

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 for analyzing cells, comprising: (a) providing a first plurality of partitions comprising a plurality of cells, wherein a first partition of said first plurality of partitions comprises: (i) a first cell of said plurality of cells, wherein said first cell comprises a nucleic acid molecule which comprises a first target region and a second target region; (ii) a first probe comprising a first probe sequence that hybridizes to said first target region and a second probe comprising a second probe sequence that hybridizes to said second target region; and (iii) a plurality of first nucleic acid barcode molecules, wherein a first nucleic acid barcode molecule of said plurality of first nucleic acid barcode molecules comprises a first barcode sequence; (b) in said first partition, (i) hybridizing said first probe sequence to said first target region and said second probe sequence to said second target region in said first cell, and (ii) forming a first barecoded nucleic acid molecule that comprises said first barcode sequence by linking said first probe to said second probe and attaching said first nucleic acid barcode molecule to said first probe or said second probe to provide said first barecoded nucleic acid molecule; (c) pooling said first cell with other cells of said plurality of cells to provide a plurality of pooled cells; (d) providing a second plurality of partitions comprising said plurality of pooled cells, wherein a second partition of said second plurality of partitions comprises: (i) said first cell comprising said first barecoded nucleic acid molecule, and (ii) a plurality of second nucleic acid barcode molecules, wherein a second nucleic acid barcode molecule of said plurality of second nucleic acid barcode molecules comprises a second barcode sequence; and (e) in said second partition, attaching said second nucleic acid barcode molecule to said first barecoded nucleic acid molecule or derivative thereof from said first cell to provide a second barecoded nucleic acid molecule, which comprises said first barcode sequence or complement thereof and said second barcode sequence or complement thereof~~ method of analyzing a sample comprising a nucleic acid molecule, comprising: (a) providing: (i) the sample comprising the 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 an additional nucleic acid molecule comprising the first probe linked to the second probe; and (d) using the additional nucleic acid molecule and the polynucleotide that comprises the second barcode sequence to generate a barcoded nucleic acid molecule comprising (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, wherein, after (c), the polynucleotide is released from the support.

~~{0005b}—In another aspect, provided here is a method for analyzing cells, comprising: (a) providing a first plurality of partitions comprising a plurality of cells, wherein a first partition of said first plurality of partitions comprises: (i) —a first cell of said plurality of cells, wherein said first cell comprises a nucleic acid molecule which comprises a first target region and a second target region, (ii) a first probe comprising a first probe sequence that hybridizes to said first target region and a second probe comprising a second probe sequence that hybridizes to said second target region, and (iii) —a plurality of first nucleic acid barcode molecules, wherein a first nucleic acid barcode molecule of said plurality of first nucleic acid barcode molecules comprises a first barcode sequence; (b) in said first partition, (i) hybridizing said first probe sequence to said first target region and said second probe sequence to said second target region in said first~~

cell to provide a probe-associated nucleic acid molecule, and (ii) forming a barcoded probe-associated nucleic acid molecule that comprises said first barcode sequence by hybridizing a nucleic acid barcode molecule to said first probe or said second probe to provide said barcoded probe-associated nucleic acid molecule; (c) pooling said first cell with other cells of said plurality of cells to provide a plurality of pooled cells; (d) providing a second plurality of partitions comprising said plurality of pooled cells, wherein a second partition of said second plurality of partitions comprises: (i) said first cell comprising said barcoded probe-associated nucleic acid molecule, and (ii) a plurality of second nucleic acid barcode molecules, wherein a second nucleic acid barcode molecule of said plurality of second nucleic acid barcode molecules comprises a second barcode sequence; and (e) in said second partition, forming a barcoded probe-linked nucleic acid molecule by linking said first probe to said second probe and attaching said second nucleic acid barcode molecule to said first probe or said second probe to provide said barcoded probe-linked nucleic acid molecule, which barcoded probe-linked nucleic acid molecule comprises said first barcode sequence or complement thereof and said second barcode sequence or complement thereof.

