

CLAIMS

WHAT IS CLAIMED:

1. A method for nucleic acid sequencing, comprising:

(a) providing a plurality of ~~partitions~~droplets, wherein a ~~partition~~droplet of ~~the said~~ plurality of ~~partitions~~droplets comprises

(i) a plurality of ribonucleic acid (RNA) molecules from a single cell, wherein the plurality of RNA molecules comprises at least one immune receptor; comprising a nucleic acid sequence, and

(ii) ~~a bead comprising a nucleic acid barcode molecules coupled thereto, wherein said nucleic acid barcode molecule each~~ comprising a barcode sequence and a sequence that hybridizes to individual RNA molecules of the plurality of RNA molecules;

(b) ~~using said RNA molecule and said hybridizing the~~ nucleic acid barcode molecules to the RNA molecules in the partition ~~generate a barcoded nucleic acid molecule comprising, from a 5' end to a 3' end, a sequence corresponding to said nucleic acid sequence of said RNA molecule and a complement of said barcode sequence; and~~

(c) ~~extending the sequencing said barcoded~~ nucleic acid barcode molecules using a reverse transcriptase having terminal transferase activity to generate barcoded cDNA molecules each comprising a 3' terminal sequence added by the reverse transcriptase or a derivative thereof

(d) hybridizing each 3' terminal sequence of the barcoded cDNA molecules to individual hybridization regions of template switching oligonucleotides (TSOs);

(e) using the TSOs as a template to extend the barcoded cDNA molecules, thereby generating a plurality of barcoded nucleic acid molecules;

(f) amplifying, outside the partition, a subset of the plurality of barcoded nucleic acid molecules generated in (e), to generate an unenriched sequencing library; and

(g) amplifying, outside the partition, a different subset of the plurality of barcoded nucleic acid molecules generated in (e), with a target specific primer to generate a target enriched sequencing library comprising amplicons of the immune receptor.

2.—~~The method of claim 1, wherein said RNA molecule is from a cell.~~

3.—~~The method of claim 2, wherein said droplet comprises said cell.~~

4.—~~The method of claim 3, further comprising releasing said RNA molecule from said cell prior to (b).~~

~~5.2. The method of claim 1, wherein a said bead comprises a plurality of nucleic acid barcode molecules of the nucleic acid barcode molecules is coupled to a particlethereto, wherein said plurality of nucleic acid molecules comprises said nucleic acid barcode molecule.~~

~~6.—The method of claim 5, wherein each of said plurality of nucleic acid molecules comprises said barcode sequence.~~

~~7.—The method of claim 6, wherein each of said plurality of nucleic acid molecules comprises an additional barcode sequence that varies across said plurality of nucleic acid molecules.~~

~~8.—The method of claim 1, wherein said nucleic acid barcode molecule comprises a template switching sequence.~~

~~9.—The method of claim 1, further comprising, prior to (c), subjecting said barcoded nucleic acid molecule or derivative thereof to nucleic acid amplification.~~

~~10.—The method of claim 9, wherein said nucleic acid amplification is performed subsequent to releasing said barcoded nucleic acid molecule or derivative thereof from said droplet.~~

~~11.—The method of claim 9, wherein said nucleic acid amplification is polymerase chain reaction.~~

~~12.—The method of claim 1, wherein said RNA molecule is a messenger ribonucleic acid (mRNA) molecule.~~

~~13.—The method of claim 1, wherein in (a) said droplet comprises (i) an additional nucleic acid molecule comprising an additional nucleic acid sequence, and (ii) an additional nucleic acid barcode molecule comprising an additional barcode sequence, and wherein in (b) said additional nucleic acid molecule and said additional nucleic acid barcode molecule are used to generate an additional barcoded nucleic acid molecule comprising, from a 5' end to a 3' end, said additional barcode sequence and an additional sequence corresponding to said additional nucleic acid sequence.~~

