

METHODS AND SYSTEMS TO MINIMIZE BARCODE EXCHANGE

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/714,476, filed August 3, 2018, which is entirely incorporated herein by reference.

BACKGROUND

[0002] A sample may be processed for various purposes, such as identification of a type of moiety within the sample. The sample may be a biological sample. Biological samples may be processed, such as for 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.

[0005] Certain applications may benefit from the amplification or sequencing of species obtained from single cells obtained from a much larger population. In some cases, the single cells of interest may be quite rare. Thus, there is a need for sample preparation techniques that allow sequencing of nucleic acids from single cells of interest.

SUMMARY

[0005a] In one aspect, provided herein is a method for nucleic acid processing, comprising: (a) contacting a plurality of biological particles comprising template nucleic acid molecules with a plurality of transposase complexes comprising adapter sequences to generate, from said template nucleic acid molecules, a plurality of adapter-flanked fragments, wherein said plurality of biological particles is a plurality of cells or a plurality of cell nuclei; (b) partitioning said plurality of biological particles into a plurality of partitions, wherein a partition of said plurality of partitions comprises: (i) a biological particle of said plurality of biological particles, wherein said biological particle comprises an adapter-flanked fragment of said plurality of adapter-flanked fragments; (ii) a plurality of nucleic acid barcode molecules comprising a common

barcode sequence; and (iii) a nucleic acid splint molecule comprising a first sequence complementary to said adapter sequence and a second sequence complementary to a sequence of said nucleic acid barcode molecule, wherein said nucleic acid splint molecule comprises a non-canonical base; and (c) using said adapter-flanked fragment, said nucleic acid barcode molecule, and said nucleic acid splint molecule to generate a barcoded nucleic acid molecule comprising a sequence of said adapter-flanked fragment and said common barcode sequence.

[0006] In an example, provided herein is a method for nucleic acid processing, comprising: (a) in a plurality of partitions, using nucleic acid barcode molecules from a plurality of nucleic acid barcode molecules and a plurality of nucleic acid molecules to generate a plurality of barcoded nucleic acid molecules; (b) recovering the plurality of barcoded nucleic acid molecules from the plurality of partitions, to yield a mixture comprising the plurality of nucleic acid barcode molecules and a remainder of the plurality of nucleic acid barcode molecules; (c) subsequent to (b), subjecting the mixture to conditions sufficient to couple a blocking oligonucleotide to the remainder of the plurality of nucleic acid barcode molecules, wherein the blocking oligonucleotide comprises (i) a complementary region comprising a sequence at least 75% complementary to a sequence in the remainder of the plurality of nucleic acid barcode molecules; and (ii) a 3' blocking group.

[0007] In some examples, the 3' blocking group is configured to prevent ligation of the remainder to a nucleic acid molecule. In some examples, the 3' blocking group is configured to prevent extension of the primer sequence when hybridized to a target nucleic acid molecule. In some examples, the plurality of partitions is a plurality of droplets. In some examples, the plurality of partitions is a plurality of wells. In some examples, the blocking oligonucleotide comprises, from 5' to 3', a first stem sequence, a loop sequence, a second stem sequence at least 75% complementary to the first stem sequence, and the complementary region. In some examples, the 3' blocking group is at a 3' terminus of the blocking oligonucleotide. In some examples, the 3' blocking group is: a 2'-3'-dideoxycytosine (ddC), a 2'-5' linked nucleoside, a 3' C3 spacer, an amino-modified C6, an inverted deoxythymidine (dT), a 3' amino modified nucleotide, or a 3' phosphate group. In some examples, the 2'-5' linked nucleoside is a 3'-deoxythymidine (3'-dT) 3'-deoxycytidine (3'-dC), 3'-deoxyadenosine (3'-dA), or a 3'-deoxyguanosine (3'-dG). In some examples, the blocking oligonucleotide is ligated to the remainder. In some examples, the blocking oligonucleotide comprises a 5' phosphate group.

