

methods that utilize a programmable microfluidic device to isolate single cells and perform scATAC-seq in nanoliter reaction chambers (see, *e.g.*, Buenrostro, *et al.*, Nature, 2015 Jul 23;523(7561):486-90) are limited by the throughput of the assay and may not generate personal epigenomic profiles on a timescale compatible with clinical decision-making.

[0010a] In one aspect, the present disclosure provides a method for processing a nucleic acid molecule, comprising: (a) providing a partition comprising (i) a biological particle comprising (I) a tagmented fragment of a deoxyribonucleic acid (DNA) molecule and (II) a ribonucleic acid (RNA) molecule (ii) a first nucleic acid barcode molecule comprising a first barcode sequence, (iii) a second nucleic acid barcode molecule comprising a second barcode sequence, and (iv) a splint sequence, wherein said first nucleic acid barcode molecule comprises an overhang sequence of said tagmented fragment, and wherein said splint molecule comprises a first sequence complementary to a sequence of said tagmented fragment and a second sequence complementary to said overhang sequence; and (b) within said partition: (i) using said tagmented fragment, said first nucleic acid barcode molecule, and said splint molecule to generate a first barcoded nucleic acid product comprising (I) said first barcode sequence or a complement thereof and (II) a sequence of said DNA molecule; and (ii) using said RNA molecule and said second nucleic acid barcode molecule to generate a second barcoded nucleic acid product comprising (I) said second barcode sequence or a complement thereof and (II) a complementary DNA (cDNA) sequence of said RNA molecule.

[0011] In an example, the present disclosure provides a method for processing a nucleic acid sample, comprising: (a) providing a biological particle comprising a deoxyribonucleic acid (DNA) molecule and a ribonucleic acid (RNA) molecule within a partition among a plurality of partitions, wherein the partition comprises (i) a first nucleic acid barcode molecule, (ii) a second nucleic acid barcode molecule, and (iii) a splint sequence, wherein the first nucleic acid barcode molecule and the second nucleic acid barcode molecule comprise a common barcode sequence, wherein the first nucleic acid barcode molecule comprises an overhang sequence, and wherein the splint sequence comprises a sequence complementary to the DNA molecule and a sequence complementary to the overhang sequence; (b) within the partition, using the DNA molecule, the first nucleic acid barcode molecule, and the splint sequence to generate a first barcoded nucleic acid product corresponding to the DNA molecule and using the RNA molecule and the second nucleic acid barcode molecule to generate a second barcoded nucleic acid product corresponding to the RNA molecule, wherein the first barcoded nucleic acid product and the second barcoded nucleic acid product comprise the common barcode sequence, or a complement thereof; and (c)

recovering the first barcoded nucleic acid product and the second barcoded nucleic acid product from the partition.

[0012] In some examples, the biological particle is a cell, cell bead, or cell nucleus. In some examples, the method further comprises lysing or permeabilizing the biological particle within the partition to provide access to the DNA molecule and the RNA molecule therein.

[0013] In some examples, the method further comprises, prior to (a), processing an open chromatin structure of the nucleic acid sample with a transposase to yield the DNA molecule. In some examples, the transposase is included in a transposase-nucleic acid complex that comprises (i) a first nucleic acid molecule comprising a first transposon end sequence and a first sequencing primer or portion thereof, or a complement thereof and (ii) a second nucleic acid molecule comprising a second transposon end sequence and a second sequencing primer or portion thereof, or a complement thereof. In some examples, the first transposon end sequence and the second transposon end sequence are the same, and wherein the first transposon end sequence and the second transposon end sequence are each hybridized to complementary sequences.

[0014] In some examples, generating the first barcoded nucleic acid product comprises (i) subjecting the DNA molecule and the splint sequence to conditions sufficient to hybridize to the sequence of the DNA molecule to the splint sequence and (ii) subjecting the first nucleic acid barcode molecule and the splint sequence to conditions sufficient to hybridize the overhang sequence of the first nucleic acid barcode molecule to the splint sequence. In some examples, the splint sequence is hybridized to the sequence of the DNA molecule outside of the partition. In some examples, the method further comprises ligating the first nucleic acid barcode molecule to the DNA molecule.

[0015] In some examples, the first nucleic acid barcode molecule comprises a flow cell sequence, or complement thereof; a barcode sequence; a sequencing primer, or portion thereof; a unique molecular identifier sequence; or a combination thereof.

[0016] In some examples, the DNA molecule comprises one or more gaps, and further comprising, prior to (c), subjecting the DNA molecule to an extension process to fill the one or more gaps.

[0017] In some examples, the second nucleic acid barcode molecule comprises a unique molecular identifier sequence, a barcode sequence, a sequencing primer or portion thereof, or a combination thereof.

[0018] In some examples, the method further comprises, prior to (b), within the partition, reverse transcribing the RNA molecule to provide a complementary DNA (cDNA) molecule. In some examples, prior to reverse transcribing the RNA molecule, the second nucleic acid barcode molecule is hybridized to a sequence of a precursor of the RNA molecule. In some examples, the precursor comprises a polyA sequence, and wherein the second nucleic acid barcode molecule comprises a polyT sequence configured to hybridize to the polyA sequence. In some examples, the method further comprises appending an additional sequence to the cDNA molecule, wherein the additional sequence is a poly(C) sequence. In some examples, the method further comprises contacting the cDNA molecule with a template-switching oligonucleotide under conditions sufficient to hybridize the template-switching oligonucleotide to the cDNA molecule, wherein the template-switching oligonucleotide hybridizes to the additional sequence of the cDNA molecule. In some examples, the method further comprises extending the cDNA molecule to generate an extended cDNA molecule, which extended cDNA molecule comprises sequences complementary to sequences of the template-switching oligonucleotide. In some examples, the template-switching oligonucleotide comprises a sequencing primer or portion thereof, or complement thereof; a unique molecular identifier sequence, or complement thereof; or a combination thereof. In some examples, the template-switching oligonucleotide is the second nucleic acid barcode molecule, and wherein the extended cDNA molecule is the second barcoded nucleic acid product.

