

be performed within a partition such as a droplet or well. The methods of the present disclosure may obviate the need for reverse transcription during analysis of ribonucleic acid molecules and may be useful, for example, in controlled analysis and processing of analytes such as biological particles, nucleic acids, and proteins.

[0005a] In one aspect, provided here in is a method of barcoding nucleic acid analytes comprising: (a) providing: (i) a sample comprising a biological particle that comprises a nucleic acid molecule, wherein said nucleic acid molecule comprises a first target region and a second target region, wherein said first target region and said second target region are disposed on a same strand of said nucleic acid molecule, (ii) a first probe comprising a first probe sequence and a first barcode sequence that identifies said sample, wherein said first probe sequence of said first probe is complementary to said first target region of said nucleic acid molecule, (iii) a second probe comprising a second probe sequence, wherein said second probe sequence of said second probe is complementary to said second target region of said nucleic acid molecule, (b) subjecting said biological particle to conditions sufficient to (i) hybridize said first probe sequence of said first probe to said first target region of said nucleic acid molecule, and (ii) hybridize said second probe sequence of said second probe to said second target region of said nucleic acid molecule to yield a probe-associated nucleic acid molecule; (c) subjecting said probe-associated nucleic acid molecule to conditions sufficient to yield a probe-linked nucleic acid molecule comprising said first probe linked to said second probe; and (d) generating a barcoded nucleic acid molecule using said probe-linked nucleic acid molecule and a nucleic acid barcode molecule that comprises a second barcode sequence that identifies said biological particle, wherein said barcoded nucleic acid molecule comprises (i) a sequence corresponding to said first target region, (ii) a sequence corresponding to said second target region, (iii) said first barcode sequence or a complement thereof, and (iv) said second barcode sequence or a complement thereof.

[0005b] In another aspect, provided herein is a method of barcoding nucleic acid analytes comprising: (a) providing: (i) a sample comprising a biological particle that comprises a nucleic acid molecule, wherein said biological particle is among a plurality of biological particles in said sample,

wherein said nucleic acid molecule comprises a first target region and a second target region, wherein said first target region and said second target region are disposed on a same strand of said nucleic acid molecule, (ii) a first probe comprising a first probe sequence and a first barcode sequence, wherein said first probe sequence of said first probe is complementary to said first target region of said nucleic acid molecule, (iii) a second probe comprising a second probe sequence, wherein said second probe sequence of said second probe is complementary to said second target region of said nucleic acid molecule, (b) subjecting said sample to conditions sufficient to (i) hybridize said first probe sequence of said first probe to said first target region of said nucleic acid molecule, and (ii) hybridize said second probe sequence of said second probe to said second target region of said nucleic acid molecule to yield a probe-associated nucleic acid molecule in said biological particle; (c) subjecting said probe-associated nucleic acid molecule to conditions sufficient to yield a probe-linked nucleic acid molecule comprising said first probe linked to said second probe in said biological particle among said plurality of biological particles, wherein said probe-linked nucleic acid molecule comprises said first barcode sequence; (d) after (c), partitioning said plurality of biological particles into a plurality of partitions; and (e) within a partition of said plurality of partitions, generating a barcoded nucleic acid molecule using said probe-linked nucleic acid molecule and a nucleic acid barcode molecule that comprises a second barcode sequence, wherein said barcoded nucleic acid molecule comprises (i) a sequence corresponding to said first target region, (ii) a sequence corresponding to said second target region, (iii) said first barcode sequence or a complement thereof, and (iv) said second barcode sequence or a complement thereof.

[0006] In an example, provided herein is a method of analyzing a sample comprising a nucleic acid molecule, comprising: (a) providing: (i) a sample comprising the nucleic acid molecule, wherein the nucleic acid molecule comprises a first target region and a second target region, wherein the first target region is adjacent to the second target region; (ii) a first probe comprising a first probe sequence and a second probe sequence, wherein the first probe sequence of the first probe is complementary to the first target region of the nucleic acid molecule, and wherein the first probe sequence comprises a first reactive moiety; and (iii) a second probe

comprising a third probe sequence, wherein the third probe sequence of the second probe is complementary to the second target region of the nucleic acid molecule, and wherein the third probe sequence comprises a second reactive moiety; (b) subjecting the sample to conditions sufficient to (i) hybridize the first probe sequence of the first probe to the first target region of the nucleic acid molecule, and (ii) hybridize the third probe sequence of the second probe to the second target region of the nucleic acid molecule, such that the first reactive moiety of the first probe sequence of the first probe is adjacent to the second reactive moiety of the third probe sequence of the second probe; (c) subjecting the first reactive moiety and the second reactive moiety to conditions sufficient to yield a probe-linked nucleic acid molecule comprising the first probe linked to the second probe; and (d) with the probe-linked nucleic acid molecule in a partition, barcoding the probe-linked nucleic acid molecule to provide a barcoded probe-linked nucleic acid molecule.