[0005c]—In another aspect, provided herein is a method of analyzing a sample comprising a nucleic acid molecule, comprising: (a) providing in a partition: (i) a cell comprising said 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 second probe 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 third probe sequence, wherein said third probe sequence of said second probe is complementary to said second target region of said nucleic acid molecule; and (iv) a plurality of nucleic acid barcode molecules comprising a nucleic acid barcode molecule which comprises a first barcode sequence; (b) subjecting said sample to conditions sufficient to hybridize (i) said first probe sequence of said first probe to said first target region of said nucleic acid molecule, (ii) said third probe sequence of said second probe to said second target region of said nucleic acid molecule, and (iii) said nucleic acid barcode molecule to said first probe or said second probe to yield a barcoded probe-associated nucleic acid molecule; (c) subjecting said barcoded probe-associated nucleic acid molecule to conditions sufficient to yield a barcoded probe-linked nucleic acid molecule comprising said first probe linked to said second probe; and (d) within an additional partition, attaching an additional barcode sequence to said barcoded probe-linked nucleic acid molecule.

[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 analyzing a sample comprising a nucleic acid molecule, comprising:
- ~~A method for analyzing cells, comprising:~~
- (a) ~~providing a first plurality of partitions comprising a plurality of cells, wherein a first partition of said first plurality of partitions comprises:~~
- ~~_____:~~
- (i) ~~a first cell of said plurality of cells, wherein said first cell comprises a~~the
sample comprising the nucleic acid molecule ~~which,~~ wherein the nucleic acid
molecule comprises a first target region and a second target region,
- ~~_____ (ii) _____ a first probe comprising a first probe sequence that~~
~~hybridizes to said~~ and wherein the first target region and ~~a second probe~~
~~comprising a second probe sequence that hybridizes to said~~ the second target
region, ~~and~~ are both disposed on a strand of the nucleic acid molecule;
- ~~(iii) a plurality of first nucleic acid barcode molecules, wherein~~ probe comprising a first
~~nucleic acid barcode molecule of said plurality of first nucleic acid barcode molecules~~
~~comprises~~ probe sequence and a first barcode sequence;
- ~~(b) in said,~~ wherein the first probe sequence of the first partition,
- ~~(i) _____ hybridizing said first probe~~ sequence is complementary to ~~said~~ the first target
region ~~and said~~ of the nucleic acid molecule;
- ~~(iii) _____ a second probe~~ sequence comprising a second probe sequence, wherein the second
probe sequence of the second probe is complementary to ~~said~~ the second target region ~~in said~~
~~first cell, and~~
- ~~(ii) _____ forming a first barecode~~ of the nucleic acid molecule ~~that comprises said first~~
~~barcode sequence by linking said first probe to said second probe and~~
~~attaching said first nucleic acid barcode molecule to said first probe or said~~
~~second probe to provide said first barecoded nucleic acid molecule;~~ ; and
- ~~(c) pooling said first cell with other cells of said plurality of cells to provide a plurality of pooled~~
~~cells;~~
- ~~(d) providing a second plurality of partitions comprising said plurality of pooled cells, wherein a~~
~~second partition of said second plurality of partitions comprises:~~
- ~~_____ (i) _____ said first cell~~ (iv) _____ a support releasably coupled to a polynucleotide

comprising ~~said first barcoded~~ 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,~~and~~

~~—— (ii) —— a plurality of second nucleic acid barcode molecules, wherein a second nucleic acid barcode molecule of said plurality of second nucleic acid barcode molecules comprises a second barcode sequence; and~~

~~(e) in said second partition, attaching said second nucleic acid barcode molecule to said first barcoded~~ yield a probe-associated nucleic acid molecule or derivative thereof from said first cell to provide a second barcoded comprising the nucleic acid molecule,~~which comprises said first barcode sequence or complement thereof~~ 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 an additional nucleic acid molecule comprising the first probe linked to the second probe; and

(d) using the additional nucleic acid molecule and said the polynucleotide that comprises the second barcode sequence ~~or complement thereof~~ to generate a barcoded nucleic acid molecule comprising (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, wherein, after (c), the polynucleotide is released from the support.