[0019] In some examples, the first nucleic acid barcode molecule and the second nucleic acid barcode molecule comprise a common nucleic acid sequence.

[0020] In some examples, the partition is a droplet among a plurality of droplets. In some examples, the partition is a droplet and wherein (c) comprises breaking or disrupting the droplet.

[0021] In some examples, the method further comprises, subsequent to (c), using (i) the first barcoded nucleic acid product to generate a first plurality of amplification products and (ii) the second barcoded nucleic acid product to generate a second plurality of amplification products. In some examples, generating the first plurality of amplification products and the second plurality of amplification products comprises performing one or more nucleic acid amplification reactions using the first barcoded nucleic acid product or the second barcoded nucleic acid product. In some examples, performing the one or more nucleic acid amplification reactions comprises attaching one or more flow cell sequences to the first barcoded nucleic acid product or the second barcoded nucleic acid product, or a derivative thereof.

[0022] In some examples, the first nucleic acid barcode molecule and the second nucleic acid barcode molecule are coupled to a bead. In some examples, the bead is a gel bead. In some examples, the first nucleic acid barcode molecule is coupled to the bead via a first labile moiety and the second nucleic acid barcode molecule is coupled to the bead via a second labile moiety. In some examples, the first nucleic acid barcode molecule and the second nucleic acid barcode molecule are releasably coupled to the bead. In some examples, the first nucleic acid barcode molecule is releasable from the bead upon application of a first stimulus and the second nucleic acid barcode molecule is releasable from the bead upon application of a second stimulus.

[0023] In another example, the present disclosure provides a method for processing a nucleic acid sample, comprising: (a) providing a biological particle comprising a deoxyribonucleic acid (DNA) molecule and a ribonucleic acid (RNA) molecule within a partition among a plurality of partitions, wherein the partition comprises a first nucleic acid barcode molecule and a second nucleic acid barcode molecule, wherein the first nucleic acid barcode molecule and the second nucleic acid barcode molecule comprise a common barcode sequence; (b) within the partition, generating (i) a first barcoded nucleic acid product from the DNA molecule and the first nucleic acid barcode molecule and (ii) a second barcoded nucleic acid product from the RNA molecule and the second nucleic acid barcode molecule, wherein the first barcoded nucleic acid product and the second barcoded nucleic acid product comprise the common barcode sequence, or a complement thereof; (c) recovering the first barcoded nucleic acid product and the second barcoded nucleic acid product from the partition; (d) processing the first barcoded nucleic acid product to generate a processed nucleic acid product comprising a dA tail; and (e) using (i) the processed nucleic acid product to generate a first plurality of amplification products and (ii) the second barcoded nucleic acid product to generate a second plurality of amplification products.

[0024] In some examples, the biological particle is a cell, cell bead, or cell nucleus. In some examples, the method further comprises lysing or permeabilizing the biological particle within the partition to provide access to the DNA molecule and the RNA molecule therein.

[0025] In some examples, the method further comprises, prior to (a), processing an open chromatin structure of the nucleic acid sample with a transposase to yield the DNA molecule. In some examples, the transposase is included in a transposase-nucleic acid complex that comprises (i) a first nucleic acid molecule comprising a first transposon end sequence and a first sequencing primer or portion thereof, or a complement thereof and (ii) a second nucleic acid molecule comprising a second transposon end sequence and a second sequencing primer or portion thereof, or a complement thereof. In some examples, the first transposon end sequence and the

second transposon end sequence are the same, and wherein the first transposon end sequence and the second transposon end sequence are each hybridized to complementary sequences.

[0026] In some examples, the first nucleic acid barcode molecule comprises a sequencing primer or portion thereof, or complement thereof. In some examples, (b) comprises subjecting the DNA molecule to conditions sufficient to hybridize the first nucleic acid barcode molecule to the DNA molecule. In some examples, the method further comprises (i) extending the first nucleic acid barcode molecule hybridized to the DNA molecule and (ii) ligating the first nucleic acid barcode molecule to the DNA molecule to generate the first barcoded nucleic acid product.

[0027] In some examples, the method further comprises ligating the first nucleic acid barcode molecule to the DNA molecule.

[0028] In some examples, the first nucleic acid barcode molecule comprises a flow cell sequence, or complement thereof; a barcode sequence; a unique molecular identifier sequence; or a combination thereof.

[0029] In some examples, the DNA molecule comprises one or more gaps, and further comprising, prior to (c), subjecting the DNA molecule to an extension process to fill the one or more gaps.

[0030] In some examples, the second nucleic acid barcode molecule comprises a unique molecular identifier sequence, a barcode sequence, a sequencing primer or portion thereof, or a combination thereof.