~~14.—The method of claim 13, wherein said additional nucleic acid barcode molecule is coupled to said bead.~~

~~15.—The method of claim 13, wherein said additional nucleic acid barcode molecule is coupled to an additional bead.~~

~~16.—The method of claim 1, wherein (b) is performed in said droplet.~~

~~17.—The method of claim 16, further comprising releasing said barcoded nucleic acid molecule or a derivative thereof from said droplet.~~

~~18.—The method of claim 1, wherein said barcoded nucleic acid molecule further comprises, towards a 3' end, a functional sequence for permitting said barcoded nucleic acid molecule or a derivative thereof to couple to a flow cell of a sequencer.~~

~~19.—The method of claim 1, wherein said sequence is a reverse complement of said nucleic acid sequence.~~

~~20.—The method of claim 1, further comprising, prior to (c), using said barcoded nucleic acid molecule or a derivative thereof and a pair of primers to generate a subset of nucleic acids having a target nucleic acid sequence.~~

~~21.—The method of claim 20, wherein said target nucleic acid sequence comprises a T cell receptor variable region sequence, a B cell receptor variable region sequence, or an immunoglobulin variable region sequence.~~

~~22.—The method of claim 20, wherein at least one of said pair of primers hybridizes to a constant region of a T cell receptor nucleic acid sequence, a constant region of a B cell receptor nucleic acid sequence, or a constant region of an immunoglobulin nucleic acid sequence.~~

~~23.—The method of claim 20, wherein said subset of nucleic acids or derivatives thereof are sequenced in (c).~~

~~24.—The method of claim 1, further comprising releasing said nucleic acid barcode molecule from said bead.~~

~~25.—The method of claim 24, wherein said nucleic acid barcode molecule is released from said bead before said barcoded nucleic acid molecule is generated.~~

~~26.—The method of claim 24, wherein said nucleic acid barcode molecule is released from said bead while said barcoded nucleic acid molecule is generated.~~

~~27.—The method of claim 24, wherein said nucleic acid barcode molecule is released from said bead after said barcoded nucleic acid molecule is generated.~~

~~28.~~3.The method of claim ~~12~~, wherein ~~said bead~~the particle is a gel bead.

4. The method of claim 2, wherein the particle is a magnetic bead.

~~29.—The method of claim 1, wherein said barcode sequence is a combinatorial assembly of a plurality of barcode segments.~~

~~30.—The method of claim 29, wherein plurality of barcode segments comprises at least three segments.~~

~~31.—A method for generating a labeled polynucleotide, comprising:~~

~~(a)—providing a reaction mixture in a reaction volume, wherein said reaction mixture comprises (i) a template polynucleotide, (ii) a primer comprising a sequence towards a 3' end of said primer that hybridizes to said template polynucleotide, and (iii) a template switching oligonucleotide;~~

~~(b)—in the reaction volume, subjecting said reaction mixture to a first reaction under conditions sufficient to generate a first nucleic acid product comprising said primer, a reverse~~

complement of a sequence of said template polynucleotide, and a sequence complementary to at least a portion of said template switching oligonucleotide; and

(c)—subjecting said first nucleic acid product to a second reaction in said reaction volume, which second reaction comprises using (i) said first nucleic acid product as a template and a barcoded oligonucleotide as a primer, which barcoded oligonucleotide comprises a sequence of at least a segment of said template switching oligonucleotide, or (ii) said barcoded oligonucleotide as a template and at least a portion of said first nucleic acid product as a primer, to generate a second nucleic acid product.

32.—The method of claim 31, wherein said second reaction comprises using said first nucleic acid product as a template and said barcoded oligonucleotide as a primer.

33.—The method of claim 31, wherein said second reaction comprises using said barcoded oligonucleotide as a template and at least a portion of said first nucleic acid product as a primer.

34.—The method of claim 31, wherein said template polynucleotide is obtained from a single cell.

35.—The method of claim 34, wherein said single cell is an immune cell.

