pairs and/or the T cells expressing the TCR pairs are selected or identified by binding to an antigen (such as a neoantigen), wherein the antigen is expressed by a B cell or an antigen presenting cell.~~

~~144. The method of any one of the methods above, wherein the antigen or neoantigen is from a tumor in a subject, and wherein a TCR alpha and a TCR beta of the TCR pairs are also each from the subject.~~

~~145. The method of any one of the methods above, wherein there are at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 50, 100, 500, 1000, 10000, 100000, or 1 million TCR pairs (or cells comprising these pairs) and there are at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 50, 100, 500, 1000, 10000, 100000, or 1 million antigens present.~~