[0031] In some examples, the method further comprises, prior to (b), within the partition, reverse transcribing the RNA molecule to provide a complementary DNA (cDNA) molecule. In some examples, prior to reverse transcribing the RNA molecule, the second nucleic acid barcode molecule is hybridized to a sequence of a precursor of the RNA molecule. In some examples, the precursor comprises a polyA sequence, and wherein the second nucleic acid barcode molecule comprises a polyT sequence configured to hybridize to the polyA sequence. In some examples, the method further comprises appending an additional sequence to the cDNA molecule, wherein the additional sequence is a poly(C) sequence. In some examples, the method further comprises contacting the cDNA molecule with a template-switching oligonucleotide under conditions sufficient to hybridize the template-switching oligonucleotide to the cDNA molecule, wherein the template-switching oligonucleotide hybridizes to the additional sequence of the cDNA molecule. In some examples, the method further comprises extending the cDNA molecule to generate an extended cDNA molecule, which extended cDNA molecule comprises sequences complementary to sequences of the template-switching oligonucleotide. In some examples, the template-switching oligonucleotide comprises a sequencing primer or portion

thereof, or complement thereof; a unique molecular identifier sequence, or complement thereof; or a combination thereof. In some examples, the template-switching oligonucleotide is the second nucleic acid barcode molecule, and wherein the extended cDNA molecule is the second barcoded nucleic acid product. In some examples, the first nucleic acid barcode molecule and the second nucleic acid barcode molecule comprise a common nucleic acid sequence.

[0032] In some examples, the partition is a droplet among a plurality of droplets. In some examples, the partition is a droplet and wherein (c) comprises breaking or disrupting the droplet.

[0033] In some examples, (e) comprises performing one or more nucleic acid amplification reactions using the first barcoded nucleic acid product or the second barcoded nucleic acid product. In some examples, (e) comprises attaching one or more flow cell sequences to the first barcoded nucleic acid product or the second barcoded nucleic acid product, or a derivative thereof.

[0034] In some examples, the first nucleic acid barcode molecule and the second nucleic acid barcode molecule are coupled to a bead. In some examples, the bead is a gel bead. In some examples, the first nucleic acid barcode molecule is coupled to the bead via a first labile moiety and the second nucleic acid barcode molecule is coupled to the bead via a second labile moiety. In some examples, the first nucleic acid barcode molecule and the second nucleic acid barcode molecule are releasably coupled to the bead. In some examples, the first nucleic acid barcode molecule is releasable from the bead upon application of a first stimulus and the second nucleic acid barcode molecule is releasable from the bead upon application of a second stimulus.

[0035] In a further example, the present disclosure provides a method for processing a nucleic acid sample, comprising: (a) providing a biological particle comprising a deoxyribonucleic acid (DNA) molecule and a ribonucleic acid (RNA) molecule within a partition among a plurality of partitions, wherein the partition comprises a first nucleic acid barcode molecule and a second nucleic acid barcode molecule, wherein the first nucleic acid barcode molecule and the second nucleic acid barcode molecule comprise a common barcode sequence, and wherein the DNA molecule comprises a first hairpin moiety at a first end and a second hairpin moiety at a second end; (b) within the partition, generating (i) a first barcoded nucleic acid product from the DNA molecule and the first nucleic acid barcode molecule and (ii) a second barcoded nucleic acid product from the RNA molecule and the second nucleic acid barcode molecule, wherein the first barcoded nucleic acid product and the second barcoded nucleic acid product comprise the common barcode sequence, or a complement thereof; (c) recovering the first barcoded nucleic acid product and the second barcoded nucleic acid product from the partition; and (d) using (i) the first barcoded nucleic acid product to generate a first plurality of amplification products and

(ii) the second barcoded nucleic acid product to generate a second plurality of amplification products.

[0036] In some examples, the biological particle is a cell, cell bead, or cell nucleus. In some examples, the method further comprises lysing or permeabilizing the biological particle within the partition to provide access to the DNA molecule and the RNA molecule therein.

[0037] In some examples, the method further comprises prior to (a), processing an open chromatin structure of the nucleic acid sample with a transposase to yield the DNA molecule. In some examples, the transposase is included in a transposase-nucleic acid complex that comprises (i) a first nucleic acid molecule comprising a first transposon end sequence and a first sequencing primer or portion thereof, or a complement thereof and (ii) a second nucleic acid molecule comprising a second transposon end sequence and a second sequencing primer or portion thereof, or a complement thereof. In some examples, the first transposon end sequence and the second transposon end sequence are the same, and wherein the first transposon end sequence and the second transposon end sequence are each hybridized to complementary sequences.

[0038] In some examples, the first nucleic acid barcode molecule comprises a sequencing primer or portion thereof, or complement thereof. In some examples, (b) comprises subjecting the DNA molecule to conditions sufficient to hybridize the first nucleic acid barcode molecule to the DNA molecule. In some examples, the method further comprises (i) extending the first nucleic acid barcode molecule hybridized to the DNA molecule and (ii) ligating the first nucleic acid barcode molecule to the DNA molecule to generate the first barcoded nucleic acid product.

[0039] In some examples, the method further comprises ligating the first nucleic acid barcode molecule to the DNA molecule.

[0040] In some examples, the first nucleic acid barcode molecule comprises a flow cell sequence, or complement thereof; a barcode sequence; a unique molecular identifier sequence; or a combination thereof.

[0041] In some examples, the DNA molecule comprises one or more gaps, and further comprising, prior to (c), subjecting the DNA molecule to an extension process to fill the one or more gaps.

[0042] In some examples, the second nucleic acid barcode molecule comprises a unique molecular identifier sequence, a barcode sequence, a sequencing primer or portion thereof, or a combination thereof.

