

barcode molecule comprises a barcode sequence; (b) using the RNA molecule and the nucleic acid barcode molecule to generate a barcoded nucleic acid molecule comprising, from a 5' end to a 3' end, a sequence corresponding to the nucleic acid sequence of the RNA molecule and a complement of the barcode sequence; and (c) sequencing the barcoded nucleic acid molecule or a derivative thereof.

[0005a] In one aspect, the present disclosure provides 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 from a cell comprising a nucleic acid sequence corresponding to a V(D)J region of a genome of said cell, (ii) a bead comprising a nucleic acid barcode molecule coupled thereto, wherein said nucleic acid barcode molecule comprises a barcode sequence, and (iii) a primer; (b) generating 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 by (i) hybridizing said primer to a region at a 3' end of said RNA molecule, (ii) extending said primer to incorporate a sequence at a 3' end that is complementary to said nucleic acid barcode molecule, to generate a nucleic acid product, and (iii) using said nucleic acid barcode molecule as a template to extend said nucleic acid product; and (c) sequencing said barcoded nucleic acid molecule or a derivative thereof.

[0006] In some embodiments, the RNA molecule is from a cell. In some embodiments, the droplet comprises the cell. In some embodiments, the method further comprises releasing the RNA molecule from the cell prior to (b).

[0007] In some embodiments, the bead comprises a plurality of nucleic acid molecules coupled thereto, wherein the plurality of nucleic acid molecules comprises the nucleic acid barcode molecule.

[0008] In some embodiments, each of the plurality of nucleic acid molecules comprises the barcode sequence. In some embodiments, each of the plurality of nucleic acid molecules comprises an additional barcode sequence that varies across the plurality of nucleic acid molecules.

[0009] In some embodiments, the nucleic acid barcode molecule comprises a template switching sequence.

[0010] In some embodiments, the method further comprises, prior to (c), subjecting the barcoded nucleic acid molecule or derivative thereof to nucleic acid amplification. In some embodiments, the nucleic acid amplification is performed subsequent to releasing the barcoded nucleic acid molecule or derivative thereof from the droplet. In some embodiments, the nucleic

acid amplification is polymerase chain reaction. In some embodiments, the RNA molecule is a messenger ribonucleic acid (mRNA) molecule.

[0011] In some embodiments, in (a) the 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) the additional nucleic acid molecule and the additional nucleic acid barcode molecule are used to generate an additional barcoded nucleic acid molecule comprising, from a 5' end to a 3' end, the additional barcode sequence and an additional sequence corresponding to the additional nucleic acid sequence. In some embodiments, the additional nucleic acid barcode molecule is coupled to the bead. In some embodiments, the additional nucleic acid barcode molecule is coupled to an additional bead.

[0012] In some embodiments, (b) is performed in the droplet.

[0013] In some embodiments, the method further comprises releasing the barcoded nucleic acid molecule or a derivative thereof from the droplet.

CLAIMS

WHAT IS CLAIMED:

1. 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 from a cell comprising a nucleic acid sequence corresponding to a V(D)J region of a genome of said cell, (ii) a bead comprising a nucleic acid barcode molecule coupled thereto, wherein said nucleic acid barcode molecule comprises a barcode sequence, and (iii) a primer;
 - (b) generating 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 by (i) hybridizing said primer to a region at a 3' end of said RNA molecule, (ii) extending said primer to incorporate a sequence at a 3' end that is complementary to said nucleic acid barcode molecule, to generate a nucleic acid product, and (iii) using said nucleic acid barcode molecule as a template to extend said nucleic acid product; and
 - (c) sequencing said barcoded nucleic acid molecule or a derivative thereof.
2. The method of claim 1, wherein said droplet comprises said cell.
3. The method of claim 2, further comprising releasing said RNA molecule from said cell prior to (b).
4. The method of claim 1, wherein said bead comprises a plurality of nucleic acid molecules coupled thereto, wherein said plurality of nucleic acid molecules comprises said nucleic acid barcode molecule comprising said barcode sequence, and wherein each of said plurality of nucleic acid molecules comprises an additional barcode sequence that varies across said plurality of nucleic acid molecules.
5. The method of claim 1, wherein said nucleic acid barcode molecule comprises a template switching sequence.
6. The method of claim 1, further comprising, prior to (c), subjecting said barcoded nucleic acid molecule or derivative thereof to nucleic acid amplification.
7. The method of claim 6, wherein (b) further comprises releasing said barcoded nucleic acid molecule or derivative thereof, and wherein said nucleic acid amplification is performed subsequent to said releasing of said barcoded nucleic acid molecule or derivative thereof from said droplet.

8. The method of claim 1, wherein in (a) said droplet comprises (i) an additional RNA molecule comprising an additional nucleic acid sequence transcribed from an additional V(D)J region of said genome of said cell, and (ii) an additional nucleic acid barcode molecule comprising an additional barcode sequence, wherein said additional barcode sequence is coupled to said bead, and wherein in (b) said additional RNA 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, a sequence complementary to said barcode sequence and a sequence complementary to said additional nucleic acid sequence.
9. The method of claim 8, wherein said additional nucleic acid barcode molecule is coupled to said bead or to an additional bead.
10. The method of claim 1, wherein (b) is performed in said droplet.
11. The method of claim 1, wherein said barcoded nucleic acid molecule further comprises one or more functional sequences selected from the group consisting of: a sequence for permitting said barcoded nucleic acid molecule, a derivative thereof to couple to a flow cell of a sequencer, a primer sequence, and a primer binding sequence.
12. The method of claim 1, wherein said sequence is a reverse complement of said nucleic acid sequence.
13. The method of claim 1, further comprising, prior to (c), using said barcoded nucleic acid molecule or derivative thereof and a pair of primers to generate a subset of nucleic acids having a target nucleic acid sequence.
14. The method of claim 13, 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.
15. The method of claim 13, 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.
16. The method of claim 1, further comprising releasing said nucleic acid barcode molecule from said bead to generate said barcoded nucleic acid molecule.
17. The method of claim 1, wherein said bead is a gel bead.

18. The method of claim 8, wherein said RNA molecule and said additional RNA molecule encode a transcript selected from the group consisting of a T cell receptor (TCR) alpha, TCR beta, TCR gamma, and TCR delta.

19. The method of claim 1, wherein said nucleic acid barcode molecule further comprises an adapter sequence 5' to said barcode sequence, and wherein said barcoded nucleic acid molecule comprises said adapter sequence.

20. The method of claim 19, further comprising, prior to (c), performing a polymerase chain reaction (PCR) on said barcoded nucleic acid molecule or derivative thereof using a first primer complementary to said adapter sequence and a second primer complementary to a constant region of a T cell receptor (TCR), B cell receptor (BCR), or immunoglobulin gene sequence.