Current Protocols in Protein Science (CPPS) (John E. Coligan, *et al*., ed., John Wiley and Sons, Inc.), Current Protocols in Immunology (CPI) (John E. Coligan, *et al*., ed. John Wiley and Sons, Inc.), Current Protocols in Cell Biology (CPCB) (Juan S. Bonifacino *et al*. ed., John Wiley and Sons, Inc.), Culture of Animal Cells: A Manual of Basic Technique by R. Ian Freshney, Publisher: Wiley-Liss; 5th edition (2005), and Animal Cell Culture Methods (Methods in Cell Biology, Vol. 57, Jennie P. Mather and David Barnes editors, Academic Press, 1st edition, 1998).

EXAMPLES

Example 1 – Immunotyping of single T-lymphocytes in a population of T-cells

[00436] The immunophenotyping methods described herein were validated by analyzing both CD4 and CD8 mRNA and surface protein expression in human T lymphocytes. Canonically, mature T-cells are expected to be either the CD4 subtype or the CD8 subtype. A CD4 subtype should express CD4 mRNA and CD4 protein together, but should express either CD8 mRNA or CD8 protein. A CD8 subtype should express CD8 mRNA and CD8 protein together, but should express either CD4 mRNA or CD4 protein.

[00437] 30,000 T-cells were incubated with both a CD4-specific antibody-oligonucleotide comprising a CD4 Antigen ID sequence and a CD8-specific antibody-oligonucleotide comprising a CD8 Antigen ID sequence. The CD4, CD8 and TCR mRNA content of the T-cells were also analyzed in a single-cell emulsion experiment. The T-cell receptor alpha, T-cell receptor beta, CD4 and CD8 mRNA were reverse transcribed to cDNA and a droplet-specific barcode sequence was fused to both the cDNA and antibody-conjugated oligonucleotides through a polymerase chain reaction. This DNA was extracted from the emulsion and analyzed with next generation sequencing.

[00438] Almost all droplet-barcodes (related to a single cell) were associated with either the CD4-specific Antigen ID or the CD8-specific Antigen ID. Substantial agreement was found between the mRNA and surface protein-based assignments (FIG. 3).

Example 2

[00439] Sequencing of the immune repertoire has wide applications in basic immunology, autoimmunity, infectious disease and oncology. While many studies have investigated BCR and TCR diversity in circulating blood, there is growing interest in the immune receptors of TILs, whose functions are highly relevant to cancer growth or regression yet variable and often uncharacterized. A critical step towards a better understanding of TILs will be recovery and functional characterization of their BCRs and TCRs, since this may allow the identification of new tumor-associated antigens. Tumor antigens are critically required to develop cancer vaccines, understand the role of checkpoint inhibitors, and advance chimeric antigen receptor T-cell (CAR-T) therapy in solid tumors. However, despite decades of technical progress in immune sequencing, no study has recovered full-length, natively paired BCRs (heavy and light chains) and TCRs (alpha and beta chains) from a heterogeneous sample such as a tumor without in vitro culture or cell sorting, steps that restrict and bias the observed repertoire. The technical challenge is particularly high since primary uncultured immune cells can contain 100-fold less receptor RNA than in vitro stimulated cells. To allow comprehensive analysis of natively paired BCRs and TCRs from complex heterogeneous samples a

microfluidic emulsion-based method was developed for parallel isolation and DNA barcoding of large numbers of single cells. Up to a million cells per hour are isolated in individual ~65 picoliter emulsion droplets. Within the droplets cells are lysed, target mRNA is reverse transcribed with target-specific primers and a two-step DNA barcoding process attaches both molecule-specific and droplet-specific barcodes to the cDNAs. After subsequent recovery and next generation sequencing, the dual barcoding strategy allows clustering of sequence reads into both their molecules and cells of origin. This allows extensive correction of errors and amplification biases, clone counting at both the mRNA and cellular levels, heavy chain isotype determination, and importantly, recovery of full-length, natively paired V(D)J sequences of BCR and TCRs simultaneously at extremely high throughput.

