FUNCTIONALIZED GEL BEADS

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/586,784, filed November 15, 2017, and U.S. Provisional Application No. 62/629,561, filed February 12, 2018, each of which applications is entirely incorporated herein by reference.

BACKGROUND

[0002] Samples may be processed for various purposes, such as identification of a type of moiety within the sample. The sample may be a biological sample. The biological samples may be processed for various purposes, such as detection of a disease (e.g., cancer) or identification of a particular species. There are various approaches for processing samples, such as polymerase chain reaction (PCR) and sequencing.

[0003] Biological samples may be processed within various reaction environments, such as partitions. Partitions may be wells or droplets. Droplets or wells may be employed to process biological samples in a manner that enables the biological samples to be partitioned and processed separately. For example, such droplets may be fluidically isolated from other droplets, enabling accurate control of respective environments in the droplets.

[0004] Biological samples in partitions may be subjected to various processes, such as chemical processes or physical processes. Samples in partitions may be subjected to heating or cooling, or chemical reactions, such as to yield species that may be qualitatively or quantitatively processed.

SUMMARY

[0005] The present disclosure provides methods for use in various sample processing and analysis applications. The methods provided herein may provide barcode molecules comprising one or more sequences such as a barcode sequence and a functional sequence. Barcode molecules may be attached to supports such as beads. Such methods may employ combinatorial (e.g., split pool) ligation reactions and may be useful, for example, in controlled analysis and processing of analytes such as biological particles, nucleic acid molecules, proteins, and perturbation agents.

[0005a] In one aspect, the present disclosure provides a method for generating nucleic acid barcode molecules, comprising:

(a) providing a plurality of nucleic acid molecules coupled to a plurality of supports; and

(b) coupling one or more additional nucleic acid molecules to nucleic acid molecules of said plurality of nucleic acid molecules coupled to said plurality of supports, thereby assembling a plurality of nucleic acid barcode molecules comprising barcode sequences coupled to said plurality of supports,

wherein said plurality of nucleic acid barcode molecules comprises (i) a first set of nucleic acid barcode molecules coupled to a support of said plurality of supports and (ii) a second set of nucleic acid barcode molecules coupled to said support, wherein a first nucleic acid barcode molecule of said first set of nucleic acid barcode molecules comprises a first nucleic acid capture sequence and a second nucleic acid barcode molecule of said second set of nucleic acid barcode molecules comprises a second nucleic acid capture sequence, wherein said first nucleic acid capture sequence and said second nucleic acid capture sequence are independently selected from SEQ ID NO:1 through SEQ ID NO:163.

[0005b] In another aspect, the present disclosure provides a composition comprising a support coupled to a nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NO: 1-163.

[0006] In an example, the present disclosure provides a method for generating a plurality of barcode molecules, comprising: (a) providing a plurality of molecules coupled to a plurality of supports; and combinatorially assembling the plurality of barcode molecules coupled to the plurality of supports by coupling one or more molecules each comprising one or more segments to each of the plurality of molecules, wherein the plurality of barcode molecules comprises (i) a

first set of barcode molecules coupled to a support of the plurality of supports and (ii) a second set of barcode molecules coupled to the support, wherein barcode molecules of the first set of barcode molecules are different than barcode molecules of the second set of barcode molecules.

[0007] In some examples, the barcode molecules of the first set of barcode molecules and the barcode molecules of the second set of barcode molecules comprise barcode sequences that are different from barcode sequences of barcode molecules coupled to other supports of the plurality of supports.

[0008] In some examples, barcode sequences of the barcode molecules of the first set of barcode molecules and the barcode molecules of the second set of barcode molecules are identical.

[0009] In some examples, the plurality of supports is a plurality of beads, and wherein the barcode molecules of the first set of barcode molecules and the barcode molecules of the second set of barcode molecules are coupled to a bead of the plurality of beads. In some examples, at least a subset of the barcode molecules of the first set of barcode molecules and/or the barcode molecules of the second set of barcode molecules is coupled to an interior of the bead. In some examples, the barcode molecules of the first set of barcode molecules and the barcode molecules of the second set of barcode molecules are releasably coupled to the bead. In some examples, the barcode molecules of the first set of barcode molecules and the barcode molecules of the second set of barcode molecules are releasably coupled to the bead through chemical cross-linkers. In some examples, the plurality of beads is a plurality of gel beads. In some examples, the plurality of beads is dissolvable or disruptable.

[0010] In some examples, (b) comprises sequentially coupling multiple molecules to each of the plurality of molecules.

