

CLAIMS

WHAT IS CLAIMED IS:

1. A method for characterizing cells comprising: performing a reaction in at least one single vessel of a plurality of vessels, the reaction comprising attaching a first vessel barcoded polynucleotide, which comprises a first vessel barcode sequence, to an oligonucleotide portion of an affinity-oligonucleotide conjugate, wherein an affinity portion of the affinity-oligonucleotide conjugate binds to a target antigen expressed by a single cell isolated in the at least one single vessel of the plurality of vessels, and wherein the oligonucleotide portion comprises an antigen identification sequence (AID).
2. The method of claim 1, wherein the single cell is from a plurality of cells.
3. The method of claim 2, wherein the plurality of cells is from a same sample.
4. The method of any one of claims 1-3, wherein the AID is barcoded to the target antigen or the affinity portion of the affinity-oligonucleotide conjugate.
5. The method of any one of claims 1-4, wherein the first vessel barcoded polynucleotide is from a template vessel barcoded polynucleotide in the at least one single vessel.
6. The method of any one of claims 1-5, wherein the method further comprises sequencing the oligonucleotide portion of the affinity-oligonucleotide conjugate, or amplicon thereof to obtain sequence information.
7. The method of claim 6, wherein the method further comprises determining at least one characteristic of the single cell in the at least one single vessel based on the sequence information.
8. The method of claims 6 or 7, wherein the sequence information comprises the antigen identification (AID) sequence or a complement thereof and/or a copy number of molecules having the AID sequence.
9. The method of claim 7, wherein the at least one characteristic is a phenotype.
10. The method of claim 9, wherein the phenotype is an immunophenotype.
11. The method of any one of claims 1-10, wherein the method further comprises contacting a plurality of cells comprising the single cell with the affinity-oligonucleotide conjugate before the single cell is isolated in the at least one single vessel.

12. The method of claim 11, wherein the method further comprises washing the plurality of cells after the contacting.
13. The method of any one of claims 1-12, wherein the method further comprises lysing the single cell.
14. The method of claim 13, wherein the lysing occurs in the at least one single vessel.
15. The method of any one of claims 1-14, wherein the single cell is from a plurality of unsorted cells.
16. The method of any one of claims 1-15, wherein the first vessel barcode sequence of the first vessel barcoded polynucleotide or an amplicon thereof in any one single vessel of the plurality of vessels is unique to the one single vessel.
17. The method of any one of claims 1-16, wherein the method further comprises attaching a second vessel barcoded polynucleotide comprising a second vessel barcode sequence to a cell polynucleotide from the single cell.
18. The method of claim 17, wherein the first vessel barcode sequence of the first vessel barcoded polynucleotide attached to the oligonucleotide portion of the affinity-oligonucleotide conjugate and the second vessel barcode sequence of the second vessel barcoded polynucleotide attached to the cell polynucleotide are the same and/or are from a same template vessel barcoded polynucleotide in the at least one single vessel.
19. The method of claim 17 or 18, further comprising attaching a molecular barcode polynucleotide comprising a molecular barcode sequence to the cell polynucleotide, wherein the molecular barcode sequence is barcoded to the cell polynucleotide and/or amplicons thereof.
20. The method of any one of claims 17-19, wherein attaching the first vessel barcoded polynucleotide to the oligonucleotide portion of the affinity-oligonucleotide conjugate and attaching the second vessel barcoded polynucleotide to the cell polynucleotide are performed simultaneously.
21. The method of any one of claims 18-21, wherein the method further comprises amplifying at least one of the first vessel barcode sequence, the second vessel barcode sequence, the oligonucleotide portion of the affinity-oligonucleotide conjugate, the cell polynucleotide, or any complements thereof.
22. The method of claim 21, comprising amplifying the oligonucleotide portion of the

affinity-oligonucleotide conjugate or a complement thereof, and the cell polynucleotide or a complement thereof simultaneously.