[00440] Tumor-infiltrating lymphocytes (TILs) are critical to anti-cancer immune responses, but are challenging to study due to their unpredictable abundance, phenotypes and function. A method for deep TIL characterization without the need for cell-sorting, stimulation or culture was developed by the inventors. The emulsion-based, single-cell barcoding method captures natively paired B-cell and T-cell receptor (BCR and TCR) sequences from lymphocytes among millions of input cells. In contrast to previous approaches the recovered variable regions are full-length and can be accompanied by additional mRNA and protein targets. The method was validated with 3 million B-cells from healthy human blood and 350,000 B-cells from an HIV patient before processing 400,000 unsorted cells from an ovarian adenocarcinoma, recovering paired BCRs and TCRs from over 11,000 TILs. Our results represent the deepest sampling of any paired BCR or TCR repertoire as well as the first demonstration of simultaneous RNA sequencing and protein measurements from single-cells in emulsion.

Example 3 – Large scale recovery of B-cell V_HV_L pairs from a healthy blood sample

[00441] The technology was initially developed and pairing capability and throughput was assessed with 3 million B-cells isolated by negative bead enrichment from peripheral blood of a healthy volunteer. The emulsion was split across six separate fractions which were processed in parallel and not remixed prior to sequencing. The emulsion was loaded at 0.2 cells per droplet, giving a Poisson expectation that ~90% of occupied droplets contain single cells, which was consistent with emulsion droplet observations (FIG. 6A). After emulsion breaking and additional library processing steps paired-end 325+300bp sequencing was performed with Illumina MiSeq. To process the sequencing data, the droplet and molecular barcodes were used together to collect PCR replicate reads from each original mRNA molecule, and determined a consensus for each mRNA keeping only mRNA sequences built from at least two reads. Forward and reverse reads were stitched to generate full-length products comprising the 5' UTR, complete V(D)J sequence, and constant region sufficient for isotype determination. Rearranged immunoglobulin heavy and light chain sequences were annotated with IMGT High-VQuest and/or IgBLAST.

[00442] The resulting dataset contained 324,988 droplet barcodes that were associated with at least one heavy chain (V_H) and one light chain (V_L) mRNA, with 229,869 distinct V_H clonal lineages present as estimated by heavy chain clustering analysis. Since this raw set includes data from multi-cell as well as single-cell droplets, data from single-cell droplets was enriched by filtering out droplet barcodes linked to non-unanimous heavy

or light chain V(D)J sequences (FIG. 6B). This step is made possible by the high diversity of typical immune repertoires, in which V_H or V_L mRNAs from two random cells will almost never match. The resulting enriched dataset comprised 259,368 V_HV_L droplet barcodes and contained 182,745 V_H clonal lineages, representing comfortably the deepest sampling of a paired immune repertoire to date. Precision of the V_HV_L pairings by identifying incidences of clonal expansion was directly estimated, since clonally related cells should show consistency in their V_HV_L pairings. 2,604 V_H clones were identified that were observed in more than one emulsion fraction with high confidence to be clonally expanded cells. The consistency of V_L sequences paired with these V_H clones across fractions was very high and indicated a pairing precision of 96.1%, allowing high confidence in the entire filtered dataset of 259,368 V_HV_L pairs. The cross-fraction V_H and V_L sequences were invariably associated with different droplet and molecular barcodes in each fraction and thus did not represent library cross- contamination. The analysis may underestimate pairing precision since some B-cells are known to express multiple light chains. 75.0% of the 259,368 filtered V_HV_L droplet barcodes or "V_HV_L pairs" contained IgM and/or IgD (FIG. 6C), which were frequently observed together as expected given the typical IgM^+IgD^+ phenotype of naïve B-cells. Lower but substantial fractions of IgA (18.3%) and IgG (6.6%) V_HV_L pairs were also found. All V_H isotypes were paired with either Igκ or Igλ in a ~3:2 ratio. Among the 182,745 V_H clonal lineages clone expansion was assessed in two ways: the number of droplet barcodes associated with a clone and observation of the clone across emulsion fractions. Clones seen in multiple droplet barcodes could reflect clonal expansion or multi-barcode droplets, which are expected in ~37% of droplets given the initial $\lambda = 1$ Poisson dispersal of barcodes into droplets. However, any clone represented by > 8 droplet barcodes is likely to be genuinely expanded (Poisson probability in a single droplet < 10-6). While overall 6.0% of clones were seen in more than one fraction, for the clones seen in more than 8 droplet barcodes (0.7% overall), 99% of them were seen in more than one fraction. The 100 most frequent clones (30-137 droplet barcodes each, FIG. 6D) were all seen in at least five of six fractions. A combination of barcode counting and independent fraction analysis thus allows detection of rare expanded lineages amongst a vast background of non-expanded clones. Notably however, even the most abundant expanded clone was present at less than one cell in a thousand, exemplifying the huge diversity of human peripheral immune repertoires.