[0011] In some examples, in (b) the one or more molecules comprise a first molecule comprising a first segment and a second molecule comprising a second segment, and wherein the combinatorially assembling comprises coupling the first molecule to a molecule of the plurality of molecules and coupling the second molecule to the first molecule, wherein the molecule is coupled to the support. In some examples, the method further comprises coupling a third molecule comprising a third segment to the second molecule. In some examples, the third segment is different than the first segment or the second segment. In some examples, the third segment is different than the first segment and the second segment. In some examples, the first segment of the first molecule is different than the second segment of the second molecule.

[0012] In some examples, wherein the first molecule comprises a double-stranded region comprising the first segment and a single-stranded region. In some examples, the single-

stranded region of the first molecule has six or fewer nucleotides. In some examples, the single-stranded region of the first molecule has two nucleotides. In some examples, the sequence of the single-stranded region of the first molecule is selected from the group consisting of CA, GT, AC, and TG. In some examples, the first molecule further comprises an additional single-stranded region configured to couple to the molecule of the plurality of molecules. In some examples, the single-stranded region and the additional single-stranded region of the first molecule are included in the same strand of the first molecule. In some examples, the second molecule comprises a double-stranded region comprising the second segment and a single-stranded region. In some examples, the single-stranded region of the second molecule comprises a sequence complementary to the sequence of the single-stranded region of the first molecule. In some examples, the single-stranded region of the second molecule has six or fewer nucleotides. In some examples, the single-stranded region of the second molecule has two nucleotides. In some examples, the second molecule further comprises an additional single-stranded region. In some examples, the single-stranded region and the additional single-stranded region of the second molecule are included in different strands of the second molecule.

[0013] In some examples, in (b) the one or more molecules comprise a third molecule comprising a third segment and a fourth molecule comprising a fourth segment, and wherein the combinatorially assembling comprises coupling the third molecule to an additional molecule of the plurality of molecules and coupling the fourth molecule to the third molecule, wherein the additional molecule is coupled to the support. In some examples, (i) the first molecule comprises a double-stranded region comprising the first segment and a single-stranded region and (ii) the third molecule comprises a double-stranded region comprising the third segment and a single-stranded region. In some examples, the first segment of the first molecule and the third segment are the same. In some examples, the second segment of the second molecule and the fourth segment of the fourth molecule are the same. In some examples, the single-stranded region of the first molecule and the single-stranded region of the third molecule are the same. In some examples, the single-stranded region of the first molecule and the single-stranded region of the third molecule are different. In some examples, the single-stranded region of the first molecule and the single-stranded region of the third molecule each have six or fewer nucleotides. In some examples, the molecule and the additional molecule coupled to the support are the same. In some examples, the molecule and the additional molecule coupled to the support are different. In some examples, the second molecule comprises a first functional sequence and the fourth molecule comprises a second functional sequence, which first functional sequence is different than the second functional sequence.

[0014] In some examples, the one or more molecules comprise a plurality of first molecules comprising the first segment, a plurality of second molecules comprising the second segment, a plurality of third molecules comprising the third segment, and a plurality of fourth molecules comprising the fourth segment, and wherein the combinatorially assembling comprises (i) coupling first molecules of the plurality of first molecules to molecules of the plurality of molecules and coupling second molecules of the plurality of second molecules to the first molecules, and (ii) coupling third molecules of the plurality of third molecules to additional molecules of the plurality of molecules and coupling fourth molecules of the plurality of fourth molecules to the third molecules, wherein the molecules and the additional molecules of the plurality of molecules are coupled to the support. In some examples, among the one or molecules, the plurality of first molecules comprises a greater number of molecules than the plurality of third molecules. In some examples, among the one or more molecules, the plurality of second molecules comprises a greater number of molecules than the plurality of fourth molecules. In some examples, the molecules of the plurality of molecules comprises a greater number of molecules than the additional molecules of the plurality of molecules.

[0015] In some examples, (b) comprises ligating the one or more molecules to each of the plurality of molecules coupled to the plurality of supports.

[0016] In some examples, (b) comprises partitioning the plurality of molecules coupled to the plurality of supports in separate partitions, and coupling at least a subset of the one or more molecules to molecules of the plurality of molecules in the separate partitions. In some examples, (b) further comprises partitioning the plurality of molecules coupled to the plurality of supports in one or more additional partitions, and coupling at least a subset of the one or more molecules to molecules of the plurality of molecules in the one or more additional partitions. In some examples, the separate partitions are wells. In some examples, the separate partitions are droplets.

[0017] In some examples, the barcode sequences are nucleic acid sequences. In some examples, the barcode sequences are amino acid sequences. In some examples, barcode molecules of the other supports of the plurality of supports comprise different barcode sequences. In some examples, each other support of the other supports of the plurality of supports comprises barcode molecules comprising a different barcode sequence.