[0043] In some examples, the method further comprises, prior to (b), within the partition, reverse transcribing the RNA molecule to provide a complementary DNA (cDNA) molecule. In some examples, prior to reverse transcribing the RNA molecule, the second nucleic acid barcode

molecule is hybridized to a sequence of a precursor of the RNA molecule. In some examples, the precursor comprises a polyA sequence, and wherein the second nucleic acid barcode molecule comprises a polyT sequence configured to hybridize to the polyA sequence. In some examples, the method further comprises appending an additional sequence to the cDNA molecule, wherein the additional sequence is a poly(C) sequence. In some examples, the method further comprises contacting the cDNA molecule with a template-switching oligonucleotide under conditions sufficient to hybridize the template-switching oligonucleotide to the cDNA molecule, wherein the template-switching oligonucleotide hybridizes to the additional sequence of the cDNA molecule. In some examples, the method further comprises extending the cDNA molecule to generate an extended cDNA molecule, which extended cDNA molecule comprises sequences complementary to sequences of the template-switching oligonucleotide. In some examples, the template-switching oligonucleotide comprises a sequencing primer or portion thereof, or complement thereof; a unique molecular identifier sequence, or complement thereof; or a combination thereof. In some examples, the template-switching oligonucleotide is the second nucleic acid barcode molecule, and wherein the extended cDNA molecule is the second barcoded nucleic acid product.

[0044] In some examples, the first nucleic acid barcode molecule and the second nucleic acid barcode molecule comprise a common nucleic acid sequence.

[0045] In some examples, the partition is a droplet among a plurality of droplets. In some examples, the partition is a droplet and wherein (c) comprises breaking or disrupting the droplet.

[0046] In some examples, (e) comprises performing one or more nucleic acid amplification reactions using the first barcoded nucleic acid product or the second barcoded nucleic acid product. In some examples, (e) comprises attaching one or more flow cell sequences to the first barcoded nucleic acid product or the second barcoded nucleic acid product, or a derivative thereof.

[0047] In some examples, the first nucleic acid barcode molecule and the second nucleic acid barcode molecule are coupled to a bead. In some examples, the bead is a gel bead. In some examples, the first nucleic acid barcode molecule is coupled to the bead via a first labile moiety and the second nucleic acid barcode molecule is coupled to the bead via a second labile moiety. In some examples, the first nucleic acid barcode molecule and the second nucleic acid barcode molecule are releasably coupled to the bead. In some examples, the first nucleic acid barcode molecule is releasable from the bead upon application of a first stimulus and the second nucleic acid barcode molecule is releasable from the bead upon application of a second stimulus.

[0048] In an example, the present disclosure provides a method for processing a nucleic acid sample, comprising: (a) providing a partition among a plurality of partitions, wherein the partition comprises a deoxyribonucleic acid (DNA) molecule derived from the nucleic acid sample; (b) transcribing the DNA molecule to generate a ribonucleic acid (RNA) molecule; (c) reverse transcribing the RNA molecule to generate a complementary DNA (cDNA) molecule; and (d) recovering the cDNA molecule or a derivative thereof from the partition.

[0049] In some examples, the partition is a well among a plurality of wells.

[0050] In some examples, the partition is a droplet among a plurality of droplets.

[0051] In some examples, the method further comprises, prior to (a), processing an open chromatin structure of the nucleic acid sample with a transposase to yield the DNA molecule. In some examples, the transposase is a Tn5 transposase. In some examples, the DNA molecule comprises one or more gaps. In some examples, the one or more gaps are filled using a reverse transcriptase. In some examples, the processing further comprises attachment of a nucleic acid molecule to the nucleic acid sample, wherein the nucleic acid molecule comprises a sequence having a first sub-sequence and a second sub-sequence, wherein the first sub-sequence is complementary to the second sub-sequence. In some examples, the nucleic acid molecule is a hairpin nucleic acid molecule. In some examples, the nucleic acid molecule comprises a promoter sequence. In some examples, the promoter sequence is a T7 promoter sequence. In some examples, the nucleic acid molecule is attached to a 3' end of the nucleic acid sample.

[0052] In some examples, (b) comprises transcribing the DNA molecule using a T7 polymerase.

[0053] In some examples, (c) comprises appending a poly(C) sequence to the RNA molecule.

[0054] In some examples, the method further comprises subsequent to (c), attaching a nucleic acid barcode molecule to a 5' end of the cDNA molecule. In some examples, the nucleic acid barcode molecule comprises a unique molecular identifier sequence. In some examples, the nucleic acid barcode molecule comprises a barcode sequence. In some examples, the nucleic acid barcode molecule comprises a primer sequence. In some examples, the nucleic acid barcode molecule comprises a capture sequence. In some examples, the capture sequence comprises a poly(G) sequence.

[0055] In some examples, the nucleic acid barcode molecule is coupled to a bead. In some examples, the bead is a gel bead. In some examples, the nucleic acid barcode molecule is releasably coupled to the bead. In some examples, the nucleic acid barcode molecule is releasable from the bead upon application of a stimulus. In some examples, the stimulus is a chemical stimulus. In some examples, the chemical stimulus is a reducing agent.

[0056] In some examples, the cDNA molecule is a single-stranded cDNA molecule. In some examples, the method further comprises using the single-stranded cDNA molecule to generate a double-stranded cDNA molecule. In some examples, the double-stranded cDNA molecule is generated using a 5' blocked primer and a Klenow fragment. In some examples, the Klenow fragment does not have exonuclease activity.