23. The method of any one of claims 1-22, wherein the method further comprises pooling the oligonucleotide portions of the affinity-oligonucleotide conjugates or amplicons thereof and the cell polynucleotides or amplicons thereof, if present, from two or more single vessels of the plurality of vessels.
24. The method of claim 23, further comprising sequencing the pooled oligonucleotide portions or amplicons thereof and/or the pooled cell polynucleotides or amplicons thereof.
25. The method of any one of claims 1-24, wherein the affinity-oligonucleotide conjugate comprises a plurality of different affinity-oligonucleotide conjugates.
26. The method of claim 25, wherein each affinity-oligonucleotide conjugate of the plurality of affinity-oligonucleotide conjugates comprises a unique antigen identification (AID) sequence.
27. The method of any one of claims 1-26, wherein the oligonucleotide portion of the affinity-oligonucleotide conjugate further comprises at least one of an affinity molecular barcode (AMB) sequence, a primer binding sequence, or a constant sequence.
28. The method of any one of claims 1-27, wherein the oligonucleotide portion of the affinity-oligonucleotide comprises a fusion sequence and the attaching comprises attaching the vessel barcoded polynucleotide to the fusion sequence.
29. The method of any one of claims 1-28, wherein the method further comprises sequencing the oligonucleotide portion of the affinity-oligonucleotide along with the attached vessel barcoded polynucleotide, complements thereof, amplified products thereof, or a combination thereof, thereby producing oligonucleotide sequence reads and/or sequencing the cell polynucleotide along with the attached vessel barcoded polynucleotide, complements thereof, amplified products thereof, or a combination thereof, if present, thereby producing cell polynucleotide sequence reads.
30. The method of claim 29, wherein the method further comprises analyzing the oligonucleotide sequence reads and/or comparing one or more first oligonucleotide sequence reads to one or more second oligonucleotide sequence reads.
31. The method of claim 30, further comprising determining a frequency of one or more

- vessel barcode sequences, one or more AID sequences, one or more affinity molecular barcode (AMB) sequences, or a combination thereof.
32. The method claim 29, wherein the method further comprises comparing oligonucleotide sequence reads to the cell polynucleotide sequence reads, vessel barcode sequences of oligonucleotide sequence reads to vessel barcode sequences of the cell polynucleotide sequence reads, antigen identification (AID) sequences of oligonucleotide sequence reads to affinity molecular barcode (AMB) sequences of oligonucleotide sequence reads, cell polynucleotide sequence reads; and/or analyzing vessel barcode sequences of the cell polynucleotide sequence reads or molecular barcode sequences of the cell polynucleotide sequence reads.
 33. The method of any one of claims 30-32, wherein the method further comprises determining a characteristic of a cell based on the analyzing or the comparing.
 34. The method of any one of claims 30-33, wherein the method further comprises selecting an antibody or TCR based on the oligonucleotide sequence reads and/or the cell polynucleotide sequence reads.
 35. The method of any one of claims 1-34, wherein the first vessel barcoded polynucleotide attached to the oligonucleotide portion of the affinity-oligonucleotide conjugate and/or the second vessel barcoded polynucleotide attached to the cell polynucleotide, if present, is an amplification product of a template vessel barcoded polynucleotide.
 36. The method of any one of claims 1-35, wherein the one single vessel comprises a solid support.
 37. The method of any one of claims 1-35, wherein the one single vessel does not comprise a solid support.
 38. The method of any one of claims 1-37, wherein any one single vessel is a well, an emulsion, or a droplet.
 39. The method of any one of claims 1-38, wherein attaching the first vessel barcoded polynucleotide to the oligonucleotide portion or attaching the second vessel barcoded polynucleotide to the cell polynucleotide, if present, comprises a ligation reaction, an enzymatic reaction, a hybridization reaction, an extension reaction, or an amplification reaction.
 40. The method of any one of claims 1-39, wherein the affinity portion of the affinity-

