

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 barcoded 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 barcoded 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 barcoded 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 nucleic acid molecule or derivative thereof from said first cell to provide a second barcoded nucleic acid molecule, which comprises said first barcode sequence or complement thereof and said second barcode sequence or complement thereof.

[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

amplification reaction is a polymerase chain reaction. In some examples, the method further comprises releasing the amplification product from the partition. In some examples, the method further comprises sequencing the amplification product.

[0008] In some examples, the second probe comprises a fourth probe sequence, and wherein (d) further comprises providing, in the partition, a nucleic acid binding molecule, wherein the nucleic acid binding molecule comprises a second binding sequence that is complementary to the fourth probe sequence of the second probe. In some examples, the nucleic acid binding molecule further comprises a third binding sequence. In some examples, the nucleic acid binding molecule further comprises a second barcode sequence. In some examples, the method further comprises hybridizing the second binding sequence to the fourth probe sequence of the second probe in the partition.

[0009] In some examples, the nucleic acid barcode molecule is attached to a bead. In some examples, the bead is a gel bead. In some examples, the bead comprises a plurality of nucleic acid barcode molecules attached thereto, wherein the plurality of nucleic acid barcode molecules comprise the nucleic acid barcode molecule. In some examples, the bead comprises at least 10,000 nucleic acid barcode molecules attached thereto. In some examples, the bead comprises at least 100,000 nucleic acid barcode molecules attached thereto. In some examples, the bead comprises at least 1,000,000 nucleic acid barcode molecules attached thereto. In some examples, the bead comprises at least 10,000,000 nucleic acid barcode molecules attached thereto. In some examples, the plurality of nucleic acid barcode molecules are releasably attached to the bead. In some examples, the plurality of nucleic acid barcode molecules are releasable from the bead upon application of a stimulus. In some examples, the stimulus is selected from the group consisting of a thermal stimulus, a photo stimulus, and a chemical stimulus. In some examples, the stimulus is a reducing agent. In some examples, the stimulus is dithiothreitol.

[0010] In some examples, the application of the stimulus results in one or more of (i) cleavage of a linkage between nucleic acid barcode molecules of the plurality of nucleic acid barcode molecules and the bead, and (ii) degradation of the bead to release nucleic acid barcode molecules of the plurality of nucleic acid barcode molecules from the bead. In some examples, (d) comprises (i) providing, in the partition, the nucleic acid barcode molecule releasably attached to the bead, wherein the nucleic acid barcode molecule comprises the binding sequence and the barcode sequence; (ii) releasing the nucleic acid barcode molecule from the bead; and (iii) hybridizing the binding sequence of the released nucleic acid barcode molecule to the second probe sequence in the partition.

[0011] In some examples, the first probe further comprises a barcode sequence or unique molecular identifier. In some examples, the second probe further comprises a barcode sequence or a unique molecular identifier.

[0012] In some examples, the first reactive moiety of the first probe comprises an azide moiety. In some examples, the second reactive moiety of the second probe comprises an alkyne moiety. In some examples, the first probe is linked to the second probe in the probe-linked nucleic acid molecule via a linker, wherein the linker comprises a triazole moiety.

[0013] In some examples, the first reactive moiety of the first probe comprises a phosphorothioate moiety. In some examples, the second reactive moiety of the second probe comprises an iodide moiety. In some examples, the first probe is linked to the second probe in the probe-linked nucleic acid molecule via a linker, wherein the linker comprises a phosphorothioate bond.

[0014] In some examples, the first reactive moiety of the first probe comprises an amine moiety. In some examples, the second reactive moiety of the second probe comprises a phosphate moiety. In some examples, the first probe is linked to the second probe in the probe-linked nucleic acid molecule via a linker, wherein the linker comprises a phosphoramidate bond.