[0057] In some examples, the cDNA molecule comprises a DNA sequence coupled to an RNA sequence, and wherein subsequent to (d), the cDNA molecule is processed with an RNase enzyme to digest the RNA sequence or remove the RNA sequence from the cDNA molecule.

[0058] In some examples, the partition is a droplet, and wherein (d) comprises breaking or disrupting the droplet.

[0059] In some examples, the method further comprises subsequent to (d), attaching one or more flow cell sequences to the cDNA molecule or derivative thereof. In some examples, the one or more flow cell sequences are attached via one or more nucleic acid amplification reactions.

[0060] In some examples, the method further comprises subsequent to (d), attaching a primer to a 3' end of the cDNA molecule or derivative thereof. In some examples, the cDNA molecule includes an A tail and wherein the primer attaches to the A tail.

[0061] In some examples, the nucleic acid sample is from a cell or cell nucleus. In some examples, the cell or the cell nucleus is in the partition. In some examples, the cell or the cell nucleus comprises the DNA molecule. In some examples, the cell or the cell nucleus further comprises a second ribonucleic acid (RNA) molecule. In some examples, the method further comprises lysing or permeabilizing the cell or the cell nucleus to provide access to the DNA molecule and/or the second RNA molecule therein.

[0062] In some examples, the method further comprises prior to (a), processing a messenger RNA (mRNA) molecule to provide the second RNA molecule. In some examples, the method further comprises, within the partition, reverse transcribing the second RNA molecule to provide an additional cDNA molecule. In some examples, prior to reverse transcribing the second RNA molecule, a primer molecule is hybridized to a sequence of a precursor of the second RNA molecule. In some examples, the precursor comprises a polyA sequence, and wherein the primer molecule comprises a polyT sequence configured to hybridize to the polyA sequence. In some examples, the primer molecule comprises a unique molecular identifier sequence. In some examples, the primer molecule comprises a barcode sequence. In some examples, the primer molecule comprises a sequencing primer or portion thereof. In some examples, the primer molecule is coupled to an additional bead. In some examples, the additional bead is a gel bead.

In some examples, the primer molecule is coupled to the additional bead via a labile moiety. In some examples, the primer molecule is releasably coupled to the additional bead. In some examples, the primer molecule is releasable from the additional bead upon application of stimulus. In some examples, the stimulus is a chemical stimulus. In some examples, the chemical stimulus is a reducing agent.

[0063] In some examples, the method further comprises appending an additional sequence to the additional cDNA molecule. In some examples, the additional sequence comprises a poly(C) sequence. In some examples, the method further comprises contacting the additional cDNA molecule with a template-switching oligonucleotide under conditions sufficient to hybridize the template-switching oligonucleotide to the additional cDNA molecule. In some examples, the template-switching oligonucleotide hybridizes to the additional sequence of the additional cDNA molecule. In some examples, the method further comprises extending the additional cDNA molecule to generate an extended cDNA molecule, which extended cDNA molecule comprises sequences complementary to sequences of the template-switching oligonucleotide. In some examples, the template-switching oligonucleotide comprises a sequencing primer, or complement thereof. In some examples, the template-switching oligonucleotide comprises a barcode sequence, or complement thereof. In some examples, the template-switching oligonucleotide comprises a unique molecular identifier sequence, or complement thereof. In some examples, the template-switching oligonucleotide is coupled to a further bead. In some examples, the further bead is a gel bead. In some examples, the template-switching oligonucleotide is coupled to the further bead via a labile moiety. In some examples, the template-switching oligonucleotide is releasably coupled to the further bead. In some examples, the template-switching oligonucleotide is releasable from the further bead upon application of stimulus. In some examples, the stimulus is a chemical stimulus. In some examples, the chemical stimulus is a reducing agent.

[0064] In some examples, the template-switching oligonucleotide is a further nucleic acid barcode molecule, and wherein the extended cDNA molecule is a barcoded nucleic acid product. In some examples, the further nucleic acid barcode molecule comprises a flow cell sequence, or complement thereof. In some examples, the method further comprises contacting the extended cDNA molecule with an additional nucleic acid barcode molecule under conditions sufficient to hybridize the additional nucleic acid barcode molecule to a sequence of the extended cDNA molecule. In some examples, the method further comprises subjecting the extended cDNA molecule hybridized to the additional nucleic acid barcode molecule to an extension reaction to generate a barcoded nucleic acid product, which barcoded nucleic acid product comprises

sequences complementary to sequences of the extended cDNA molecule. In some examples, the additional nucleic acid barcode molecule comprises a sequencing primer or portion thereof, or complement thereof. In some examples, the additional nucleic acid barcode molecule comprises a flow cell sequence, or complement thereof. In some examples, the additional nucleic acid barcode molecule comprises a barcode sequence, or complement thereof. In some examples, the additional nucleic acid barcode molecule is coupled to another bead. In some examples, the other bead is a gel bead. In some examples, the additional nucleic acid barcode molecule is coupled to the other bead via a labile moiety. In some examples, the additional nucleic acid barcode molecule is releasably coupled to the other bead. In some examples, the additional nucleic acid barcode molecule is releasable from the other bead upon application of stimulus. In some examples, the stimulus is a chemical stimulus. In some examples, the chemical stimulus is a reducing agent.