36.—The method of claim 35, wherein said immune cell is a T-cell.

37.—The method of claim 35, wherein said immune cell is a B-cell.

38.—The method of claim 34, further comprising lysing said single cell in said reaction volume to obtain said template polynucleotide prior to generating said first nucleic acid product in said first reaction.

39.—The method of claim 31, wherein said template polynucleotide comprises a T-cell receptor gene or gene product.

40.—The method of claim 31, wherein said template polynucleotide comprises a B-cell receptor gene or gene product.

41.—The method of claim 31, wherein said template polynucleotide is among a plurality of template polynucleotides.

42.—The method of claim 31, wherein a concentration of said template switching oligonucleotide in said reaction volume is at least two times that of a concentration of said barcoded oligonucleotide in said reaction volume.

43.—The method of claim 31, wherein said primer comprises a sequence towards a 5' end that does not specifically hybridize to the template polynucleotide.

44.—The method of claim 31, wherein said first reaction and said second reaction are performed sequentially in the absence of an intervening purification step.

45.—The method of claim 31, wherein said template switching oligonucleotide is not available for primer extension during said second reaction.

~~46.—The method of claim 31, further comprising degrading said template-switching oligonucleotide prior to said second reaction.~~

~~47.—The method of claim 46, wherein said template-switching oligonucleotide comprises ribonucleotide bases.~~

~~48.—The method of claim 31, further comprising degrading said template-switching oligonucleotide during said second reaction.~~

~~49.—The method of claim 48, wherein said template-switching oligonucleotide comprises ribonucleotide bases.~~

~~50.—The method of claim 31, wherein said first nucleic acid product and said barcoded oligonucleotide has a higher melting temperature as compared to a melting temperature of said first nucleic acid product and said template-switching oligonucleotide.~~

~~51.—The method of claim 31, wherein a primer annealing temperature of said second reaction is at least 0.5 °C greater than a primer annealing temperature of said first reaction.~~

~~52.—The method of claim 31, wherein said template-switching oligonucleotide comprises modified nucleotides.~~

~~53.—The method of claim 31, wherein said barcoded oligonucleotide comprises modified nucleotides.~~

~~54.—The method of claim 31, wherein said reaction volume is a droplet or a well.~~

~~55.—The method of claim 31, wherein said sequence towards said 3' end of said primer comprises a polyA sequence.~~

~~56.—The method of claim 31, wherein said barcoded oligonucleotide is coupled to a bead.~~

~~57.—The method of claim 56, wherein said barcoded oligonucleotide is releasably coupled to said bead.~~

~~58.—The method of claim 56, wherein said bead is a gel bead.~~

~~59.—The method of claim 56, further comprising releasing said barcoded oligonucleotide from said bead.~~

~~60.5.~~ The method of any one of claims 2-459, wherein the nucleic acid barcode molecule said barcoded oligonucleotide is released from the particle said bead upon application of a stimulus.

~~61.6.~~ The method of claim 560, wherein thesaid stimulus ~~includes one or more members selected from the group consisting of~~ comprises a biological stimulus, a chemical stimulus, a thermal stimulus, an electrical stimulus, a magnetic stimulus, ~~or~~ and a photo stimulus.

7. The method of claim 5, wherein the nucleic acid barcode molecule is released from the particle prior to step (b).

8. The method of any one of claims 1-7, wherein a nucleic acid barcode molecule of the nucleic

acid barcode molecules further comprises a unique molecule identifier (UMI) sequence.

9. The method of any one of claims 1-7, wherein a nucleic acid barcode molecule of the nucleic acid barcode molecules further comprises a sequencing primer sequence.

10. The method of any one of claims 1-9, wherein, for at least a subset of the nucleic acid barcode molecules, the sequence that hybridizes to the RNA molecule comprises a polyT sequence.

11. The method of any one of claims 1-9, wherein the sequence that hybridizes to the RNA molecule comprises a sequence complementary to a region of the immune receptor of the RNA molecule.

