bias and sequencing error, with the consequences that clonotype abundances can be drastically distorted and that non-existent clonotypes can be recorded, thus falsely increasing the observed diversity. Moreover, selection and/or synthesis of cognate pairs of the TCR chains from the sequencing data for downstream applications such as cloning, functional study and therapeutic use can be time consuming. And the library of selected and synthesized cognate pairs of the TCR chains is small or has low diversity.

SUMMARY

[0003a] In one aspect, provided herein is a method of identifying a plurality of target-reactive T-cell receptors (TCRs), comprising:

(a) providing a mixture comprising a plurality of cells expressing a plurality of TCRs, each cell of the plurality of cells expressing a TCR of the plurality of TCRs, wherein the plurality of TCRs comprises at least 50 different cognate pairs from a sample from a subject and comprises V regions from a plurality of V genes, wherein each cognate pair of the at least 50 different cognate pairs comprises a TCR alpha (TCRα) chain paired with a TCR beta (TCRβ) chain, and wherein the plurality of TCRs are exogenous to the plurality of cells;

(b) contacting the mixture comprising the plurality of cells from (a) with one or more target peptide antigens in complex with a major histocompatibility complex (MHC) molecule, wherein a subset of the plurality of cells expressing the plurality of target-reactive TCRs bind to the one or more target peptide antigens in complex with the MHC molecule; and

(c) identifying at least two cells of the subset of the plurality of cells from (b) that express at least two target-reactive TCRs of the plurality of target-reactive TCRs, thereby identifying the at least two target-reactive TCRs of the plurality of target-reactive TCRs.

[0004] Provided herein are compositions and methods for high throughput cloning of natively paired TCRs which can be used for various applications. The compositions and methods provided herein can surpass sequencing and synthesis and provide fused nucleic acid molecules encoding cognate pairs of TCRs for direct expression in host cells. The compositions and methods can also be applied for other bipartite immunoreceptors, such as B-cell receptors (BCRs).

[0005] According to an example, provided herein is a composition comprising a plurality of fused T-cell receptor (TCR) polynucleotides, wherein each fused TCR polynucleotide of the plurality comprises: a first nucleic acid sequence and a second nucleic acid sequence, wherein (1)

CLAIMS

What is claimed is:

1. A method of identifying a plurality of target-reactive T-cell receptors (TCRs), comprising:

   (a) providing a mixture comprising a plurality of cells expressing a plurality of TCRs, each cell of the plurality of cells expressing a TCR of the plurality of TCRs, wherein the plurality of TCRs comprises at least 50 different cognate pairs from a sample from a subject and comprises V regions from a plurality of V genes, wherein each cognate pair of the at least 50 different cognate pairs comprises a TCR alpha (TCRα) chain paired with a TCR beta (TCRβ) chain, and wherein the plurality of TCRs are exogenous to the plurality of cells;

   (b) contacting the mixture comprising the plurality of cells from (a) with one or more target peptide antigens in complex with a major histocompatibility complex (MHC) molecule, wherein a subset of the plurality of cells expressing the plurality of target-reactive TCRs bind to the one or more target peptide antigens in complex with the MHC molecule; and

   (c) identifying at least two cells of the subset of the plurality of cells from (b) that express at least two target-reactive TCRs of the plurality of target-reactive TCRs, thereby identifying the at least two target-reactive TCRs of the plurality of target-reactive TCRs.
2. The method of claim 1, wherein the plurality of V genes comprises at least 10 different V genes.
3. The method of claim 1 or 2, wherein the plurality of cells is isolated from a sample from a subject, and wherein the sample is a tissue sample, a blood sample, a peripheral blood mononuclear cell (PBMC) sample, or a combination thereof.
4. The method of any one of claims 1-3, wherein the plurality of TCRs comprises at least 100 different cognate pairs, wherein each cognate pair of the at least 100 different cognate pairs comprises a TCRα chain paired with a TCRβ chain.
5. The method of any one of claims 1-4, wherein (b) comprises contacting the plurality of cells with one or more cells presenting the one or more target peptide antigens in complex with the MHC molecule.

6. The method of claim 5, wherein the one or more cells are one or more tumor cells, tumorspheres, tumor lysate-pulsed antigen-presenting cells (APCs), or APCs engineered to present the one or more target peptide antigens.
7. The method of claim 6, wherein the one or more APCs engineered to present the one or more target peptide antigens comprise a DNA or RNA encoding the one or more target peptide antigens.
8. The method of claim 6, wherein the one or more APCs engineered to present the one or more target peptide antigens exogenously expresses an MHC molecule from a subject.
9. The method of any one of claims 1-8, wherein the plurality of TCRs comprises a TCR from an exhausted T cell.
10. The method of any one of claims 1-4, wherein the MHC molecule is an MHC tetramer.
11. The method of any one of claims 1-10, wherein the sequence or identity of the one or more target peptide antigens is unknown.
12. The method of any one of claims 1-11, wherein each cell of the plurality of cells comprises a reporter gene, which reporter gene is regulated to send a signal when a TCR of the cell binds to a target peptide antigen of the one or more target peptide antigens, and wherein identifying in (c) comprises selecting the at least two cells based on the reporter gene.
13. The method of any one of claims 1-12, wherein identifying in (c) comprises selecting the at least two cells based on expression of a cell surface marker.
14. The method of any one of claims 1-13, wherein identifying in (c) comprises selecting the at least two cells based on calcium influx.
15. The method of any one of claims 1-14, wherein the plurality of cells is cell line cells.
16. The method of any one of claims 1-15, wherein the plurality of TCRs comprises at least 100 different VJ combinations.
17. The method of any one of claims 1-16, wherein the plurality of TCRs comprises TCRs from a sample from a subject with a disease or a condition.
18. The method of any one of claims 1-17, wherein the plurality of TCRs comprises TCRs from a sample comprising tumor-infiltrating lymphocytes from a subject.
19. The method of any one of claims 1-18, further comprising, prior to (a), physically linking a first polynucleotide encoding a first TCR chain and a second polynucleotide encoding a second TCR chain of each TCR of the plurality of TCRs, thereby

generating a plurality of fused polynucleotides; and delivering the plurality of fused polynucleotides or copies thereof into the plurality of cells.