2. The method of claim 1, wherein ~~said first plurality of partitions or said second plurality of partitions is a plurality of wells.~~ (d) is performed in a partition.

3. The method of claim 1,2, wherein ~~said first~~ the partition is selected from a well among a plurality of ~~partitions or said second plurality of partitions is wells and a droplet among~~ a plurality of droplets.

~~4. The method of any one of claims 1-3, wherein said linking of (b)(ii) comprises ligating said first probe to said second probe.~~

4. The method of claim 2, wherein (d) 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 to the first sequence or the second sequence.

5. The method of ~~any one of claims 1-4,~~claim 4, further comprising subjecting ~~said second~~the partition to conditions sufficient to conduct a nucleic acid extension reaction to generate a barcoded nucleic acid molecule.

~~5.6.~~ 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, ~~which amplification product comprises nucleic acid molecules comprising said first barcode sequence or complement thereof and said second barcode sequence or complement thereof.~~

~~6.—The method of any one of claims 1-5, wherein said first probe or said second probe further comprises an additional probe sequence, wherein said first partition further comprises a splint oligonucleotide comprising (i) a first sequence complementary to said additional probe sequence and (ii) a second sequence, and wherein (b)(ii) comprises subjecting said first partition to conditions sufficient to hybridize said first sequence of said splint oligonucleotide to said additional probe sequence.~~

~~7.—The method of claim 6, wherein said first nucleic acid barcode molecule further comprises a binding sequence, wherein said binding sequence is complementary to said second sequence of said splint oligonucleotide, and wherein (b)(ii) comprises subjecting said partition to conditions sufficient to hybridize said binding sequence to said second sequence of said splint oligonucleotide.~~

~~8.—The method of any one of claims 1-7, wherein said plurality of second nucleic acid barcode molecules is coupled to a bead.~~

7. The method of claim 6, wherein the amplification reaction comprises use of a primer comprising one or more functional sequences, and wherein the amplification product comprises nucleic acid molecules further comprising the one or more functional sequences or complements thereof.

~~9.8.~~ The method of claim 8,6, wherein ~~said bead~~the amplification reaction is ~~a gel bead~~an isothermal amplification reaction.

~~10.9.~~ The method of claim 8-or-9,6, wherein ~~said plurality of second nucleic acid barcode molecules~~the amplification reaction is ~~releasably coupled to said bead~~performed within the partition.

~~11. The method of any one of claims 1-10, wherein said first probe sequence of said first probe comprises a first reactive moiety and said second probe sequence of said second probe comprises a second reactive moiety, wherein, subsequent to (b)(i), said first reactive moiety is adjacent to said second reactive moiety.~~

~~12. The method of claim 11, wherein said linking in (b)(ii) comprises subjecting said first reactive moiety and said second reactive moiety to conditions sufficient to link said first probe sequence to said second probe sequence.~~

~~13. The method of claim 11 or 12, wherein said first reactive moiety of said first probe or said second reactive moiety of said second probe comprises an azide moiety, an alkyne moiety, a phosphorothioate moiety, an iodide moiety, an amine moiety, or a phosphate moiety.~~

~~14. The method of any one of claims 11-13, wherein said first probe is linked to said second probe in said probe-linked nucleic acid molecule via a linker, wherein said linker comprises a triazole moiety, a phosphorothioate bond, or a phosphoroamidate bond.~~

~~15. The method of any one of claims 1-10, wherein said linking in (b)(ii) comprises performing an enzymatic ligation reaction and/or an extension reaction.~~

~~16. The method of any one of claims 1-15, further comprising, subsequent to (a), lysing or permeabilizing said first cell, thereby providing access to said nucleic acid molecule.~~

10. The method of claim 9, further comprising recovering the amplification product from the partition.

11. The method of claim 6, wherein the amplification reaction is performed outside of the partition.

~~17. The method of claim 16, wherein said first cell is selected from the group consisting of a prokaryotic cell, a eukaryotic cell, and a human cell.~~