[0065] In some examples, the method further comprises recovering the barcoded nucleic acid product or a derivative thereof from the partition. In some examples, the method further comprises attaching one or more flow cell sequences to the barcoded nucleic acid product or derivative thereof. In some examples, the one or more flow cell sequences are attached via one or more nucleic acid amplification reactions.

[0066] In another example, the present disclosure provides a method for processing a nucleic acid sample, comprising: (a) providing a biological particle comprising a deoxyribonucleic acid (DNA) molecule and a ribonucleic acid (RNA) molecule within a partition among a plurality of partitions, wherein the partition comprises a first plurality of nucleic acid barcode molecules and a second plurality of nucleic acid barcode molecules, wherein the first plurality of nucleic acid barcode molecules and the second plurality of nucleic acid barcode molecules comprise a common barcode sequence; (b) within the partition, generating a first barcoded nucleic acid product corresponding to the DNA molecule and a second barcoded nucleic acid product corresponding to the RNA molecule, wherein the first barcoded nucleic acid product and the second barcoded nucleic acid product comprise the common barcode sequence, or a complement thereof; (c) recovering the first barcoded nucleic acid product and the second barcoded nucleic acid product from the partition; and (d) using (i) the first barcoded nucleic acid product to generate a first plurality of amplification products and (ii) the second barcoded nucleic acid product to generate a second plurality of amplification products, wherein in (b), the generating does not comprise performing an exponential amplification reaction.

[0067] In some examples, the partition is a droplet among a plurality of droplets.

- [0068]** In some examples, the partition is a well among a plurality of wells.
- [0069]** In some examples, the biological particle is a cell, cell bead, or cell nucleus.
- [0070]** In some examples, the method further comprises lysing or permeabilizing the biological particle within the partition to provide access to the DNA molecule and the RNA molecule therein.
- [0071]** In some examples, the method further comprises, prior to (a), processing an open chromatin structure of the nucleic acid sample with a transposase to yield the DNA molecule. In some examples, the transposase is a Tn5 transposase. In some examples, the transposase is included in a transposase-nucleic acid complex. In some examples, the transposase-nucleic acid complex comprises (i) a first nucleic acid molecule comprising a first transposon end sequence and a first sequencing primer or portion thereof, or a complement thereof and (ii) a second nucleic acid molecule comprising a second transposon end sequence and a second sequencing primer or portion thereof, or a complement thereof. In some examples, the first transposon end sequence and the second transposon end sequence are the same, and wherein the first transposon end sequence and the second transposon end sequence are each hybridized to complementary sequences. In some examples, the first sequencing primer or portion thereof, or complement thereof, is different than the second sequencing primer or portion thereof, or complement thereof.
- [0072]** In some examples, the first plurality of nucleic acid barcode molecules each comprise a sequencing primer or portion thereof, or complement thereof. In some examples, (b) comprises subjecting the DNA molecule to conditions sufficient to hybridize a first nucleic acid barcode molecule of the first plurality of nucleic acid barcode molecules to the DNA molecule. In some examples, the method further comprises extending the first nucleic acid barcode molecule hybridized to the DNA molecule to generate the first barcoded nucleic acid product. In some examples, the method further comprises ligating the first nucleic acid barcode molecule to the DNA molecule.
- [0073]** In some examples, the first plurality of nucleic acid barcode molecules each comprise an overhang sequence. In some examples, (b) comprises subjecting the DNA molecule to conditions sufficient to hybridize to a splint sequence, wherein the splint sequence comprises a sequence complementary to the DNA molecule and a sequence complementary to the overhang sequence. In some examples, the splint sequence comprises a 3' blocking group. In some examples, (b) comprises subjecting the DNA molecule hybridized to the splint sequence to conditions sufficient to hybridize the overhang sequence of a first nucleic acid barcode molecule of the first plurality of nucleic acid barcode molecules to the sequence complementary to the

overhang sequence. In some examples, the method further comprises ligating the first nucleic acid barcode molecule to the DNA molecule. In some examples, the split sequence is hybridized to the overhang sequence outside of the partition.

[0074] In some examples, the first plurality of nucleic acid barcode molecules each comprise a flow cell sequence, or complement thereof.

[0075] In some examples, the DNA molecule comprises one or more gaps. In some examples, the method further comprises, prior to (c), subjecting the DNA molecule to an extension process to fill the one or more gaps. In some examples, filling of the one or more gaps is blocked by the presence of a transposase. In some examples, the method further comprises subsequent to (c), subjecting the DNA molecule to an extension process to fill the one or more gaps.

[0076] In some examples, the method further comprises, prior to (a), processing a messenger RNA (mRNA) molecule to provide the RNA molecule. In some examples, the method further comprises, prior to (b), within the partition, reverse transcribing the RNA molecule to provide an additional cDNA molecule. In some examples, prior to reverse transcribing the RNA molecule, a primer molecule is hybridized to a sequence of a precursor of the second RNA molecule. In some examples, the precursor comprises a polyA sequence, and wherein the primer molecule comprises a polyT sequence configured to hybridize to the polyA sequence. In some examples, the primer molecule comprises a unique molecular identifier sequence. In some examples, the primer molecule comprises a barcode sequence. In some examples, the primer molecule comprises a sequencing primer or portion thereof. In some examples, the primer molecule is a second nucleic acid barcode molecule of the plurality of second nucleic acid barcode molecules. In some examples, the method further comprises appending an additional sequence to the additional cDNA molecule. In some examples, the additional sequence is a poly(C) sequence. In some examples, the method further comprises contacting the additional cDNA molecule with a template-switching oligonucleotide under conditions sufficient to hybridize the template-switching oligonucleotide to the additional cDNA molecule. In some examples, the template-switching oligonucleotide hybridizes to the additional sequence of the additional cDNA molecule. In some examples, the method further comprises extending the additional cDNA molecule to generate an extended cDNA molecule, which extended cDNA molecule comprises sequences complementary to sequences of the template-switching oligonucleotide. In some examples, the template-switching oligonucleotide comprises a sequencing primer or portion thereof, or complement thereof. In some examples, the template-switching oligonucleotide comprises a unique molecular identifier sequence, or complement thereof. In some examples,