[0018] In some examples, the one or more segments are at least one nucleotide in length.

[0019] In some examples, each of the barcode molecules of the first set of barcode molecules and the barcode molecules of the second set of barcode molecules comprises a unique identifier that is different than unique identifiers of other barcode molecules coupled to the support.

[0020] In some examples, the barcode molecules of the first set of barcode molecules and the barcode molecules of the second set of barcode molecules comprise at least 100,000 barcode molecules.

[0021] In some examples, each of the barcode molecules of the first set of barcode molecules and the barcode molecules of the second set of barcode molecules comprise a segment separated from the one or more segments by a sequence that is the same across at least a subset of the plurality of barcode molecules.

[0022] In some examples, the one or more segments are different for at least a subset of the plurality of barcode molecules.

[0023] In some examples, barcode molecules of the first set of barcode molecules include a first sequence for use with a first assay and barcode molecules of the second set of barcode molecules include a second sequence for use with a second assay, wherein the first assay is different than the second assay.

[0024] In some examples, barcode molecules of the first set of barcode molecules and barcode molecules of the second set of barcode molecules include both identical barcode sequences and different barcode sequences.

[0025] In some examples, barcode molecules of the first set of barcode molecules include first functional sequences and barcode molecules of the second set of barcode molecules include second functional sequences, wherein the first functional sequences and the second functional sequences are different. In some examples, the first set of barcode molecules coupled to the support comprises a greater number of barcode molecules than the second set of barcode molecules coupled to the support. In some examples, the first set of barcode molecules coupled to the support comprises at least 10,000 barcode molecules. In some examples, the second set of barcode molecules coupled to the support comprises at least 10,000 barcode molecules.

[0026] In some examples, the method further comprises contacting a solution comprising the first molecule and the second molecule coupled to the molecule of the plurality of molecules with an exonuclease. In some examples, the second molecule comprises a phosphorothioate moiety.

[0027] In another example, the present disclosure provides a method of processing a plurality of barcode molecules, comprising: (a) providing a plurality of molecules coupled to a plurality of supports; (b) subjecting a subset of molecules of the plurality of molecules to conditions sufficient to couple first molecules to the subset of molecules, wherein the subset of molecules are coupled to a support of the plurality of supports; (c) subjecting the first molecules coupled to the subset of molecules to conditions sufficient to couple second molecules to the first

molecules, thereby generating the plurality of barcode molecules and by-products, which plurality of barcode molecules comprise the second molecules coupled to the first molecules; and (d) contacting the products and by-products with a molecule capable of degrading the by-products.

[0028] In some examples, the molecule capable of degrading the by-products is an exonuclease.

[0029] In some examples, the second molecules comprise phosphorothioate moieties. In some examples, the phosphorothioate moieties are disposed at an end of the second molecules.

[0030] In some examples, the plurality of barcode molecules comprises a plurality of barcode sequences. In some examples, the plurality of barcode sequences of the plurality of barcode molecules coupled to the support are identical. In some examples, the plurality of barcode sequences comprises a plurality of nucleic acid barcode sequences.

[0031] In some examples, the plurality of supports comprises a plurality of beads, and wherein the plurality of barcode molecules are coupled to a bead of the plurality of beads. In some examples, the plurality of beads is a plurality of gel beads. In some examples, the plurality of beads is dissolvable or disruptable. In some examples, the plurality of barcode molecules is releasably coupled to the bead.

[0032] In some examples, (b) comprises ligating the first molecules to the molecules.

[0033] In some examples, (c) comprises ligating the second molecules to the first molecules.

[0034] In some examples, the method further comprises partitioning the plurality of molecules coupled to the plurality of supports in separate partitions. In some examples, (b) comprises coupling the first molecules to the subset of molecules within a partition of the separate partitions. In some examples, the method further comprises, subsequent to (b), partitioning the plurality of molecules coupled to the plurality of supports in one or more additional partitions. In some examples, (c) comprises coupling the second molecules to the first molecules within a partition of the one or more additional partitions. In some examples, the separate partitions are wells. In some examples, the separate partitions are droplets. In some examples, prior to (d), the plurality of barcode molecules coupled to the support and the by-products are recovered from the separate partitions.

[0035] In some examples, the first molecules and the second molecules are nucleic acid molecules, which nucleic acid molecules each comprise at least two nucleotides.

[0036] In some examples, the plurality of barcode molecules coupled to the support comprises at least 10,000 barcode molecules.