[0015] In some examples, the first reactive moiety of the first probe comprises an amine moiety. In some examples, the second reactive moiety of the second probe comprises a phosphate moiety. In some examples, the first probe is linked to the second probe in the probe-linked nucleic acid molecule via a linker, wherein the linker comprises a phosphoroamidate bond. In some examples, the sample comprises a cell, and wherein the nucleic acid molecule is contained within the cell. In some examples, the method further comprises, subsequent to (a), permeabilizing the cell, thereby providing access to the nucleic acid molecule. The cell may be alive or dead (e.g., fixed). In some examples, the method further comprises, subsequent to (a), lysing the cell, thereby releasing the nucleic acid molecule from the cell. In some examples the cell is a prokaryotic cell. In some examples, the cell is a eukaryotic cell. In some examples, the cell is a lymphocyte. In some examples, the cell is a B cell. In some examples, the cell is a T cell. In some examples, the cell is a human cell. In some examples, the cell is provided within the partition.

[0016] In some examples, the nucleic acid molecule is a single-stranded nucleic acid molecule. In some examples, the nucleic acid molecule comprises a polyA sequence at a terminus of the nucleic acid molecule. In some examples, the nucleic acid molecule comprises an untranslated region (UTR). In some examples, the nucleic acid molecule comprises a 5' cap structure. In some examples, the nucleic acid molecule is a ribonucleic

acid (RNA) molecule. In some examples, the nucleic acid molecule is a messenger RNA (mRNA) molecule.

[0017] In some examples, the nucleic acid molecule is a deoxyribonucleic acid (DNA) molecule.

[0018] In some examples, the partition further comprises one or more reagents selected from the group consisting of fluorophores, oligonucleotides, primers, nucleic acid barcode molecules, barcodes, buffers, deoxynucleotide triphosphates, DNA splints, detergents, reducing agents, chelating agents, oxidizing agents, nanoparticles, antibodies, and enzymes.

[0019] In some examples, the partition further comprises one or more reagents selected from the group consisting of temperature-sensitive enzymes, pH-sensitive enzymes, light-sensitive enzymes, proteases, ligase, polymerases, restriction enzymes, nucleases, protease inhibitors, and nuclease inhibitors.

[0020] In some examples, the sample comprises a cell bead, and wherein the nucleic acid molecule is contained within the cell bead.

[0021] In some examples, (a)-(c) are performed without reverse transcription.

[0022] In some examples, the first probe and the second probe are parts of the same nucleic acid molecule.

[0023] In another 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, a gap region, and a second target region, wherein the gap region is disposed between the first target region and 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 (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; (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, (ii) hybridize the third probe sequence of the second probe to the second target region of the nucleic acid molecule, and (iii) yield a probe-linked nucleic acid molecule comprising the first probe linked to the second probe; and (c) 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.

[0024] In some examples, the partition is a well. In some examples, the partition is a droplet.

[0025] In some examples, (b) comprises performing a nucleic acid reaction. In some examples, (b) comprises performing an enzymatic ligation reaction or an extension reaction. In some examples, (b) comprises performing the extension reaction and the enzymatic ligation reaction. In some examples, the nucleic acid reaction comprises using an enzyme selected from the group consisting of T4 RNL2, SplintR, PBCV1, DNA polymerase, and Mu polymerase, or a derivative thereof. In some examples, the gap region comprises a length of at least one base.

[0026] In some examples, (c) 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 examples, the binding sequence is hybridized to the second probe sequence in a partition among a plurality of partitions.

[0027] In some examples, the method further comprises, subsequent to (b), co-partitioning the probe-linked nucleic acid molecule and 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) is 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 amplification reaction is a polymerase chain reaction. In some examples, the method further comprises releasing the amplification product from the partition. In some examples, the method further comprises sequencing the amplification product. In some examples, the second probe comprises a fourth probe sequence, and wherein (d) further comprises providing, in the partition, a nucleic acid binding molecule, wherein the nucleic acid binding molecule comprises a second binding sequence that is complementary to the fourth probe sequence of the second probe.