[0007] In some examples, the partition is a well. In some examples the partition is a droplet. In some examples, (d) comprises (i) providing, in the partition, a nucleic acid barcode molecule comprising a binding sequence and a barcode sequence, wherein the binding sequence is complementary to the second probe sequence of the first probe, and (ii) hybridizing the binding sequence to the second probe sequence in the partition. In some examples, the nucleic acid barcode molecule further comprises an additional binding sequence. In some cases, the binding sequence is hybridized to the second probe sequence in a partition among a plurality of partitions. In some examples, subsequent to (c), the probe-linked nucleic acid molecule is co-partitioned with the nucleic acid barcode molecule. In some examples, subsequent to (a), the nucleic acid molecule is co-partitioned with the first probe, the second probe, and the nucleic acid barcode molecule. In some examples, (b) and (c) are performed in the partition. In some examples, the method further comprises subjecting the partition to conditions sufficient to

CLAIMS

WHAT IS CLAIMED IS:

1. A method of barcoding nucleic acid analytes comprising:

(a) providing:

(i) a sample comprising a biological particle that comprises a nucleic acid molecule, wherein said nucleic acid molecule comprises a first target region and a second target region, wherein said first target region and said second target region are disposed on a same strand of said nucleic acid molecule,

(ii) a first probe comprising a first probe sequence and a first barcode sequence that identifies said sample, wherein said first probe sequence of said first probe is complementary to said first target region of said nucleic acid molecule,

(iii) a second probe comprising a second probe sequence, wherein said second probe sequence of said second probe is complementary to said second target region of said nucleic acid molecule,

(b) subjecting said biological particle to conditions sufficient to (i) hybridize said first probe sequence of said first probe to said first target region of said nucleic acid molecule, and (ii) hybridize said second probe sequence of said second probe to said second target region of said nucleic acid molecule to yield a probe-associated nucleic acid molecule;

(c) subjecting said probe-associated nucleic acid molecule to conditions sufficient to yield a probe-linked nucleic acid molecule comprising said first probe linked to said second probe; and

(d) generating a barcoded nucleic acid molecule using said probe-linked nucleic acid molecule and a nucleic acid barcode molecule that comprises a second barcode sequence that identifies said biological particle, wherein said barcoded nucleic acid molecule comprises (i) a sequence corresponding to said first target region, (ii) a sequence corresponding to said second target region, (iii) said first barcode sequence or a complement thereof, and (iv) said second barcode sequence or a complement thereof.

2. The method of claim 1, wherein (d) is performed in a partition among a plurality of partitions.

3. A method of barcoding nucleic acid analytes comprising:

(a) providing:

(i) a sample comprising a biological particle that comprises a nucleic acid molecule, wherein said biological particle is among a plurality of biological particles in said sample, wherein said nucleic acid molecule comprises a first target region and a second target region, wherein said first target region and said second target region are disposed on a same strand of said nucleic acid molecule,

(ii) a first probe comprising a first probe sequence and a first barcode sequence, wherein said first probe sequence of said first probe is complementary to said first target region of said nucleic acid molecule,

(iii) a second probe comprising a second probe sequence, wherein said second probe sequence of said second probe is complementary to said second target region of said nucleic acid molecule,

(b) subjecting said sample to conditions sufficient to (i) hybridize said first probe sequence of said first probe to said first target region of said nucleic acid molecule, and (ii) hybridize said second probe sequence of said second probe to said second target region of said nucleic acid molecule to yield a probe-associated nucleic acid molecule in said biological particle;

(c) subjecting said probe-associated nucleic acid molecule to conditions sufficient to yield a probe-linked nucleic acid molecule comprising said first probe linked to said second probe in said biological particle among said plurality of biological particles, wherein said probe-linked nucleic acid molecule comprises said first barcode sequence;

(d) after (c), partitioning said plurality of biological particles into a plurality of partitions; and

(e) within a partition of said plurality of partitions, generating a barcoded nucleic acid molecule using said probe-linked nucleic acid molecule and a nucleic acid barcode molecule that comprises a second barcode sequence, wherein said barcoded nucleic acid molecule comprises (i) a sequence corresponding to said first target region, (ii) a sequence corresponding to said second target region, (iii) said first barcode sequence or a complement thereof, and (iv) said second barcode sequence or a complement thereof.