[0037] In some examples, the plurality of barcode molecules coupled to the support comprises a plurality of first barcode molecules and a plurality of second barcode molecules, which plurality of first barcode molecules and plurality of second barcode molecules are different. In some examples, the plurality of first barcode molecules comprise a plurality of first barcode sequences and the plurality of second barcode molecules comprise a plurality of second barcode sequences, which first barcode sequences are different than the second barcode sequences. In some examples, the plurality of first barcode molecules comprise first functional sequences and the plurality of second barcode molecules comprise second functional sequences, which first functional sequences are different than the second functional sequences.

[0038] In some examples, the plurality of barcode molecules comprise a plurality of functional sequences. In some examples, the functional sequences are poly(T) sequences.

[0039] In a further example, the present disclosure provides a composition comprising a support comprising a plurality of nucleic acid barcode molecules coupled thereto, which plurality of nucleic acid barcode molecules comprise one or more sequences selected from the sequences included in Table 1 or Table 2.

[0040] In some examples, the plurality of nucleic acid barcode molecules comprise one or more sequences from the sequences included in Table 1.

[0041] In some examples, the plurality of nucleic acid barcode molecules comprise one or more sequences from the sequences included in Table 2. In some examples, the plurality of nucleic acid barcode molecules comprise the sequence CCTTAGCCGCTAATAGGTGAGC. In some examples, the plurality of nucleic acid barcode molecules comprise the sequence TTGCTAGGACCGGCCTTAAAGC.

[0042] In some examples, each nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules comprises one or more sequences selected from the sequences included in Table 1 or Table 2.

[0043] In some examples, the plurality of nucleic acid barcode molecules comprise a first plurality of nucleic acid barcode molecules and a second plurality of nucleic acid barcode molecules, which first plurality of nucleic acid barcode molecules comprise one or more sequences selected from the sequences included in Table 1 or Table 2 and which second plurality of nucleic acid barcode molecules do not comprise a sequence included in Table 1 or Table 2. In some examples, the second plurality of nucleic acid barcode molecules are configured to interact with ribonucleic acid molecules. In some examples, the second plurality of nucleic acid barcode molecules comprise a poly(T) sequence. In some examples, the first plurality of nucleic acid barcode molecules are configured to interact with deoxyribonucleic acid molecules.

[0044] In some examples, the first plurality of nucleic acid barcode molecules comprises at least 10,000 nucleic acid barcode molecules. In some examples, the second plurality of nucleic acid barcode molecules comprises at least 10,000 nucleic acid barcode molecules. In some examples, among the plurality of nucleic acid barcode molecules, the first plurality of nucleic acid barcode molecules comprises a greater number of nucleic acid barcode molecules than the second plurality of nucleic acid barcode molecules. In some examples, among the plurality of nucleic acid barcode molecules, the second plurality of nucleic acid barcode molecules comprises a greater number of nucleic acid barcode molecules than the first plurality of nucleic acid barcode molecules.

[0045] In some examples, the support is a bead. In some examples, the bead is a gel bead. In some examples, the bead is dissolvable or disruptable. In some examples, the plurality of nucleic acid barcode molecules is releasably coupled to the bead.

[0046] In some examples, the plurality of nucleic acid barcode molecules coupled to the support comprise a plurality of barcode sequences. In some examples, the plurality of barcode sequences is the same for each of the plurality of nucleic acid barcode molecules. In some examples, the plurality of barcode sequences is a plurality of nucleic acid barcode sequences.

[0047] In some examples, the plurality of nucleic acid barcode molecules comprises at least 10,000 nucleic acid barcode molecules.

[0048] In another example, the present disclosure provides a kit comprising a plurality of barcode molecules, comprising: a plurality of supports; and a plurality of barcode molecules coupled to the plurality of supports, wherein the plurality of barcode molecules comprises (i) a first set of barcode molecules coupled to a support of the plurality of supports and (ii) a second set of barcode molecules coupled to the support, wherein first barcode molecules of the first set of barcode molecules are different than second barcode molecules of the second set of barcode molecules, and wherein the first barcode molecules are configured to interact with different target molecules than the second barcode molecules.

[0049] In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules comprise barcode sequences that are different from barcode sequences of barcode molecules coupled to other supports of the plurality of supports.

[0050] In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules comprise barcode sequences, wherein the barcode sequences of the first barcode molecules of the first set

of barcode molecules and the second barcode molecules of the second set of barcode molecules are identical.