[0028] In some examples, the nucleic acid binding molecule further comprises a third binding sequence. In some examples, the nucleic acid binding molecule further comprises a second barcode sequence. In some examples, the method further comprises hybridizing the second binding sequence to the fourth probe sequence of the second probe in the partition. In some examples, the nucleic acid barcode molecule is attached to a bead. In some examples, the bead is a gel bead. In some examples, the bead comprises a plurality of nucleic acid barcode molecules attached thereto, wherein the plurality of nucleic acid barcode

molecules comprise the nucleic acid barcode molecule. In some examples, the bead comprises at least 10,000 nucleic acid barcode molecules attached thereto. In some examples, the bead comprises at least 100,000 nucleic acid barcode molecules attached thereto. In some examples, the bead comprises at least 1,000,000 nucleic acid barcode molecules attached thereto. In some examples, the bead comprises at least 10,000,000 nucleic acid barcode molecules attached thereto. In some examples, the plurality of nucleic acid barcode molecules are releasably attached to the bead. In some examples, the plurality of nucleic acid barcode molecules are releasable from the bead upon application of a stimulus. In some examples, the stimulus is selected from the group consisting of a thermal stimulus, a photo stimulus, and a chemical stimulus. In some examples, the stimulus is a reducing agent. In some examples, the stimulus is dithiothreitol.

[0029] In some examples, the application of the stimulus results in one or more of (i) cleavage of a linkage between nucleic acid barcode molecules of the plurality of nucleic acid barcode molecules and the bead, and (ii) degradation of the bead to release nucleic acid barcode molecules of the plurality of nucleic acid barcode molecules from the bead.

[0030] In some examples, (c) comprises (i) providing, in the partition, the nucleic acid barcode molecule releasably attached to the bead, wherein the nucleic acid barcode molecule comprises the binding sequence and the barcode sequence; (ii) releasing the nucleic acid barcode molecule from the bead; and (iii) hybridizing the binding sequence of the released nucleic acid barcode molecule to the second probe sequence in the partition. In some examples, the first probe further comprises a barcode sequence or unique molecular identifier. In some examples, the second probe further comprises a barcode sequence or a unique molecular identifier. In some examples, the sample comprises a cell, and wherein the nucleic acid molecule is contained within the cell. In some examples, the method further comprises, subsequent to (a), permeabilizing the cell, thereby providing access to the nucleic acid molecule. In some examples, the method further comprises, subsequent to (a), lysing the cell, thereby releasing the nucleic acid molecule from the cell.

[0031] In some examples, the cell is a prokaryotic cell. In some examples, the cell is a eukaryotic cell. In some examples, the cell is a lymphocyte. In some examples, the cell is a B cell. In some examples, the cell is a T cell. In some examples, the cell is a human cell. In some examples, the cell is provided within the partition. In some examples, the nucleic acid molecule is a single-stranded nucleic acid molecule. In some examples, the nucleic acid molecule comprises a polyA sequence at a terminus of the nucleic acid molecule.

[0032] In some examples, the nucleic acid molecule comprises an untranslated region (UTR). In some examples, the nucleic acid molecule comprises a 5' cap structure. In some

examples, the nucleic acid molecule is a ribonucleic acid (RNA) molecule. In some examples, the nucleic acid molecule is a messenger RNA (mRNA) molecule. In some examples, the nucleic acid molecule is a deoxyribonucleic acid (DNA) molecule.

[0033] In some examples, the partition further comprises one or more reagents selected from the group consisting of fluorophores, oligonucleotides, primers, nucleic acid barcode molecules, barcodes, buffers, deoxynucleotide triphosphates, ribonucleoside triphosphates, DNA splints, detergents, reducing agents, chelating agents, oxidizing agents, nanoparticles, antibodies, and enzymes.