4. The method of claim 3, wherein said barcode sequence identifies said sample.

5. The method of claim 3 or 4, wherein said barcode sequence identifies said biological particle.

6. The method of any one of claims 3-5, wherein said partition of the plurality of partitions is a well among a plurality of wells.

7. The method of any one of claims 3-5, wherein said partition of said plurality of partitions is a droplet among a plurality of droplets.

8. The method of any one of claims 1-7, wherein, in (a), said first probe sequence and said first barcode sequence are disposed on a same strand of said first probe.

9. The method of any one of claims 1-7, wherein, in (a), said first probe sequence and said first barcode sequence are disposed on different strands of said first probe.

10. The method of claim 9, wherein the first probe comprises (I) a first oligonucleotide comprising said first probe sequence and a non-hybridizing segment; and (II) a second oligonucleotide comprising said first barcode sequence, wherein a portion of said second oligonucleotide is hybridized to said non-target hybridizing segment of said first oligonucleotide.

11. The method of claim 9, wherein the first probe comprises:

(I) an first oligonucleotide comprising said first probe sequence and a first adapter sequence;

(II) a second oligonucleotide comprising said first barcode sequence and a second adapter sequence; and

(III) a splint oligonucleotide comprising a first sequence complementary to at least a portion of said first adapter sequence of said first oligonucleotide and a second sequence complementary to at least a portion of said second adapter sequence of said second oligonucleotide;

wherein said first sequence of said splint oligonucleotide is hybridized to said first adapter sequence of said first oligonucleotide, and said second sequence of said splint oligonucleotide is hybridized to said second adapter sequence of said second oligonucleotide.

12. The method of any one of claims 1 to 11, wherein (c) comprises subjecting said probe-associated nucleic acid molecule to conditions sufficient to ligate said first probe to said second probe to yield said probe-linked nucleic acid molecule.

13. The method of any one of claims 1 to 11, wherein a gap region of one or more nucleotides occurs between said first probe and said second probe when said first probe and said second probe are hybridized to said nucleic acid molecule in said probe-associated nucleic acid molecule.

14. The method of any one of claims 1 to 13, wherein generating said barcoded nucleic acid molecule comprises conducting a nucleic acid extension reaction using said probe-linked nucleic acid molecule and said nucleic acid barcode molecule.

15. The method of any one of claims 1 to 14, wherein generating said barcoded nucleic acid molecule comprises ligating said probe-linked nucleic acid molecule and said nucleic acid barcode molecule.

16. The method of any one of claims 1 to 15, wherein said biological particle comprises a member selected from the group consisting of a cell, an organelle, and a cell bead.

17. The method of any one of claims 1 to 16, wherein said biological particle is a cell.

18. The method of claim 17, further comprising, subsequent to (a), lysing or permeabilizing said cell, thereby providing access to said nucleic acid molecule.

19. The method of claim 17 or claim 18, wherein said cell is a fixed cell.

20. The method of any one of claims 16 to 19, wherein said cell is a permeabilized cell.

21. The method of any one of claims 16 to 20, wherein said cell is a eukaryotic cell.

22. The method of claim 3, wherein said plurality of biological particles comprises a plurality of barcoded nucleic acid molecules generated in (e).

23. The method of claim 22, further comprising, after (e), pooling said plurality of biological particles from said plurality of partitions to provide a pooled mixture of biological particles comprising barcoded nucleic acid molecules.

24. The method of claim 23, wherein said pooled mixture of biological particles is partitioned into additional partitions, wherein a single partition of said additional partitions comprises one or more biological particles of said pooled mixture of biological particles and a plurality of additional nucleic acid barcode molecules.

25. The method of claim 24, in said single partition of said additional partitions, further comprising barcoding said barcoded nucleic acid molecule of (e) with an additional nucleic acid barcode molecule of said plurality of additional nucleic acid barcode molecules.

26. The method of claim 25, wherein said single partition of said additional partitions comprises two or more biological particles from said pooled mixture of biological particles.

27. The method of any one of claims 24 to 26, wherein said additional partitions are droplets.

28. The method of any one of claims 24 to 26, wherein said additional partitions are wells.