[0051] In some examples, the plurality of supports is a plurality of beads, and wherein the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules are coupled to a bead of the plurality of beads. In some examples, at least a subset of the first barcode molecules of the first set of barcode molecules and/or the second barcode molecules of the second set of barcode molecules is coupled to an interior of the bead. In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules are releasably coupled to the bead. In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules are releasably coupled to the bead through chemical cross-linkers. In some examples, the plurality of beads is a plurality of gel beads. In some examples, the plurality of beads is dissolvable or disruptable.

[0052] In some examples, the first barcode molecules or the second barcode molecules are configured to interact with deoxyribonucleic acid molecules. In some examples, the first barcode molecules or the second barcode molecules are configured to interact with ribonucleic acid molecules. In some examples, the first barcode molecules or the second barcode molecules are configured to interact with amino acids, polypeptides or proteins.

[0053] In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules comprise barcode sequences, which barcode sequences are nucleic acid sequences. In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules comprise barcode sequences, which barcode sequences are amino acid sequences.

[0054] In some examples, each of the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules comprises a unique identifier that is different than unique identifiers of other barcode molecules coupled to the support.

[0055] In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules comprise at least 100,000 barcode molecules.

[0056] In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules include both identical barcode sequences and different barcode sequences.

[0057] In some examples, the first barcode molecules of the first set of barcode molecules include a first sequence for use with a first assay and the second barcode molecules of the second set of barcode molecules include a second sequence for use with a second assay, wherein the first assay is different than the second assay. In some examples, the first sequences of the first barcode molecules of the first set of barcode molecules are configured to interact with first target molecules and the second sequences of the second barcode molecules of the second set of barcode molecules are configured to interact with second target molecules. In some examples, the first target molecules are deoxyribonucleic acid molecules and the second target molecules are ribonucleic acid molecules.

[0058] In some examples, the first set of barcode molecules coupled to the support comprises a greater number of barcode molecules than the second set of barcode molecules coupled to the support.

[0059] In a further example, the present disclosure provides a method for processing a plurality of analytes, comprising: (a) providing a plurality of barcode molecules coupled to a plurality of supports, wherein the plurality of barcode molecules comprises (i) a first set of barcode molecules coupled to a support of the plurality of supports and (ii) a second set of barcode molecules coupled to the support, wherein first barcode molecules of the first set of barcode molecules are different than second barcode molecules of the second set of barcode molecules; (b) partitioning the support of the plurality of supports in a partition, wherein subsequent to partitioning, the partition comprises the plurality of analytes; (c) using (i) a first barcode molecule from the first set of barcode molecules and a first analyte from the plurality of analytes to generate a first barcoded analyte, and (ii) a second barcode molecule from the second set of barcode molecules and a second analyte from the plurality of analytes to generate a second barcoded analyte; and (d) recovering (i) the first barcoded analyte or a derivative thereof and (ii) the second barcoded analyte or a derivative thereof from the partition.

[0060] In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules comprise barcode sequences that are different from barcode sequences of barcode molecules coupled to other supports of the plurality of supports.

[0061] In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules comprise barcode sequences, wherein the barcode sequences of the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules are identical. In some examples, the plurality of supports is a plurality of beads, and wherein the first barcode molecules of the first set of barcode molecules and the second barcode

molecules of the second set of barcode molecules are coupled to a bead of the plurality of beads. In some examples, at least a subset of the first barcode molecules of the first set of barcode molecules and/or the second barcode molecules of the second set of barcode molecules is coupled to an interior of the bead. In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules are releasably coupled to the bead. In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules are releasably coupled to the bead through chemical cross-linkers. In some examples, the plurality of beads is a plurality of gel beads. In some examples, the plurality of beads is dissolvable or disruptable.

[0062] In some examples, the plurality of analytes comprises a plurality of deoxyribonucleic acid molecules. In some examples, the plurality of analytes comprises a plurality of ribonucleic acid molecules. In some examples, the plurality of analytes comprises a plurality of amino acids, polypeptides or proteins.

[0063] In some examples, the first analyte is a deoxyribonucleic acid molecule. In some examples, the second analyte is a ribonucleic acid molecule.

[0064] In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules comprise barcode sequences, which barcode sequences are nucleic acid sequences. In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules comprise barcode sequences, which barcode sequences are amino acid sequences.

[0065] In some examples, each of the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules comprises a unique identifier that is different than unique identifiers of other barcode molecules coupled to the support.

[0066] In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules comprise at least 100,000 barcode molecules.

[0067] In some examples, the first barcode molecules of the first set of barcode molecules and the second barcode molecules of the second set of barcode molecules include both identical barcode sequences and different barcode sequences.