[0034] In some examples, the partition further comprises one or more reagents selected from the group consisting of temperature-sensitive enzymes, pH-sensitive enzymes, light-sensitive enzymes, proteases, ligase, polymerases, restriction enzymes, nucleases, protease inhibitors, and nuclease inhibitors.

[0035] In some examples, the sample comprises a cell bead, and the nucleic acid molecule is contained within the cell bead. In some examples, (b) is performed without reverse transcription.

[0036] In some examples, the first probe or the second probe comprises a known sequence.

[0037] In some examples, the first probe or the second probe comprises a degenerate sequence.

[0038] In some examples, the first probe or the second probe comprises a Phi-29 based rolling circle amplification sequence.

[0039] In another 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 target region; (ii) a probe comprising a probe sequence and an adapter sequence, wherein the probe sequence is complementary to the target region; and (iii) an adapter comprising a binding sequence, wherein the binding sequence is complementary to the adapter sequence; (b) subjecting the sample to conditions sufficient to (i) hybridize the probe sequence of the probe to the target region, and (ii) hybridize the adapter sequence of the probe to the binding sequence of the adapter, to yield an adapter-bound probe; and (c) with the adapter-bound probe in a partition, barcoding the adapter-bound probe to provide a barcoded nucleic acid molecule.

[0040] In some examples, the adapter sequence comprises between 5 to 10 nucleotides.

[0041] In another example, the present disclosure provides 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, a gap

region, and a second target region, wherein the gap region is disposed between the first target region and 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 (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; (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, (ii) hybridize the third probe sequence of the second probe to the second target region of the nucleic acid molecule, and (iii) 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.

[0042] In another example, the present disclosure provides a method of analyzing a sample comprising a nucleic acid molecule, comprising: (a) providing: (i) a sample 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; and (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; (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 third 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) within a partition, attaching a barcode sequence to said probe-linked nucleic acid molecule.

[0043] In some examples, said partition is a well among a plurality of wells.

[0044] In some examples, said partition is a droplet among a plurality of droplets.

[0045] In some examples, (d) comprises (i) providing, in said partition, a nucleic acid barcode molecule comprising a binding sequence and a barcode sequence, wherein said binding sequence is complementary to said second probe sequence of said first probe, and (ii) subjecting said partition to conditions sufficient to hybridize said binding sequence to said second probe sequence. In some examples, the method further comprises subjecting said partition to

conditions sufficient to conduct a nucleic acid extension reaction to generate a barcoded nucleic acid molecule comprising a sequence corresponding to said first probe, a sequence corresponding to said second probe, and a sequence corresponding to said barcode sequence. In some examples, the method further comprises subjecting said partition to conditions sufficient to ligate said probe-linked nucleic acid molecule to said nucleic acid barcode molecule to generate a barcoded nucleic acid molecule comprising a sequence corresponding to said first probe, a sequence corresponding to said second probe, and a sequence corresponding to said barcode sequence. In some examples, the method further comprises subjecting said 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 sequence corresponding to said first probe, said sequence corresponding to said second probe, and said sequence corresponding to said barcode sequence. In some examples, the amplification reaction comprises use of a primer comprising one or more functional sequences and wherein said amplification product comprises nucleic acid molecules further comprising said one or more functional sequences. In some examples, said amplification is isothermal amplification. In some examples, said amplification reaction is performed within said partition. In some examples, the method further comprises recovering said amplification product from said partition. In some examples, said amplification reaction is performed outside of said partition. In some examples, the method further comprises sequencing said amplification product or a derivative thereof.