[00443] The number of captured mRNAs of each V_H and V_L chain within pairs as an estimate of expression level (FIG. 6E). Generally less than ten heavy chain (mean 2.0) and light chain (mean 4.0) mRNAs were captured per droplet barcode, a small population of droplet barcodes with dozens to hundreds of captured heavy and light chain mRNAs per cell was observed, almost exclusively from IgG and IgA expressing cells. Interestingly the degree of V_H and V_L mutation within pairs was strongly correlated both within each isotype (*e.g.*, V_H vs V_L for IgG) and between isotypes (*e.g.*, IgG vs IgM) (FIG. 6F). Furthermore, IgG and IgA pairs were almost all substantially mutated in both their V_H and V_L chains, whereas IgM and IgD pairs mostly showed little V_H or V_L mutation. These results are consistent with the mechanism of B-cell activation leading to class-switching from IgM and IgD to IgG or IgA, increased immunoglobulin expression and somatic hypermutation that affects both heavy and light chain loci in the cell. In addition to this observation that

highly mutated V_H chains tend to be paired with highly mutated V_L chains, this method is capable of generating large numbers of full-length, natively paired BCRs from resting human B-cell repertoires.

Example 4 - Recovery of known low frequency V_HV_L pairs from an HIV elite controller.

[00444] As a further validation of the pairing sensitivity and accuracy of the assay, a sample was processed where several rare (< 1 cell in 10,000) native V_HV_L pairings are already and publically known. Peripheral B-cells from an HIV elite controller patient were obtained whose memory B cells have been mined heavily in recent years for antibodies displaying HIV neutralization activity. 350,000 B-cells were processed to generate a total of 38,620 filtered V_HV_L pairs. Interestingly, this individual showed a greater proportion of IgG than the previous healthy sample (FIG. 7A) or typical healthy peripheral B-cell repertoires. V_H sequences from this dataset were compared to all reported broadly neutralizing antibodies (bNAbs) from this individual including PGT121 and found eight close or identical V_H sequences, indicating that this family of bNAbs represents less than 0.03% of circulating B-cells. Crucially, all light chains paired to these heavy chains were of the expected and similarly rare bNAb lineage, displaying the same Igλ-V3-21/J3 rearrangement and hallmark triple codon insertion as previously reported, supporting the high accuracy and sensitivity of our method. Furthermore, on a phylogenetic tree of all known and newly generated PGT121-like V_HV_L pairs from this individual (FIG. 7B), the V_H and V_L trees show strikingly similar topology with paired V_H and V_L sequences occupying mirror-like positions, likely reflecting shared phylogenetic history. The variant pairs discovered here fit well with this rule. Interestingly, two published antibodies PGT122 and PGT123 appear as exceptions; support for these two pairings was not found, but instead PGT122V_H:PGT123V_L-like, and PGT123V_H:PGT122V_L-like pairs were found, addressing the unverified pairing in the original report. DNA encoding the complete V(D)J regions of 8 novel PGT-like V_HV_L pairs were synthesized, expressed the antibodies as full IgG and tested their ability to neutralize multiple pseudostrains of HIV (FIG. 7C). The antibodies expressed well and all showed strong neutralizing activity against the virus, demonstrating the utility of our approach in rapidly generating natively paired functional antibody variants from a relevant biological sample.

Example 5 -B-cell and T-cell receptor pairs from tumor infiltrating lymphocytes

[00445] Having validated emulsion barcoding for high throughput recovery of paired receptors, immune receptors were recovered directly from a tumor. A protease-dissociated resected ovarian adenocarcinoma sample was taken and entered 400,000 unsorted cells into emulsion. CD3/CD19 staining of a separate aliquot of the sample suggested substantial numbers of infiltrating B (~5%) and T cells (~20%) among the material. Single cell dispersal in the emulsion was similar to purified cells albeit with some limited clumping visible, and extensive variation in cell size and shape within the droplets as expected given the cell type heterogeneity of the sample (FIG. 8A).