[0068] In some examples, the first barcode molecules of the first set of barcode molecules include first functional sequences and the second barcode molecules of the second set of barcode molecules include second functional sequences, wherein the first functional

sequences and the second functional sequences are different. In some examples, the first functional sequences of the first barcode molecules of the first set of barcode molecules are configured to interact with first analytes of the plurality of analytes and the second functional sequences of the second barcode molecules of the second set of barcode molecules are configured to interact with second analytes of the plurality of analytes. In some examples, the first analytes are deoxyribonucleic acid molecules and the second analytes are ribonucleic acid molecules.

[0069] In some examples, the first set of barcode molecules coupled to the support comprises a greater number of barcode molecules than the second set of barcode molecules coupled to the support.

[0070] In some examples, barcode molecules of the other supports of the plurality of supports comprise different barcode sequences.

[0071] In some examples, the partition is a well. In some examples, the partition is a droplet.

[0072] In some examples, (c) comprises performing one or more nucleic acid extension reactions.

[0073] In some examples, (i) the first barcode molecule or a sequence thereof and (ii) the second barcode molecule or a sequence thereof are released from the support.

[0074] In some examples, the plurality of analytes is components of a cell. In some examples, subsequent to (b), the cell is lysed or permeabilized to provide access to the plurality of analytes.

[0075] In some examples, subsequent to (c), the first barcoded analyte and the second barcoded analyte are in solution in the partition.

[0076] 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

[0077] 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

CLAIMS

WHAT IS CLAIMED IS:

1. A method for generating nucleic acid barcode molecules, comprising:

   (a) providing a plurality of nucleic acid molecules coupled to a plurality of supports; and

   (b) coupling one or more additional nucleic acid molecules to nucleic acid molecules of said plurality of nucleic acid molecules coupled to said plurality of supports, thereby assembling a plurality of nucleic acid barcode molecules comprising barcode sequences coupled to said plurality of supports,

   wherein said plurality of nucleic acid barcode molecules comprises (i) a first set of nucleic acid barcode molecules coupled to a support of said plurality of supports and (ii) a second set of nucleic acid barcode molecules coupled to said support, wherein a first nucleic acid barcode molecule of said first set of nucleic acid barcode molecules comprises a first nucleic acid capture sequence and a second nucleic acid barcode molecule of said second set of nucleic acid barcode molecules comprises a second nucleic acid capture sequence, wherein said first nucleic acid capture sequence and said second nucleic acid capture sequence are independently selected from SEQ ID NO:1 through SEQ ID NO:163.

2. The method of claim 1, wherein nucleic acid barcode molecules of said first set of nucleic acid barcode molecules and nucleic acid barcode molecules of said second set of nucleic acid barcode molecules comprise barcode sequences that are different from barcode sequences of nucleic acid barcode molecules coupled to other supports of said plurality of supports.

3. The method of claim 1, wherein said first set of nucleic acid barcode molecules and said second set of nucleic acid barcode molecules comprise an identical barcode sequence.

4. The method of claim 1, wherein said plurality of supports is a plurality of beads, and wherein said support is a bead of said plurality of beads.

5. The method of claim 4, wherein said nucleic acid barcode molecules of said first set of nucleic acid barcode molecules and said nucleic acid barcode molecules of said second set of nucleic acid barcode molecules are releasably coupled to said bead.

6. The method of claim 4, wherein said plurality of beads is a plurality of gel beads.

7. The method of claim 1, wherein in (b) said one or more additional nucleic acid molecules comprise a first nucleic acid molecule comprising a first barcode segment and a second nucleic acid molecule comprising a second barcode segment, and wherein said assembling comprises coupling said first nucleic acid molecule to a nucleic acid molecule of said plurality of nucleic acid molecules and coupling said second nucleic acid molecule to said first nucleic acid molecule.
8. The method of claim 7, further comprising coupling a third nucleic acid molecule comprising a third barcode segment to said second nucleic acid molecule.
9. The method of claim 7, wherein said first barcode segment of said first nucleic acid molecule comprises a third nucleic acid sequence and said second barcode segment of said second nucleic acid molecule comprises a fourth nucleic acid sequence, wherein said third nucleic acid sequence and said fourth nucleic acid sequence are different.
10. The method of claim 7, wherein said first nucleic acid molecule comprises a first double-stranded region comprising said first barcode segment and a first single-stranded region.
11. The method of claim 10, wherein said first single-stranded region of said first nucleic acid molecule comprises six or fewer nucleotides.
12. The method of claim 10, wherein said first nucleic acid molecule further comprises an additional single-stranded region configured to couple to said nucleic acid molecule of said plurality of nucleic acid molecules.
13. The method of claim 10, wherein said second nucleic acid molecule comprises a second double-stranded region comprising said second barcode segment and a second single-stranded region, wherein said second single-stranded region of said second nucleic acid molecule comprises a sequence complementary to a sequence of said first single-stranded region of said first nucleic acid molecule.
14. The method of claim 7, wherein in (b) said one or more additional nucleic acid molecules comprise a third nucleic acid molecule comprising a third barcode segment and a fourth nucleic acid molecule comprising a fourth barcode segment, and wherein said assembling comprises coupling said third nucleic acid molecule to an additional nucleic acid molecule of said plurality of nucleic acid molecules and coupling said fourth nucleic acid molecule to said third nucleic acid molecule, wherein said additional nucleic acid molecule is coupled to said support.