[0046] In some examples, the method further comprises (i) providing a splint oligonucleotide comprising a first sequence complementary to said second probe sequence and a second sequence, and (ii) subjecting said partition to conditions sufficient to hybridize said first sequence of said splint oligonucleotide to said second probe sequence. In some examples, said first sequence of said splint oligonucleotide hybridizes to said second probe sequence prior to (c). In some examples, said first sequence of said splint oligonucleotide hybridizes to said second probe sequence after (c). In some examples, (d) comprises (i) providing, in said partition, a nucleic acid barcode molecule comprising a binding sequence and a barcode sequence, wherein said binding sequence is complementary to said second sequence of said splint oligonucleotide, and (ii) subjecting said partition to conditions sufficient to hybridize said binding sequence to said second sequence of said splint oligonucleotide. In some examples, said binding sequence of said nucleic acid barcode molecule comprises one or more ribobases. In some examples, said method further comprises subjecting (i) said splint oligonucleotide hybridized to said second probe sequence and (ii) said nucleic acid barcode molecule to conditions sufficient to ligate said probe-linked nucleic acid molecule to said nucleic acid

barcode molecule. In some examples, said method further comprises subjecting (i) said splint oligonucleotide hybridized to said second probe sequence and (ii) said nucleic acid barcode molecule to conditions sufficient to conduct a nucleic acid extension reaction to generate a barcoded nucleic acid molecule comprising a sequence corresponding to said first probe, a sequence corresponding to said second probe, and a sequence corresponding to said barcode sequence. In some examples, said method further comprises subjecting said 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 sequence corresponding to said first probe, said sequence corresponding to said second probe, and said sequence corresponding to said barcode sequence. In some examples, said amplification reaction is a polymerase chain reaction. In some examples, said amplification reaction is performed within said partition. In some examples, said method further comprises recovering said amplification product from said partition. In some examples, said amplification reaction is performed outside of said partition. In some examples, said amplification reaction is isothermal amplification. In some examples, the method further comprises sequencing said amplification product or derivative thereof.

[0047] In some examples, said nucleic acid barcode molecule further comprises a unique molecular identifier sequence, a sequencing primer sequence, and/or a partial sequencing primer sequence. In some examples, subsequent to (c), said probe-associated nucleic acid molecule is co-partitioned with said nucleic acid barcode molecule. In some examples, subsequent to (a), said nucleic acid molecule is co-partitioned with said first probe, said second probe, and said nucleic acid barcode molecule. In some examples, (c) is performed within said partition. In some examples, (b) and (c) are performed within said partition.

[0048] In some examples, said second probe comprises a fourth probe sequence, and wherein said method further comprises providing a nucleic acid binding molecule in said partition, wherein said nucleic acid binding molecule comprises a second binding sequence that is complementary to said fourth probe sequence of said second probe. In some examples, the method further comprises hybridizing said second binding sequence to said fourth probe sequence of said second probe within said partition.

[0049] In some examples, said nucleic acid barcode molecule is coupled to a bead. In some examples, said bead is a gel bead. In some examples, said nucleic acid barcode molecule is coupled to said bead via a labile moiety. In some examples, said bead comprises a plurality of nucleic acid barcode molecules coupled thereto, wherein said plurality of nucleic acid barcode molecules comprise said nucleic acid barcode molecule. In some examples, said bead comprises at least 100,000 nucleic acid barcode molecules coupled thereto. In some

examples, said plurality of nucleic acid barcode molecules are releasably coupled to said bead. In some examples, said plurality of nucleic acid barcode molecules are releasable from said bead upon application of a stimulus. In some examples, said stimulus is selected from the group consisting of a thermal stimulus, a photo stimulus, a biological stimulus, and a chemical stimulus. In some examples, said stimulus is a reducing agent. In some examples, the application of said stimulus results in one or more of (i) cleavage of a linkage between nucleic acid barcode molecules of said plurality of nucleic acid barcode molecules and said bead, and (ii) degradation of said bead to release nucleic acid barcode molecules of said plurality of nucleic acid barcode molecules from said bead. In some examples, said bead is provided in said partition, and wherein said nucleic acid barcode molecule is released from said bead within said partition.

[0050] In some examples, (c) is performed before (d). In some examples, (d) is performed before (c).

[0051] In some examples, said first probe or said second probe further comprises a barcode sequence or unique molecular identifier.