[00446] Primers targeting the constant regions of T-cell receptor alpha and beta chains together were used with the BCR primers used previously, and following sequencing and stringent filtering recovered thousands of droplet barcodes linked to BCR or TCR products. To assess single cell precision all possible combinations of the four target loci (V_H, V_L, Vα, Vβ) within droplet barcodes were counted (FIG. 8B). The vast majority (97.9%) of droplet barcodes with more than one target chain contained biologically expected pairings of BCR

$V_H^+V_L$ or TCR $V\alpha^+V\beta$ with only 2.1% containing mixed BCR-TCR combinations. Since barcoding of products is unbiased with respect to target chain, this result allows a high degree of confidence in the resulting 6,056 BCR V_HV_L and 5,217 TCR VαVβ pairs. The BCRs showed striking dominance of IgG (>80%) compared to other isotypes (FIG. 8C), although all were present (IgE < 0.05% only). Kappa and lambda light chains were present in similar ratios to the peripheral blood datasets.

[00447] Similarly to peripheral blood a correlation was observed between BCR isotype and mutation level of both V_H and V_L chains, with IgG and IgA pairs showing greater V_H and V_L mutation than IgD and IgM, and a general correlation of mutation between V_H and V_L within each isotype (FIG. 8D). Interestingly, while IgD, IgM and IgA pairs showed very similar mutational distributions between the tumor and peripheral blood datasets, the tumor IgG fraction also contained a substantial proportion of little-to non-mutated sequences that was not observed in the peripheral blood. For TCRs, and for BCRs containing IgD, similar numbers of captured mRNAs were observed per droplet barcode to the BCR results from peripheral blood (FIG. 8E, mostly <10 per droplet barcode). In stark contrast, the tumor-derived IgM, IgA and IgG pairs showed a 10 to 100-fold increased average expression level with hundreds or thousands of target mRNAs captured in many of the droplet barcodes. The diversity of the captured TIL- TCR and BCR repertoires was then assessed (FIG. 8F). Among the 5,217 total TCR pairs 2,423 distinct TCR beta clones were observed. Seven clones were present at a frequency >1% with the top clone representing 16.9% of all droplet barcodes. Among the 6,056 total BCR pairs 1,518 distinct heavy chain clones were observed, with 15 clones at >1% frequency but none >5%. While this represents substantially more restricted diversity than the healthy peripheral BCR repertoire (where no clone was present in greater than 0.06% frequency), the presence of so many class-switched, mutated and highly expressed clones in the tumor sample demonstrates the necessity of a deep and sensitive sampling approach for TIL characterization. These method allows rapid retrieval of large numbers of TIL immune receptor pairs, from both B and T cells simultaneously, without the need for prior sorting or exogenous activation of defined TIL populations.

Example 6 -Capture of additional phenotypic markers of interest

[00448] Pairing of receptor chains by droplet barcoding potentially allows capture of additional targets besides immune receptors. To investigate this possibility healthy T-cells into $CD4^+$ and $CD8^+$ populations were separated by magnetic bead enrichment and entered 20,000 cells of each type into separate emulsion runs, with primers targeting TCR alpha and beta chains and CD4 and CD8 mRNAs. After sequencing, 47.0% of 3,861 droplet barcodes containing TCR Vα and Vβ ("TCR pairs") from $CD4^+$ isolated cells were linked to CD4 mRNA, while only 0.3% were linked to CD8 mRNA. Conversely, 50.6% of 2,235 TCR pairs from $CD8^+$ isolated cells were associated with CD8 mRNA, while only 0.6% were linked to CD4 mRNA. This demonstrates the high specificity but limited sensitivity of an mRNA-based approach to cell phenotyping, similar to a previous report. In contrast, proteins such as cell surface receptors are usually present in far higher numbers (1,000-100,000 per cell) than their coding mRNAs, potentially making them easier to detect as well as being potentially more directly relevant to cell phenotype. To measure target protein levels on each cell custom oligonucleotide DNA labels were conjugated to anti-human CD4 and CD8 antibodies, and incubated