- oligonucleotide conjugate binds to an extracellular antigen of the single cell.
41. The method of claim 40, wherein the extracellular antigen of the single cell is an antigen specific to an immune cell.
 42. The method of claim 41, wherein the extracellular antigen of the single cell is an antigen specific to or expressed by a T-cell.
 43. The method of claim 42, wherein the extracellular antigen is selected from the group consisting of CD154, CD4, CD8, CD137, CD40L, CD80, CD86, CD11c, CD25, CD69, CD44, CD125, CD2, CD3, CDS, CD14 and CD19.
 44. The method of claim 41, wherein the extracellular antigen of the single cell is an antigen specific to or expressed by a B-cell.
 45. The method of claim 44, wherein the extracellular antigen is an immunoglobulin.
 46. The method of any one of claims 1-45, wherein the oligonucleotide portion of the affinity-oligonucleotide conjugate is double stranded.
 47. The method of any one of claims 1-45, wherein the oligonucleotide portion of the affinity-oligonucleotide conjugate is single stranded.
 48. The method of any one of claims 1-47, wherein the oligonucleotide portion of the affinity-oligonucleotide conjugate is DNA.
 49. The method of any one of claims 1-48, wherein the oligonucleotide portion of the affinity-oligonucleotide conjugate is RNA.
 50. The method of any one of claims 17-49, wherein the cell polynucleotide comprises a variable region sequence.
 51. The method of any one of claims 1-50, wherein the method further comprises pairing native chain sequences containing a variable region sequence.
 52. The method of any one of claims 1-43 wherein the single cell is a T-cell.
 53. The method of any one of claims 1-41 and 44-45, wherein the single cell is a B-cell.
 54. The method of any one of claims 17-53, wherein the cell polynucleotide is DNA.
 55. The method of any one of claims 17-53, wherein the cell polynucleotide is RNA.
 56. The method of claim 55, wherein the RNA is mRNA.
 57. The method of any one of claims 1-56, wherein the affinity portion of the affinity oligonucleotide conjugate is an antibody or fragment thereof, a peptide, a protein, an aptamer, a small molecule, a drug, or a cell.
 58. The method of claim 57, wherein the cell is an antigen presenting cell (APC).

59. The method of any one of claims 1-56, wherein the affinity portion of the affinity oligonucleotide conjugate comprises a major histocompatibility complex (MHC) or functional or binding portion thereof.
60. The method of claim 59, wherein the affinity portion comprises a multimer of the MHC, optionally a tetramer of the MHC.
61. The method of claim 59 or 60, wherein the MHC is in a soluble form.
62. The method of any one of claims 59-61, wherein the MHC is bound to a peptide and/or contains a peptide within a groove of the MHC.
63. The method of claim 62 wherein the peptide is a synthetic peptide.
64. The method of any one of claims 59-63, wherein the MHC is bound to a T-cell receptor (TCR) of the single cell.
65. The method of any of claims 1-56, wherein the affinity portion comprises a peptide that binds to an antibody or a chimeric antigen receptor (CAR) and/or wherein the target is an antibody or a CAR.
66. A composition comprising a plurality of vessels, wherein at least one single vessel of the plurality of vessels comprises:
 - (a) a single cell from a sample comprising a plurality of cells, and
 - (b) a vessel barcoded polynucleotide or complement thereof comprising a vessel barcode sequence; and
 - (c) an affinity-oligonucleotide conjugate that binds to a target antigen of the single cell.
67. The composition of claim 66, wherein the plurality of cells is from a same sample.
68. The composition of claims 66 or 67, wherein the vessel barcoded polynucleotide or a complement thereof is attached to the oligonucleotide portion of the affinity-oligonucleotide conjugate.
69. The composition of any one of claims 66-58, wherein the single cell is lysed.
70. A composition comprising a plurality of vessels, wherein a vessel of the plurality of vessels comprises:
 - (a) a single lysed cell from a sample comprising a plurality of cells, and
 - (b) an affinity-oligonucleotide conjugate comprising an affinity portion that binds to a target antigen of the single lysed cell, or an oligonucleotide portion of the affinity-oligonucleotide conjugate; wherein the oligonucleotide portion of the affinity-

oligonucleotide conjugate comprises a vessel barcode sequence, and wherein a cell polynucleotide from the single lysed cell comprises the same vessel barcode sequence.

71. A kit, comprising:
 - (a) a first container comprising a first oligonucleotide comprising a first antigen identification (AID) sequence, wherein the first AID sequence is a known sequence;
 - (b) a second container comprising a second oligonucleotide comprising a second antigen identification (AID) sequence, wherein the second AID sequence is a known sequence and is different than the first AID sequence;
 - (c) one or more third containers comprising reagents capable of conjugating the first oligonucleotide to a first affinity molecule and reagents capable of conjugating the second oligonucleotide to a second affinity molecule;
 - (d) a set of instructions describing how to conjugate the first oligonucleotide to the first affinity molecule and the second oligonucleotide to the second affinity molecule.
72. The kit of claim 71, further comprising:
 - (e) a third container comprising a plurality of vessel barcoded polynucleotides, wherein each vessel barcoded polynucleotide of the plurality of vessel barcoded polynucleotides comprises a unique and distinct vessel barcoded sequence; and
 - (f) reagents and a set of instructions to attach a vessel barcoded polynucleotide of the plurality of vessel barcoded polynucleotides to the first or the second oligonucleotide when conjugated to the first or second affinity molecule.