[0052] In some examples, said second probe comprises a fourth probe sequence, which fourth probe sequence hybridizes to a third target region of said nucleic acid molecule. In some examples, said second target region is not adjacent to said third target region, and wherein said third probe sequence and said fourth probe sequence of said second probe are separated by a linker sequence.

[0053] In some examples, said first probe sequence of said first probe comprises a first reactive moiety and said third probe sequence of said second probe comprises a second reactive moiety, wherein, subsequent to (b), said first reactive moiety is adjacent to said second reactive moiety. In some examples, wherein (c) comprises subjecting said first reactive moiety and said second reactive moiety to conditions sufficient to link said first probe sequence to said third probe sequence. In some examples, 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. In some examples, 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 phosphoroamidatephosphoramidate bond.

[0054] In some examples, (c) comprises performing an enzymatic ligation reaction and/or an extension reaction. In some examples, said enzymatic ligation reaction and/or said extension reaction comprises use of an enzyme selected from the group consisting of T4 RNL2, SplintR, T4 DNA ligase, PBCV1, DNA polymerase, and Mu polymerase, or a derivative thereof.

In some examples, prior to (a), said first probe is linked to said second probe via one or more linking sequences. In some examples, said one or more linking sequences comprise one or more of a spacer sequence, a sequencing primer or complement thereof, a capture sequence, a restriction site, a transposition site, and a unique molecular identifier sequence. In some examples, said one or more linking sequences comprise a thermolabile, photocleavable, or enzymatically cleavable site.

[0055] In some examples, said first target region is adjacent to said second target region.

[0056] In some examples, said first target region and said second target region are separated by a gap region disposed between said first target region and said second target region. In some examples, said gap region is at least one nucleotide long. In some examples, said gap region is at least 10 nucleotides long. In some examples, said gap region is at least 100 nucleotides long.

[0057] In some examples, the method further comprises digesting one or more nucleic acid molecules or portions thereof using an exonuclease.

[0058] In some examples, said first probe or said second probe comprises a known sequence or a degenerate sequence.

[0059] In some examples, said first probe or said second probe comprises a Phi-29 based rolling circle amplification sequence. In some examples, said first probe or said second probe comprises a cleavable site, wherein said cleavable site is cleavable using a thermal, photo-, chemical, or biological stimulus. In some examples, the method further comprises contacting said first probe or said second probe with a transposase.

[0060] In some examples, said sample comprises a cell, and wherein said nucleic acid molecule is contained within said cell. In some examples, the method further comprises, subsequent to (a), lysing or permeabilizing said cell, thereby providing access to said nucleic acid molecule. In some examples, said cell is a prokaryotic cell. In some examples, said cell is a eukaryotic cell. In some examples, said cell is a human cell. In some examples, said cell is a fixed suspension cell or a formalin-fixed paraffin-embedded cell. In some examples, said cell is provided within said partition. In some examples, said cell is a single cell.

[0061] In some examples, said nucleic acid molecule is a ribonucleic acid (RNA) molecule. In some examples, said nucleic acid molecule is a messenger RNA (mRNA) molecule. In some examples, said nucleic acid molecule comprises a poly-A sequence at a terminus of said nucleic acid molecule.

[0062] In some examples, said nucleic acid molecule is a deoxyribonucleic acid (DNA) molecule.

[0063] In some examples, said partition further comprises one or more reagents selected from the group consisting of fluorophores, oligonucleotides, primers, nucleic acid barcode molecules, barcodes, buffers, deoxynucleotide triphosphates, DNA splints, detergents, reducing agents, chelating agents, oxidizing agents, nanoparticles, antibodies, temperature-sensitive enzymes, pH-sensitive enzymes, light-sensitive enzymes, proteases, ligases, polymerases, reverse transcriptases, restriction enzymes, nucleases, protease inhibitors, and nuclease inhibitors. In some examples, said polymerase is a polymerase selected from the group of DNA polymerase, RNA polymerase, Hot Start polymerase, and Warm start polymerase. In some examples, said sample comprises a cell bead, and wherein said nucleic acid molecule is contained within said cell bead. In some examples, (a)-(c) are performed without reverse transcription.