12. The method of any one of claims 1-11, wherein the immune receptor comprises a T cell receptor (TCR) variable region, a B cell receptor (BCR) variable region, or an immunoglobulin (Ig) variable region.

13. The method of claim 11, wherein the region of the immune receptor sequence corresponds to a constant region of the immune receptor.

14. The method of any one of claims 1-13, wherein the partition comprises the single cell, and wherein the method further comprises lysing the single cell to release the RNA molecules.

62. ~~The method of claim 56, wherein said barcoded oligonucleotide is releasably coupled to said bead through a chemical cross-linker.~~

63. ~~The method of claim 62, wherein said chemical cross-linker is a disulfide bond.~~

64. ~~A method, comprising:~~

~~(a) providing a reaction volume comprising (i) a cell or cell derivative, and (ii) a bead comprising a barcoded oligonucleotide releasably coupled thereto, wherein said barcoded oligonucleotide is a template-switching oligonucleotide; and~~

~~(b) releasing said barcoded oligonucleotide from said bead to provide said barcoded oligonucleotide in said reaction volume at a concentration of at least about 0.20 μ M; and~~

~~(c) subjecting said reaction volume to an amplification reaction to generate an amplification product, wherein during said amplification reaction, said reaction volume comprises a template polynucleotide from said cell or cell derivative, said barcoded oligonucleotide and a primer having a sequence towards a 3' end that hybridizes to said template polynucleotide, and wherein said amplification product has sequence complementarity with said template polynucleotide and said barcoded oligonucleotide.~~

65. ~~The method of claim 64, wherein the template polynucleotide from the cell is an mRNA molecule.~~

66. ~~The method of claim 64, wherein said barcoded oligonucleotide does not hybridize to said template polynucleotide.~~

- ~~67.—The method of claim 64, wherein said cell is a mammalian cell.~~
- ~~68.—The method of claim 64, wherein said cell is an immune cell.~~
- ~~69.—The method of claim 68, wherein said immune cell is a B-cell.~~
- ~~70.—The method of claim 68, wherein said immune cell is a T-cell.~~
- ~~71.—The method of claim 68, wherein the template polynucleotide comprises a T-cell receptor gene sequence, a B-cell receptor gene sequence, or an immunoglobulin gene sequence.~~
- ~~72.—The method of claim 68, wherein the template polynucleotide is a T-cell receptor mRNA molecule, a B-cell receptor mRNA molecule, or an immunoglobulin mRNA molecule.~~
- ~~73.—The method of claim 64, wherein said bead is a gel bead.~~
- ~~74.—The method of claim 64, wherein said bead comprises a chemical cross-linker.~~
- ~~75.—The method of claim 74, wherein said chemical cross-linker is a disulfide bond.~~
- ~~76.—The method of claim 64, wherein said dissociation condition is at least one of a biological stimulus, a chemical stimulus, a thermal stimulus, an electrical stimulus, a magnetic stimulus, a photo-stimulus, or any combination thereof.~~
- ~~77.15. The method of any one of claims 1-1464, wherein said same reaction volume comprises an emulsion, the partition is a droplet, ~~or a microwell~~ or well.~~
- ~~78.—A non-transitory computer-readable medium comprising machine-executable code that, upon execution by one of more computer processors, implements a method for nucleic acid sequencing, comprising:~~
- ~~(a)—providing a plurality of droplets, wherein a droplet of said plurality of droplets comprises (i) a ribonucleic acid (RNA) molecule comprising a nucleic acid sequence, and (ii) a bead comprising a nucleic acid barcode molecule coupled thereto, wherein said nucleic acid barcode molecule comprises a barcode sequence;~~
- ~~(b)—using said RNA molecule and said nucleic acid barcode molecule to generate a barcoded nucleic acid molecule comprising, from a 5' end to a 3' end, a sequence corresponding to said nucleic acid sequence of said RNA molecule and a complement of said barcode sequence; and~~
- ~~(c)—sequencing said barcoded nucleic acid molecule or a derivative thereof.~~
- ~~79.—A non-transitory computer-readable medium comprising machine-executable code that, upon execution by one of more computer processors, implements a method for generating a labeled polynucleotide, the method comprising:~~
- ~~(a)—subjecting a reaction mixture to a first reaction under conditions sufficient to generate a first nucleic acid product, wherein said reaction mixture comprises (i) a template polynucleotide, (ii) a primer having a sequence towards a 3' end that hybridizes to said template polynucleotide, and (iii) a template-switching oligonucleotide, wherein said first~~