15. The method of claim 14, wherein said first barcode segment of said first nucleic acid molecule and said third barcode segment of said third nucleic acid molecule comprise an identical nucleic acid sequence, or wherein said second barcode segment of said second nucleic acid molecule and said fourth barcode segment of said fourth nucleic acid molecule comprise an identical nucleic acid sequence.

16. The method of claim 14, wherein said first nucleic acid molecule comprises a single-stranded region and said third nucleic acid molecule comprises an additional single-stranded region, and wherein said single-stranded region of said first nucleic acid molecule and said additional single-stranded region of said third nucleic acid molecule comprise different nucleic acid sequences.

17. The method of claim 14, wherein said nucleic acid molecule and said additional nucleic acid molecule coupled to said support comprise different nucleic acid sequences.

18. The method of claim 14, wherein said second nucleic acid molecule comprises a first functional sequence and said fourth nucleic acid molecule comprises a second functional sequence, which first functional sequence comprises a different nucleic acid sequence than said second functional sequence.

19. The method of claim 1, wherein (b) comprises ligating said one or more additional nucleic acid molecules to said nucleic acid molecules of said plurality of nucleic acid molecules coupled to said plurality of supports.

20. The method of claim 1, wherein (b) comprises partitioning said plurality of nucleic acid molecules coupled to said plurality of supports in separate partitions, and coupling at least a subset of said one or more additional nucleic acid molecules to said nucleic acid molecules of said plurality of nucleic acid molecules in said separate partitions.

21. The method of claim 20, wherein (b) further comprises partitioning said plurality of nucleic acid molecules coupled to said plurality of supports in one or more additional partitions, and coupling at least an additional subset of said one or more additional nucleic acid molecules to additional nucleic acid molecules of said plurality of nucleic acid molecules in said one or more additional partitions.

22. The method of claim 20, wherein said separate partitions are wells.

23. The method of claim 1, wherein nucleic acid barcode molecules of said first set of nucleic acid barcode molecules and nucleic acid barcode molecules of said second set of nucleic acid barcode molecules comprise a unique identifier having a different nucleic acid

sequence than unique identifiers of other nucleic acid barcode molecules coupled to said support.

24. The method of claim 1, wherein nucleic acid barcode molecules of said first set of nucleic acid barcode molecules include first functional sequences and nucleic acid barcode molecules of said second set of nucleic acid barcode molecules include second functional sequences, wherein said first functional sequences and said second functional sequences comprise different nucleic acid sequences.
25. The method of claim 1, wherein nucleic acid barcode molecules of said first set of nucleic acid barcode molecules include first functional sequences and nucleic acid barcode molecules of said second set of nucleic acid barcode molecules include second functional sequences, wherein said first functional sequences and said second functional sequences comprise identical nucleic acid sequences.
26. The method of claim 1, wherein said first nucleic acid capture sequence or said second nucleic acid capture sequence is SEQ ID NO: 155.
27. The method of claim 1, wherein said first nucleic acid capture sequence or said second nucleic acid capture sequence is SEQ ID NO: 156.
28. The method of claim 4, wherein said bead is dissolvable or degradable.
29. The method of claim 28, wherein said bead comprises a disulfide linkage.
30. The method of claim 4, wherein a nucleic acid molecule of said plurality of nucleic acid molecules is coupled to said bead via a labile moiety.
31. The method of claim 30, wherein said labile moiety is selected from the group consisting of an ester linkage, a vicinal diol linkage, a Diels-Alder linkage, a sulfone linkage, a silyl ether linkage, a glycosidic linkage, a peptide linkage, a phosphodiester linkage, a disulfide linkage, and a photo-labile linkage.
32. The method of claim 5, wherein said nucleic acid barcode molecules of said first set of nucleic acid barcode molecules or said nucleic acid barcode molecules of said second set of nucleic acid barcode molecules are releasable from said bead upon application of a stimulus to said bead.
33. The method of claim 32, wherein said stimulus is a thermal stimulus, a photo stimulus, an enzymatic stimulus, or a chemical stimulus.
34. The method of claim 32, wherein said stimulus is a reducing agent.