the method further comprises contacting the extended cDNA molecule with a second nucleic acid barcode molecule of the second plurality of nucleic acid barcode molecules under conditions sufficient to hybridize the second nucleic acid barcode molecule to a sequence of the extended cDNA molecule. In some examples, the method further comprises subjecting the extended cDNA molecule hybridized to the second nucleic acid barcode molecule to an extension reaction to generate the second barcoded nucleic acid product, which second barcoded nucleic acid product comprises sequences complementary to sequences of the extended cDNA molecule. In some examples, the second nucleic acid barcode molecule comprises a sequencing primer or portion thereof, or complement thereof. In some examples, the second nucleic acid barcode molecule comprises a flow cell sequence, or complement thereof.

[0077] In some examples, the first plurality of nucleic acid barcode molecules and the second plurality of nucleic acid barcode molecules are the same.

[0078] In some examples, the template-switching oligonucleotide is a second nucleic acid barcode molecule of the second plurality of nucleic acid barcode molecules, and wherein the extended cDNA molecule is the second barcoded nucleic acid product. In some examples, the second nucleic acid barcode molecule comprises a flow cell sequence, or complement thereof.

[0079] In some examples, the partition is a droplet and wherein (c) comprises breaking or disrupting the droplet.

[0080] In some examples, (d) comprises performing one or more nucleic acid amplification reactions using the first barcoded nucleic acid product or the second barcoded nucleic acid product. In some examples, the one or more nucleic acid amplification reactions comprise one or more polymerase chain reactions (PCR). In some examples, (d) comprises attaching one or more flow cell sequences to the first barcoded nucleic acid product or the second barcoded nucleic acid product, or a derivative thereof.

[0081] In some examples, the first plurality of nucleic acid barcode molecules are coupled to a first bead and the second plurality of nucleic acid barcode molecules are coupled to a second bead. In some examples, the first bead and the second bead are gel beads. In some examples, the first bead is linked to the second bead. In some examples, the first plurality of nucleic acid barcode molecules are coupled to the first bead via a first labile moiety and the second plurality of nucleic acid barcode molecules are coupled to the second bead via a second labile moiety. In some examples, the first labile moiety and the second labile moiety are the same. In some examples, the first plurality of nucleic acid barcode molecules are releasably coupled to the first bead and the second plurality of nucleic acid barcode molecules are releasably coupled to the second bead. In some examples, the first plurality of nucleic acid barcode molecules are

releasable from the first bead upon application of a first stimulus and the second plurality of nucleic acid barcode molecules are releasable from the second bead upon application of a second stimulus. In some examples, the first stimulus and the second stimulus are the same. In some examples, the first stimulus and the second stimulus are chemical stimuli.

[0082] In some examples, the first plurality of nucleic acid barcode molecules and the second plurality of nucleic acid barcode molecules are coupled to a bead. In some examples, the bead is a gel bead. In some examples, the first plurality of nucleic acid barcode molecules are coupled to the bead via a first labile moiety and the second plurality of nucleic acid barcode molecules are coupled to the bead via a second labile moiety. In some examples, the first labile moiety and the second labile moiety are the same. In some examples, the first plurality of nucleic acid barcode molecules and the second plurality of nucleic acid barcode molecules are releasably coupled to the bead. In some examples, the first plurality of nucleic acid barcode molecules are releasable from the bead upon application of a first stimulus and the second plurality of nucleic acid barcode molecules are releasable from the bead upon application of a second stimulus. In some examples, the first stimulus and the second stimulus are the same. In some examples, the first stimulus and the second stimulus are chemical stimuli.

[0083] In some examples, the method further comprises, subsequent to (d), sequencing the first plurality of amplification products and the second plurality of amplification products to identify sequences of the DNA molecule and the RNA molecule, respectively.

[0084] Another example of the present disclosure provides a non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0085] Another example of the present disclosure provides a system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0086] 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

[0087] 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

[0088] 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:

[0089] **FIG. 1** shows an example of a microfluidic channel structure for partitioning individual biological particles.

[0090] **FIG. 2** shows an example of a microfluidic channel structure for delivering barcode carrying beads to droplets.

[0091] **FIG. 3** shows an example of a microfluidic channel structure for co-partitioning biological particles and reagents.

[0092] **FIG. 4** shows an example of a microfluidic channel structure for the controlled partitioning of beads into discrete droplets.

[0093] **FIG. 5** shows an example of a microfluidic channel structure for increased droplet generation throughput.

