

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 herein is a method of generating a barcoded nucleic acid molecule, comprising: (a) providing: (i) a sample comprising a nucleic acid molecule, wherein the nucleic acid molecule comprises a first target region and a second target region, and wherein the first target region and the second target region are both disposed on a strand of the nucleic acid molecule; (ii) a first probe comprising a first probe sequence and a first barcode sequence, wherein the first probe sequence of the first probe is complementary to the first target region of the nucleic acid molecule; (iii) a second probe comprising a second probe sequence, wherein the second probe sequence of the second probe is complementary to the second target region of the nucleic acid molecule; and (iv) a support releasably coupled to a polynucleotide comprising a second barcode sequence; (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 second probe sequence of the second probe to the second target region of the nucleic acid molecule to yield a probe-associated nucleic acid molecule comprising the nucleic acid molecule hybridized to the first probe and the second probe; (c) subjecting the probe-associated nucleic acid molecule to conditions sufficient to link the first probe to the second probe, thereby generating a probe-linked nucleic acid molecule comprising the first probe linked to the second probe; (d) releasing the polynucleotide comprising a second barcode sequence from the support; and (e) after (d), generating a barcoded nucleic acid molecule using the probe-linked nucleic acid molecule and the polynucleotide comprising the second barcode sequence, wherein the barcoded nucleic acid molecule comprises (i) a sequence corresponding to the first target region and the second target regions, (ii) the first barcode sequence or reverse complement thereof, and (iii) the second barcode sequence or reverse 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 conduct an amplification reaction using the barcoded probe-linked nucleic acid molecule, thereby generating an amplification product within the partition. In some examples, the

CLAIMS

WHAT IS CLAIMED IS:

1. A method of generating a barcoded nucleic acid molecule, comprising:
 - (a) providing:
 - (i) a sample comprising a nucleic acid molecule, wherein the nucleic acid molecule comprises a first target region and a second target region, and wherein the first target region and the second target region are both disposed on a strand of the nucleic acid molecule;
 - (ii) a first probe comprising a first probe sequence and a first barcode sequence, wherein the first probe sequence of the first probe is complementary to the first target region of the nucleic acid molecule;
 - (iii) a second probe comprising a second probe sequence, wherein the second probe sequence of the second probe is complementary to the second target region of the nucleic acid molecule; and
 - (iv) a support releasably coupled to a polynucleotide comprising a second barcode sequence;
 - (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 second probe sequence of the second probe to the second target region of the nucleic acid molecule to yield a probe-associated nucleic acid molecule comprising the nucleic acid molecule hybridized to the first probe and the second probe;
 - (c) subjecting the probe-associated nucleic acid molecule to conditions sufficient to link the first probe to the second probe, thereby generating a probe-linked nucleic acid molecule comprising the first probe linked to the second probe;
 - (d) releasing the polynucleotide comprising a second barcode sequence from the support; and
 - (e) after (d), generating a barcoded nucleic acid molecule using the probe-linked nucleic acid molecule and the polynucleotide comprising the second barcode sequence, wherein the barcoded nucleic acid molecule comprises (i) a sequence corresponding to the first target region and the second target regions, (ii) the first barcode sequence or reverse

- complement thereof, and (iii) the second barcode sequence or reverse complement thereof.
2. The method of claim 1, wherein (e) is performed in a partition.
 3. The method of claim 2, wherein the partition is selected from a well among a plurality of wells and a droplet among a plurality of droplets.
 4. The method of claim 2, wherein (e) comprises (i) providing, in the partition, the polynucleotide comprising the second barcode sequence and a binding sequence, wherein the binding sequence is complementary to a first sequence of the first probe or a second sequence of the second probe, and (ii) subjecting the partition to conditions sufficient to hybridize the binding sequence of the polynucleotide to the first sequence of the first probe in the probe-linked nucleic acid molecule or the second sequence of the second probe in the probe-linked nucleic acid molecule.
 5. The method of claim 4, further comprising subjecting the partition to conditions sufficient to conduct a nucleic acid extension reaction.
 6. The method of claim 5, further comprising subjecting the barcoded nucleic acid molecule to conditions sufficient to conduct an amplification reaction to generate an amplification product.
 7. The method of claim 6, wherein the amplification reaction comprises use of a primer comprising one or more functional sequences selected from the group consisting of a binding sequence for an additional primer, a partial sequencing primer sequence, and a sequence configured to attach to a flow cell of a sequencer, and wherein the amplification product comprises nucleic acid molecules further comprising the one or more functional sequences or complements thereof.
 8. The method of claim 6, wherein the amplification reaction is an isothermal amplification reaction.
 9. The method of claim 6, wherein the amplification reaction is performed within the partition.
 10. The method of claim 9, further comprising recovering the amplification product from the partition.
 11. The method of claim 6, wherein the barcoded nucleic acid molecule is released from the partition and the amplification reaction is performed outside of the partition.

12. The method of claim 6, further comprising sequencing the nucleic acid molecules or derivatives thereof.
13. The method of claim 1, wherein the polynucleotide further comprises a unique molecular identifier sequence, a sequencing primer sequence, or a partial sequencing primer sequence.
14. The method of claim 1, wherein, after (b), a gap region comprising one or more nucleotides occurs between the first probe and the second probe hybridized to the nucleic acid molecule.
15. The method of claim 14, wherein the gap region comprises at least 10 nucleotides.
16. The method of claim 14, wherein the gap region comprises at least 100 nucleotides.
17. The method of claim 14, wherein the gap region is filled with nucleotides during (c).
18. The method of claim 1, wherein the support is a bead.
19. The method of claim 18, wherein the bead is a gel bead.
20. The method according to claim 1, wherein the first barcode sequence and the second barcode sequence are different from one another.
21. The method according to claim 1, wherein the sample comprises a single cell or a nucleus comprising the nucleic acid molecule.
22. The method according to claim 21, wherein the single cell is fixed.
23. The method according to claim 22, wherein the single cell is fixed with a fixative selected from the group consisting of formaldehyde, paraformaldehyde, and formalin.
24. The method according to claim 22, wherein the single cell is further permeabilized.
25. The method according to claim 1, further comprising, in (c), linking the first probe to the second probe using ligation.
26. The method according to claim 4, wherein the second barcode sequence identifies the barcoded nucleic acid molecule as having been generated in the partition.
27. The method according to claim 1, wherein the polynucleotide comprising the second barcode sequence is coupled to the support via a labile moiety.
28. The method according to claim 27, wherein the labile moiety is selected from a thermolabile moiety, a photocleavable moiety, and an enzymatically cleavable moiety.
29. The method of claim 1, further comprising ligating the probe-linked nucleic acid molecule and the polynucleotide comprising the second barcode sequence.

30. The method of claim 1, further comprising (i) providing a splint oligonucleotide comprising a first sequence complementary to an additional probe sequence of the first probe and a second sequence, and (ii) subjecting the partition to conditions sufficient to hybridize the first sequence of the splint oligonucleotide to the additional probe sequence of the first probe in the probe-linked nucleic acid molecule.
31. The method of claim 30, wherein (e) comprises (I) providing the polynucleotide comprising the second barcode sequence and a binding sequence, wherein the binding sequence is complementary to the second sequence of the splint oligonucleotide, and (II) subjecting the partition to conditions sufficient to hybridize the binding sequence of the polynucleotide to the second sequence of the splint oligonucleotide.