

CLAIMS

WHAT IS CLAIMED:

1. A method for nucleic acid sequencing, comprising:
 - (a) providing a plurality of partitions, wherein a partition of the plurality of partitions 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;
 - (ii) nucleic acid barcode molecules each comprising a barcode sequence and a sequence that hybridizes to individual RNA molecules of the plurality of RNA molecules;
 - (b) hybridizing the nucleic acid barcode molecules to the RNA molecules in the partition;
 - (c) extending the 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;
 - (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 a nucleic acid barcode molecule of the nucleic acid barcode molecules is coupled to a particle.
3. The method of claim 2, wherein the particle is a gel bead.
4. The method of claim 2, wherein the particle is a magnetic bead.

5. The method of any one of claims 2-4, wherein the nucleic acid barcode molecule is released from the particle upon application of a stimulus.
6. The method of claim 5, wherein the stimulus comprises a biological stimulus, a chemical stimulus, a thermal stimulus, an electrical stimulus, a magnetic stimulus, or 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.
15. The method of any one of claims 1-14, wherein the partition is a droplet, microwell or well.

- 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.