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, 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.

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

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

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

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

9. The method of any one of claims 1-8, 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).

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

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

12. The method of any one of claims 1-4, 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.

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.

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.

15. The method of claim 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.

16. The method of claim 14 or 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.

17. The method of any one of claims 14 to 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.

18. The method of any one of claims 14-17, 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.

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

20. The method of any one of claims 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.

21. The method of claim 20, wherein the second population of cells comprises antigen-presenting cells.

22. The method of any one of claims 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.

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

24. The method of claim 22 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.

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

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.

27. The method of claim 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.

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.

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

30. The method of claim 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.

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.

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.

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.

34. The method of claim 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.

35. A nucleotide library comprising the repertoire of T cell receptors recovered according to any one of claims 1-13 and 34.

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

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

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.

39. The method of claim 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.

40. The method of claim 37 or 39, 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

41. The method of any one of claims 22-24, 26, 28, 32 and 39 , wherein the control is a second population of cells that is below a second threshold.

42. The method of any one of claims 22-24, 26, 28, 32 and 39 , 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,

43. The method of claims 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.

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

45. The method of any one of claims 37-44, 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.

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.

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.

48. The method of any one of claims 26, 32-33 and 38, 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.

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

50. The method of claim 48, wherein one employs a top-bottom comparison to evaluate reactivity.

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

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

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

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

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.

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.

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 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

providing the combination of at least two TCR pairs to be administered to the subject.

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 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 the combination of at least two TCR pairs to be administered to the subject.

59. A pharmaceutical composition comprising:

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

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

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

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.

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

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.