35. The method of claim 3, wherein said identical barcode sequence comprises between 6 and 20 nucleotides.
36. The method of claim 11, wherein said first single-stranded region of said first molecule is a sequence of two nucleotides.
37. The method of claim 36, wherein said sequence is selected from the group consisting of CA, GT, AC, and TG.
38. The method of claim 13, wherein said second single-stranded region of said second nucleic acid molecule has six or fewer nucleotides.
39. The method of claim 38, wherein said second single-stranded region of said second nucleic acid molecule is a sequence of two nucleotides.
40. The method of claim 1, wherein said first nucleic acid barcode molecule or said second nucleic acid barcode molecule comprises a uracil base.
41. The method of claim 24, wherein said first functional sequences or said second functional sequences comprise sequencing primer sequences or sequencing primer binding sequences.
42. The method of claim 25, wherein said first functional sequences or said second functional sequences comprise sequencing primer sequences or sequencing primer binding sequences.
43. The method of claim 5, wherein said nucleic acid barcode molecules of said first set of nucleic acid barcode molecules and said nucleic acid barcode molecules of said second set of nucleic acid barcode molecules are coupled to said bead via a disulfide bond.
44. A composition comprising a support coupled to a nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NO: 1-163.
45. The composition of claim 44, wherein said nucleic acid molecule further comprises a nucleic acid barcode sequence.
46. The composition of claim 44, wherein said sequence is SEQ ID NO: 155.
47. The composition of claim 44, wherein said sequence is SEQ ID NO: 156.
48. The composition of claim 44, wherein said support is a bead.
49. The composition of claim 48, wherein said bead is a gel bead.
50. The composition of claim 44, wherein said nucleic acid molecule is coupled to said support via a labile moiety.
51. The composition of claim 50, wherein said labile moiety is a disulfide bond.
52. The composition of claim 44, wherein said support comprises a labile moiety.
53. The composition of claim 52, wherein said labile moiety is a disulfide bond.

54. The composition of claim 44, wherein said support is coupled to an additional nucleic acid molecule.

55. The composition of claim 54, wherein said additional nucleic acid molecule comprises an additional sequence selected from the group consisting of SEQ ID NO: 1-163.

56. The composition of claim 55, wherein said sequence is different than said additional sequence.

57. The composition of claim 56, wherein said sequence is SEQ ID NO: 155 and said additional sequence is SEQ ID NO: 156.

58. The composition of claim 54, wherein said nucleic acid molecule comprises a first nucleic acid barcode sequence and said additional nucleic acid molecule comprises a second nucleic acid barcode sequence.

59. The composition of claim 58, wherein said first nucleic acid barcode sequence and said second nucleic acid barcode sequence are identical.

60. The composition of claim 58, wherein said first nucleic acid barcode sequence and said second nucleic acid barcode sequence are different.

61. The composition of claim 58, wherein said first nucleic acid barcode sequence and said second nucleic acid barcode sequence comprise identical barcode sequence segments.

62. The composition of claim 54, wherein said nucleic acid molecule is free of an exonuclease-resistant bond.

63. The composition of claim 62, wherein said additional nucleic acid molecule comprises an exonuclease-resistant bond.

64. The composition of claim 63, wherein said exonuclease-resistant bond is a phosphorothioate bond.

65. The composition of claim 63, wherein said exonuclease-resistant bond is disposed at an end of said additional nucleic acid molecule.

66. The composition of claim 44, wherein said nucleic acid molecule comprises a unique molecular identifier sequence.

67. The composition of claim 44, further comprising an additional nucleic acid molecule that is different from said nucleic acid molecule.

68. The composition of claim 67, wherein said additional nucleic acid molecule comprises an additional sequence selected from the group consisting of SEQ ID NO: 1-163.

69. The composition of claim 67, wherein said nucleic acid molecule comprises SEQ ID NO: 155 and said additional nucleic acid molecule comprises SEQ ID NO: 156.

70. The composition of claim 44, wherein said nucleic acid molecule is releasably coupled to said support.

71. The composition of claim 44, wherein said support is coupled to said nucleic acid molecule via a cleavable linkage.

72. The composition of claim 71, wherein said cleavable linkage is a disulfide bond or said cleavable linkage comprises a restriction recognition site.

73. The composition of claim 71, wherein said cleavable linkage is cleavable chemically, thermally, enzymatically, via a change in pH, or via light.