the labeled antibodies with an unseparated mixture of $CD4^+$ and $CD8^+$ T-cells before entry of 30,000 cells into an emulsion (FIG. 3A). The DNA labels carry antibody-specific sequence tags as well as molecular barcodes and sequence complementarity to the amplified droplet barcodes, allowing emulsion droplet barcoding and molecular counting similarly to that done for mRNAs. The DNA labels were targeted as well as TCR, CD4 and CD8 mRNAs simultaneously. After sequencing and filtering 3,682 droplet barcodes were identified with high confidence TCR VαVβ pairs. Consistent with the previous experiment, roughly half (52%) of the TCR pairs could be assigned CD4 or CD8 status based on mRNA (FIG. 3B-3C). However, over 95% of droplet barcodes could be assigned CD4 or CD8 based on protein status, with average molecular counts per droplet considerably higher for CD4/8 proteins (mean 20.5) than CD4/8 mRNAs (mean 1.0). Concordance between mRNA and protein signals was high (FIG. 3D): 96.0% of droplets given both mRNA and protein calls were in agreement. In some rare instances, both CD4 and CD8 proteins were detected, likely a result of droplets that contained two or more cells. Emulsion barcoding allows, for the first time, direct linking of single cell immune receptors to mRNA and protein markers of interest, all at high throughput. Application of this approach to TILs with an expanded, immune-oncology relevant marker set such as anti-PD-1 and anti-CTLA-4 is warranted.

Example 7 - Human Samples

[00449] The blood sample for healthy repertoire validation was collected under the approval of the Personal Genome Project. PBMCs for the HIV bNAb experiment were obtained from donor 17, an HIV-1 infected donor from the IAVI Protocol G cohort. All human HIV samples were collected with written informed consent under clinical protocols approved by the Republic of Rwanda National Ethics Committee, the Emory University Institutional Review Board, the University of Zambia Research Ethics Committee, the Charing Cross Research Ethics Committee, the UVRI Science and Ethics Committee, the University of New South Wales Research Ethics Committee. St. Vincent's Hospital and Eastern Sydney Area Health Service, Kenyatta National Hospital Ethics and Research Committee, University of Cape Town Research Ethics Committee, the International Institutional Review Board, the Mahidol University Ethics Committee, the Walter Reed Army Institute of Research (WRAIR) Institutional Review Board, and the Ivory Coast Comite "National d'Ethique des Sciences de la Vie et de la Sante" (CNESVS). Cryopreserved, dissociated resected ovarian adenocarcinoma from a single donor was obtained from Conversant Biologics with written informed consent under an IRB approved protocol.

Example 8 - Cell preparation

[00450] For the study of 3 million healthy B-cells, 50 mL blood was drawn into Vacutainer CPT Cell Preparation Tubes with sodium heparin (BD), centrifuged for 20 min at 1800 × g, washed twice in cell preparation buffer (1x PBS supplemented with 2% fetal bovine serum and 2 mM EDTA), using spins at 200 × g to remove platelets, and the resulting PBMCs were cryopreserved in RPMI-1640 medium (Life Technologies) + 20% fetal bovine serum + 10% DMSO at -80 °C until needed. Prior to emulsion generation, PBMCs were thawed, washed twice in cell preparation buffer and counted. B-cells were isolated using a negative selection-based human B-cell enrichment kit (Stem Cell Technologies) according to the

a higher binding signal. T-cells with TCRs that have a higher affinity for the MHC-peptide of the tetramer-oligonucleotide conjugate are expected to exhibit a higher binding signal as compared to the T-cells with TCRs that had a lower affinity for the MHC-peptide of the tetramer-oligonucleotide conjugate at the same concentration of tetramer-oligonucleotide conjugate within the workable range of the assay. For example, in FIG. 19 between the concentrations of log[Tetramer] -8 and -4, the binding signal for TCR1 is above that of TCR2, indicating that in this assay TCR1 would be expected to have a higher affinity for the tetramer reagent used.