~~18. The method of any one of claims 1-17, wherein said first cell is a fixed cell.~~

~~19. A method for analyzing cells, comprising:~~

12. (a) providing a first plurality of partitions ~~6, further~~ comprising a plurality of cells, wherein a first partition of said first plurality of partitions comprises:

~~———— (i) ——— a first cell of said plurality of cells, wherein said first cell comprises a nucleic acid molecule which comprises a first target region and a second target region,~~

~~———— (ii) ——— a first probe comprising a first probe sequence that hybridizes to said first target region and a second probe comprising a second probe sequence that hybridizes to~~

- ~~said second target region, and~~sequencing the nucleic acid molecules or derivatives thereof.
13. ~~(iii) — a plurality~~The method of ~~first~~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 ~~barcode molecules, wherein a first nucleic acid barcode molecule of said plurality.~~
- The method of ~~first nucleic acid barcode molecules comprises a first barcode sequence;~~
(b) ~~in said first partition,~~
15. ~~(i) — hybridizing said first probe sequence to said first target region and said second probe sequence to said second target~~claim 14, wherein the gap region ~~in said first~~comprises at least about 10 nucleotides.
16. The method of claim 14, wherein the gap region comprises at least about 100 nucleotides.
17. The method of claim 14, wherein the gap region is filled with nucleotides prior to or 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 ~~to provide a probe associated~~or an isolated nucleus comprising the nucleic acid molecule, ~~and.~~
22. ~~(ii) — forming a~~The method according to claim 21, wherein the single cell or the isolated nucleus is fixed.
23. The method according to claim 22, wherein the single cell or the isolated nucleus 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 or the isolated nucleus 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 ~~probe-associated~~ nucleic acid molecule ~~that comprises said first barcode sequence~~
~~by hybridizing aas having been generated in the partition.~~

27. The method according to claim 1, wherein the nucleic acid barcode molecule ~~to said first~~
~~probe or said second probe to provide said barcoded probe-associated nucleic acid molecule;~~
is coupled to the support via a labile moiety.

~~(c) pooling said first cell with other cells of said plurality of cells to provide a plurality of pooled cells;~~

~~(d) providing a second plurality of partitions comprising said plurality of pooled cells, wherein a second partition of said second plurality of partitions comprises:~~

~~—— (i) —— said first cell comprising said barcoded probe-associated nucleic acid molecule, and~~

~~—— (ii) —— a plurality of second nucleic acid barcode molecules, wherein a second nucleic acid barcode molecule of said plurality of second nucleic acid barcode molecules comprises a second barcode sequence; and~~

~~(e) in said second partition, forming a barcoded probe-linked nucleic acid molecule by linking said first probe to said second probe and attaching said second nucleic acid barcode molecule to said first probe or said second probe to provide said barcoded probe-linked nucleic acid molecule, which barcoded probe-linked nucleic acid molecule comprises said first barcode sequence or complement thereof and said second barcode sequence or complement thereof.~~

20. — A method of analyzing a sample comprising a nucleic acid molecule, comprising:

~~(a) providing in a partition:~~

~~(i) a cell comprising said 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 second probe 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 third probe sequence, wherein said third probe sequence of said second probe is complementary to said second target region of said nucleic acid molecule; and~~

~~(iv) a plurality of nucleic acid barcode molecules comprising a nucleic acid barcode molecule which comprises a first barcode sequence;~~

~~(b) subjecting said sample to conditions sufficient to hybridize (i) said first probe sequence of said first probe to said first target region of said nucleic acid molecule, (ii) said third probe sequence of said second probe to said second target region of said nucleic acid molecule, and (iii) said nucleic acid barcode molecule to said first probe or said second probe to yield a barcoded probe-associated nucleic acid molecule;~~

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

~~(d) within an additional partition, attaching an additional barcode sequence to said barcoded probe-linked nucleic acid molecule.~~

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.