[0094] **FIG. 6** shows another example of a microfluidic channel structure for increased droplet generation throughput.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for processing a nucleic acid molecule, comprising:
 - (a) providing a partition comprising (i) a biological particle comprising (I) a tagmented fragment of a deoxyribonucleic acid (DNA) molecule and (II) a ribonucleic acid (RNA) molecule (ii) a first nucleic acid barcode molecule comprising a first barcode sequence, (iii) a second nucleic acid barcode molecule comprising a second barcode sequence, and (iv) a splint sequence, wherein said first nucleic acid barcode molecule comprises an overhang sequence of said tagmented fragment, and wherein said splint molecule comprises a first sequence complementary to a sequence of said tagmented fragment and a second sequence complementary to said overhang sequence; and
 - (b) within said partition: (i) using said tagmented fragment, said first nucleic acid barcode molecule, and said splint molecule to generate a first barcoded nucleic acid product comprising (I) said first barcode sequence or a complement thereof and (II) a sequence of said DNA molecule; and (ii) using said RNA molecule and said second nucleic acid barcode molecule to generate a second barcoded nucleic acid product comprising (I) said second barcode sequence or a complement thereof and (II) a complementary DNA (cDNA) sequence of said RNA molecule.
2. The method of claim 1, wherein said biological particle is a cell, cell bead, or cell nucleus.
3. The method of claim 1, further comprising lysing or permeabilizing said biological particle within said partition to provide access to said tagmented fragment and said RNA molecule.
4. The method of any one of claims 1-3, further comprising, prior to (a), contacting an open chromatin structure of said biological particle with a transposase-nucleic acid complex to yield said biological particle comprising said tagmented fragment.
5. The method of claim 4, wherein said transposase-nucleic acid complex comprises a first adapter and a second adapter and wherein said tagmented fragment comprises a sequence of said DNA molecule flanked by said first adapter and said second adapter.
6. The method of claim 5, wherein (b)(i) comprises hybridizing said splint molecule to (A) said first adapter or said second adapter of said tagmented fragment and (B) said first nucleic acid barcode molecule, and ligating said first nucleic acid barcode molecule and said tagmented fragment to generate said first barcoded nucleic acid product.

7. The method of claim 5, wherein said first adapter comprises a first transposon end sequence and a first primer sequence and wherein said second adapter comprises a second transposon end sequence and a second primer sequence.
8. The method of claim 7, wherein (b)(i) comprises:
hybridizing said splint molecule to (A) said first primer sequence or said second primer sequence of said tagmented fragment and (B) said first nucleic acid barcode molecule, and
ligating said first nucleic acid barcode molecule and said tagmented fragment to generate said first barcoded nucleic acid product.
9. The method of claim 8, further comprising, prior to said ligating, using a kinase to phosphorylate said tagmented fragment or said first nucleic acid barcode molecule
10. The method of claim 9, wherein said kinase is a polynucleotide kinase (PNK).
11. The method of claim 7, wherein said first primer sequence or said second primer sequence is single stranded.
12. The method of claim 1, further comprising subjecting said first barcoded nucleic acid product to one or more nucleic acid extension reactions.
13. The method of claim 12, wherein said one or more nucleic acid extension reactions comprise attaching one or more functional sequences to said first barcoded nucleic acid product.
14. The method of claim 13, wherein said one or more functional sequences comprise one or more flow cell adapter sequences, one or more sequencing primer sequences, one or more sequencing primer binding sequences, or one or more sample index sequences.
15. The method of claim 1, wherein said second nucleic acid barcode molecule comprises a capture sequence complementary to said RNA molecule and wherein (b)(ii) comprises hybridizing said capture sequence to said RNA molecule and reverse transcribing said RNA molecule to generate said second barcoded nucleic acid product.
16. The method of claim 15, wherein said capture sequence comprises a polythymine (poly-T) sequence.
17. The method of claim 1, wherein said partition further comprises a primer comprising a sequence complementary to said RNA molecule and wherein (b)(ii) comprises hybridizing said primer to said RNA molecule, reverse transcribing said RNA molecule to generate a cDNA molecule, performing a template switching reaction onto said second nucleic acid barcode molecule, and extending said cDNA molecule to generate said second barcoded nucleic acid product.

18. The method of claim 17, wherein said sequence complementary to said RNA molecule comprises a polythymine (poly-T) sequence.
19. The method of claim 17, wherein said reverse transcribing comprises use of an enzyme with terminal transferase activity that appends a polynucleotide sequence to a 3' end of said cDNA molecule and wherein said second nucleic acid barcode molecule comprises a sequence complementary to said polynucleotide sequence to facilitate said template switching reaction.
20. The method of claim 19, wherein said polynucleotide sequence comprises a polycytosine (polyC) sequence and wherein said sequence complementary to said polynucleotide sequence comprises a polyguanine (polyG) sequence.
21. The method of claim 20, wherein said polyG sequence comprises a ribonucleotide.
22. The method of claim 1, wherein said partition is a droplet.
23. The method of claim 1, wherein said partition is a well.
24. The method of claim 1, wherein said first barcode sequence and said second barcode sequence are a same sequence.
25. The method of claim 1, wherein said first barcode sequence and said second barcode sequence are different.
26. The method of claim 1, wherein said tagmented fragment or said first nucleic acid barcode molecule comprises a 5' phosphate.
27. The method of any one of claims 1-26, wherein said first nucleic acid barcode molecule and said second nucleic acid barcode molecule are coupled to a bead.
28. The method of claim 27, wherein said bead is a gel bead.
29. The method of claim 27 or 28, wherein said first nucleic acid barcode molecule and said second nucleic acid barcode molecule are releasable from said bead upon application of a stimulus.
30. The method of claim 27, wherein said first nucleic acid barcode molecule and said second nucleic acid barcode molecule are each coupled to said bead via a labile bond.