Example 21 – Tetramer-Oligonucleotide Conjugate Staining Compared With Conventional MHC-peptide Tetramer Staining

[00465] Target-specific T cells were generated to provide a positive control for testing binding specificity of exemplary tetramer-oligonucleotide conjugate reagents as described herein. An exemplary method for generating target-specific T cells is generally described by Ho et al., *J. Immunol. Methods*, 310:1-2, 40-52. Briefly, dendritic cells are derived from adherent fractions of peripheral blood mononuclear cell (PBMC) samples obtained from normal human donors with HLA-A02:01 by culturing over two days in the presence of GM-CSF and IL-4, followed by incubation beginning at day 3 in the presence of pro-inflammatory cytokines to produce mature dendritic cells. On Day 4, the resulting mature dendritic cells are harvested, washed and pulsed with the desired target-derived peptide to be used in the MHC-peptide tetramer (pMHC). On Day 5, autologous CD8+ T-cells from normal human donors are incubated with the peptide-pulsed dendritic cells. On Day 8, IFNγ in the cultures is measured as an indicator for cultures containing antigen-specific T-cells. Cells from reactive co-cultures are selected and re-stimulated two or three times with peptide-pulsed dendritic cells to enrich for antigen-specific T-cells. In some embodiments, these antigen specific T-cells are generated from such enriched populations by cell sorting and/or limiting dilution cloning essentially as described by Ho et al. 2006. CD8+ T-cells which are not target specific were used as a T-cell control population.

[00466] The target specific CD8+ T-cells and the non- specific CD8+ T-cell control population were incubated with either a fluor-labeled standard MHC-peptide tetramer or various decreasing concentrations of a fluor-labeled tetramer-oligonucleotide conjugate. After incubation, the presence of cells staining positive for standard MHC-peptide tetramer or tetramer-oligonucleotide conjugate were identified by flow cytometry. Both the standard MHC-peptide tetramer and the tetramer-oligonucleotide conjugate incubated with the non-specific CD8+ T-cells showed little to no positive staining (FIG. 20, top row flow cytometry plots). The standard MHC-peptide tetramer incubated with the target specific CD8+ T-cells showed a strong double positive MHC-peptide tetramer+ CD8+ cell population (FIG. 20, bottom row, left flow cytometry plot). The tetramer-oligonucleotide conjugate incubated with the target specific CD8+ T-cells showed a strong double positive tetramer-oligonucleotide conjugate+ CD8+ cell population, in which this double positive population decreased with decreased tetramer-oligonucleotide conjugate concentration (FIG. 20, bottom row, right three flow cytometry plots). This indicated that tetramer-oligonucleotide conjugates bound to target specific T-cells

CLAIMS

1. A method of selecting a T cell receptor (TCR), comprising:

(a) forming a plurality of vessels, wherein a vessel of the plurality of vessels comprises at least one pMHC-oligonucleotide conjugate molecule and a single T cell of a plurality of T cells;

(b) attaching a vessel barcoded polynucleotide or complement thereof to:

(i) an oligonucleotide portion of the pMHC-oligonucleotide conjugate molecule within the vessel of the plurality of vessels, and

(ii) a TCR polynucleotide from the single T cell; and

(c) sequencing the oligonucleotide portion or a complement thereof and the TCR polynucleotide or a complement thereof, thereby generating sequence information, wherein the attaching of step (b) occurs within the vessel.

2. The method of claim 1, wherein a pMHC portion of the pMHC-oligonucleotide conjugate molecule comprises a multimer of the pMHC.

3. The method of claim 1, wherein the pMHC oligonucleotide conjugate molecule comprises a detection portion.

4. The method of claim 1, wherein the method further comprises prior to step (a), obtaining an enriched T cell population comprising T cells bound to pMHC-oligonucleotide conjugate molecules.

5. The method of claim 4, wherein the method further comprises washing the enriched T cell population.

6. The method of claim 1, wherein the method further comprises isolating the single T cell in the vessel.

7. The method of claim 4, wherein the T cells are bound to the pMHC- oligonucleotide conjugate molecules before obtaining the enriched T cell population.

8. The method of claim 6, wherein the method further comprises lysing the single T cell after the single T cell is isolated in the vessel.

9. The method of claim 1, wherein the vessel barcoded polynucleotide or complement thereof comprises a vessel barcode sequence, and wherein the vessel barcode sequence of each vessel barcoded polynucleotide or complement thereof in any single vessel of the plurality of vessels is unique.