nucleic acid product comprises said primer, a reverse complement of a sequence of said template polynucleotide, and a sequence complementary to at least a portion of said template switch oligonucleotide; and

(b)—subjecting said first nucleic acid product to a second reaction in the presence of a barcoded oligonucleotide under conditions sufficient to generate a second nucleic acid product, wherein said barcoded oligonucleotide comprises a sequence of at least a segment of said template switching oligonucleotide, wherein (i) said second reaction uses said first nucleic acid as a template and said barcoded oligonucleotide as a primer, or (ii) said second reaction uses said barcoded oligonucleotide as a template and at least a portion of said first nucleic acid as a primer, to generate said second nucleic acid product,

wherein said first reaction and said second reaction are performed within a same reaction volume.

16. The method of any one of claims 1-15, wherein amplifying the different subset of the plurality of barcoded nucleic acid molecules with the target specific primer to generate the target enriched sequencing library comprises using the target specific primer to amplify an immune receptor variable region of the immune receptor.

17. The method of claim 16, wherein the immune receptor variable region is from a T cell receptor, a B cell receptor, or an immunoglobulin sequence.

18. The method of any one of claims 1-17, wherein a hybridization region of the TSOs comprises a polyG sequence and the sequence that is added by the reverse transcriptase comprises a polyC sequence.

19. The method of any one of claims 1-18, wherein amplifying the different subset of the plurality of barcoded nucleic acid molecules with the target specific primer to generate the target enriched sequencing library comprises adding a functional sequence to the barcoded cDNA molecule.

20. The method of claim 19, wherein the functional sequence comprises a flow cell attachment sequence and/or a sequencing primer binding sequence.

21. The method of any one of claims 1-20, wherein the sequence that hybridizes to the RNA molecule is towards the 3' end of the nucleic acid barcode molecule.

22. The method of any one of claims 1-21, wherein the method further comprises:

(h) determining sequences from the unenriched sequencing library from (f) and sequences of the target enriched sequencing library from (g); and

(i) analyzing the sequences from step (h) thereby associating expression of sequences from the target enriched sequencing library and the unenriched sequencing library with the single cell.

23. The method of claim 22, wherein the target enriched sequencing library comprises sequences comprising an immune receptor variable region.
24. The method of claim 23, wherein the immune receptor variable region is from a T cell receptor or a B cell receptor sequence.
25. The method of any one of claims 1-24, wherein the method further comprises:
(g) determining sequences from the unenriched sequencing library from (e) and sequences of the target enriched sequencing library from (f); and
(h) analyzing the sequences from step (g) thereby associating expression of the target enriched library and the unenriched library with the single cell.
26. The method of any one of claims 1-25, wherein the unenriched sequencing library is a transcriptome sequencing library.
27. The method of any one of claims 1-26, wherein the sequence that hybridizes to the RNA molecule comprises a polyT sequence and the target enriched sequencing library comprises sequences comprising an immune receptor variable region.
28. The method of claim 27, wherein the immune receptor variable region is from a T cell receptor or a B cell receptor sequence.
29. The method of any one of claims 1-28, wherein step (b) is carried out in the partition after cell lysis.
30. The method of any one of claims 1-29, wherein in step (h) the analyzing comprises associating immune repertoire and transcriptome with the single cell.