[0064] In yet another example, the present disclosure provides a method of analyzing a sample comprising a nucleic acid molecule, comprising: (a) providing: (i) a sample comprising said nucleic acid molecule, wherein said nucleic acid molecule comprises a target region; (ii) a probe comprising a probe sequence and a binding sequence, wherein said probe sequence is complementary to said target region; and (iii) an adapter comprising a first sequence and a second sequence, wherein said first sequence of said adapter is complementary to said binding sequence of said probe; (b) subjecting said sample to conditions sufficient to hybridize (i) said probe sequence of said probe to said target region, and (ii) said binding sequence of said probe to said first sequence of said adapter, to yield an adapter-bound probe; and (c) within a partition, barcoding said adapter-bound probe to provide a barcoded nucleic acid molecule.

[0065] In a further example, the present disclosure provides a method of analyzing a sample comprising a nucleic acid molecule, comprising: (a) providing: (i) a sample comprising said nucleic acid molecule, wherein said nucleic acid molecule comprises a target region; (ii) a probe comprising a probe sequence and a first reactive moiety, wherein said probe sequence is complementary to said target region; and (iii) a nucleic acid barcode molecule comprising a second reactive moiety and a barcode sequence; (b) subjecting said sample to conditions sufficient to hybridize said probe sequence of said probe to said target region to provide a probe-associated nucleic acid molecule; and (c) within a partition, subjecting said first reactive moiety of said probe-associated nucleic acid molecule and said second reactive moiety of said nucleic acid barcode molecule to conditions sufficient to link said probe-associated nucleic acid molecule and said nucleic acid barcode molecule to provide a barcoded nucleic acid product.

[0066] Additional examples and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative examples of the present disclosure are shown and described. As will be realized,

the present disclosure is capable of other and different examples, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0067] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0068] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also “Figure” and “FIG.” herein), of which:

[0069] **FIG. 1** shows an example of a microfluidic channel structure for partitioning individual biological particles.

[0070] **FIG. 2** shows an example of a microfluidic channel structure for delivering barcode carrying beads to droplets.

[0071] **FIG. 3** shows an example of a microfluidic channel structure for co-partitioning biological particles and reagents.

[0072] **FIG. 4** shows an example of a microfluidic channel structure for the controlled partitioning of beads into discrete droplets.

[0073] **FIG. 5** shows an example of a microfluidic channel structure for increased droplet generation throughput.

[0074] **FIG. 6** shows another example of a microfluidic channel structure for increased droplet generation throughput.

[0075] **FIG. 7A** shows a cross-section view of another example of a microfluidic channel structure with a geometric feature for controlled partitioning. **FIG. 7B** shows a perspective view of the channel structure of **FIG. 7A**.

[0076] **FIG. 8** illustrates an example of a barcode carrying bead.

CLAIMS

WHAT IS CLAIMED IS:

1. 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 barcoded 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 barcoded 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 barcoded 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 nucleic acid molecule or derivative thereof from said first cell to provide a second

barcoded nucleic acid molecule, which comprises said first barcode sequence or complement thereof and said second barcode sequence or complement thereof.

2. The method of claim 1, wherein said first plurality of partitions or said second plurality of partitions is a plurality of wells.

3. The method of claim 1, wherein said first plurality of partitions or said second plurality of partitions is 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.

5. The method of any one of claims 1-4, further comprising subjecting said second 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.

9. The method of claim 8, wherein said bead is a gel bead.

10. The method of claim 8 or 9, wherein said plurality of second nucleic acid barcode molecules is releasably coupled to said bead.

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.

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:

(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.

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.