10. The method of claim 1, wherein the method further comprises amplifying the oligonucleotide portion or complement thereof.

11. The method of claim 1, wherein the method further comprises amplifying the TCR polynucleotide or complement thereof.

12. The method of claim 1, wherein the vessel barcoded polynucleotide or complement thereof comprises a vessel barcode sequence, and wherein the vessel barcode sequence of the vessel barcoded polynucleotide or complement thereof attached to the TCR polynucleotide and the vessel barcode sequence of the vessel barcoded polynucleotide or complement thereof attached to the oligonucleotide portion are the same.

13. The method of claim 1, wherein the method further comprises pooling oligonucleotide portions, amplified products thereof or complements thereof, and TCR polynucleotides or complements thereof, from two or more vessels of the plurality of vessels.

14. The method of claim 1, further comprising determining a binding affinity of a TCR encoded by the TCR polynucleotide to the pMHC-oligonucleotide conjugate molecule based on the sequence information.

15. The method of claim 1, wherein the method further comprises determining the number of pMHC-oligonucleotide conjugate molecules bound to the single T cell.

16. The method of claim 1, wherein the sequence information comprises TCR polynucleotide sequence reads, vessel barcode sequence reads, and oligonucleotide sequence reads.

17. The method of claim 16, wherein the method further comprises comparing the oligonucleotide sequence reads to the TCR polynucleotide sequence reads.

18. The method of claim 16, wherein the method further comprises selecting a TCR based on the oligonucleotide sequence reads.

19. The method of claim 1, wherein the oligonucleotide portion of the pMHC-oligonucleotide conjugate molecule comprises a tetramer molecular barcode (TMB) sequence that is unique to a single pMHC-oligonucleotide conjugate molecule of a plurality of pMHC-oligonucleotide conjugate molecules.

20. The method of claim 19, wherein a number of pMHC-oligonucleotide conjugate molecules bound to the single T cell is determined from the sequence information.

21. The method of claim 19, wherein the sequence information comprises tetramer molecular barcode sequence reads.

22. The method of claim 21, wherein the number of pMHC-oligonucleotide conjugate molecules bound to the single T cell is determined from the tetramer molecular barcode sequence reads.

23. The method of claim 16, wherein the method further comprises analyzing TMB sequences of the oligonucleotide sequence reads.

24. The method of claim 23, wherein the method further comprises analyzing tetramer identification (TID) sequences of the oligonucleotide sequence reads.

25. The method of claim 1, wherein the oligonucleotide portion comprises a tetramer identification (TID) sequence.

26. The method of claim 25, wherein the TID sequence is barcoded to a peptide or a pMHC portion of the pMHC-oligonucleotide conjugate molecule.

27. The method of claim 25, wherein the sequence information comprises the TID sequence or a complement thereof and a copy number of molecules having the TID sequence.

28. The method of claim 24, wherein the analyzing comprises determining a frequency of one or more vessel barcode sequences, one or more TID sequences, one or more TMB sequences, or a combination thereof.

29. The method of claim 24, wherein the method further comprises comparing TID sequences of oligonucleotide sequence reads to TMB sequences of oligonucleotide sequence reads.

30. A method of determining the binding affinity of a T cell receptor (TCR) comprising

(a) incubating a plurality of pMHC-oligonucleotide conjugate molecules with a plurality of T cells;

(b) forming a vessel comprising at least one of the plurality of pMHC-oligonucleotide conjugate molecules and at least one of the plurality of T cells;

(c) attaching a vessel barcoded polynucleotide or complement thereof to an oligonucleotide portion of the pMHC-oligonucleotide conjugate molecules within the vessel;

(d) quantifying the pMHC-oligonucleotide conjugate molecules within the vessel by sequencing the oligonucleotide portion or a complement thereof of the pMHC-oligonucleotide conjugate molecules, thereby generating sequence information; and

(e) determining a binding affinity of a TCR to a pMHC oligonucleotide conjugate molecule based on the sequence information.

31. A composition comprising a plurality of vessels, wherein a vessel of the plurality of vessels comprises

(a) a single T cell from a sample comprising a plurality of T cells;

(b) a vessel barcoded polynucleotide comprising a vessel barcode sequence; and

(c) a pMHC-oligonucleotide conjugate that binds to a TCR of the single T cell.