[0008] In another example, disclosed herein is a composition, comprising: a nucleic acid molecule comprising, from 5' to 3': (a) a first region comprising a barcode sequence and a primer sequence; and (b) a second region comprising (i) a sequence complementary to the primer sequence; and (ii) 3' a blocking group. In some examples, the 3' blocking group is at a 3'

terminus of the blocking oligonucleotide. In some examples, the 3' blocking group is a 2'-3'-dideoxycytosine (ddC), a 2'-5' linked nucleoside, a 3' C3 spacer, an amino-modified C6, an inverted deoxythymidine (dT), a 3' amino modified nucleotide, or a 3' phosphate group. In some examples, the 2'-5' linked nucleoside is a 3'-deoxythymidine (3'-dT) 3'-deoxycytidine (3'-dC), 3'-deoxyadenosine (3'-dA), or a 3'-deoxyguanosine (3'-dG). In some examples, the second region comprises, from 5' to 3': a first stem sequence, a loop sequence, a second stem sequence at least 75% complementary to the first stem sequence, the sequence complementary to the primer sequence, and the 3' blocking group.

[0009] In yet another example, disclosed herein is a method for nucleic acid processing, comprising: (a) providing a reaction mixture comprising: (i) a template nucleic acid molecule; (ii) an RNase enzyme; and (iii) a plurality of nucleic acid barcode molecules, wherein each nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules comprises: (A) a common barcode sequence; (B) a sequence capable of hybridizing to a target nucleic acid; (C) at least one ribonucleotide; and (D) a 3' blocking group; (b) subjecting the reaction mixture to conditions sufficient for the template nucleic acid molecule to hybridize to a nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules; (c) subjecting the reaction mixture to conditions sufficient for the RNase enzyme to degrade the ribonucleotide, thereby releasing the 3' blocking group; and (d) using the template nucleic acid molecule and the nucleic acid barcode molecule to generate a barcoded template nucleic acid molecule. In some examples, the 3' blocking group is configured to prevent ligation of the remainder to a nucleic acid molecule. In some examples, the 3' blocking group is configured to prevent extension of the primer sequence when hybridized to a target nucleic acid molecule. In some examples, the common barcode sequence is 5' to the at least one ribonucleotide and the 3' blocking group. In some examples, the 3' blocking group is at a 3' terminus of the plurality of nucleic acid barcode molecules. In some embodiments, the 3' blocking group is a 2'-3'-dideoxycytosine (ddC), a 2'-5' linked nucleoside, a 3' C3 spacer, an amino-modified C6, an inverted deoxythymidine (dT), a 3' amino modified nucleotide, or a 3' phosphate group. In some embodiments, the 2'-5' linked nucleoside is a 3'-deoxythymidine (3'-dT) 3'-deoxycytidine (3'-dC), 3'-deoxyadenosine (3'-dA), or a 3'-deoxyguanosine (3'-dG). In some embodiments, the method further comprises co-partitioning the reaction mixture into a partition. In some examples, the partition is a well. In some examples, the partition is an aqueous droplet in an emulsion. In some examples, the plurality of nucleic acid barcode molecules is attached to a bead. In some examples, the plurality of nucleic acid barcode molecules is releasably attached to the bead. In some examples, the bead is a gel bead. In some examples, the gel bead is a degradable gel bead. In some examples, the degradable gel bead is degradable by a chemical stimulus. In some examples, the chemical

stimulus is a reducing agent. In some examples, the reaction mixture comprises the reducing agent. In some examples, the RNase enzyme is an RNase H2 enzyme. In some examples, the barcoded template nucleic acid molecule is generated by primer extension. In some examples, the barcoded template nucleic acid molecule is generated by nucleic acid amplification. In some examples, the barcoded template nucleic acid molecule is generated by ligation. In some examples, prior to (b), an adapter sequence is added to the template nucleic acid molecule, and wherein the sequence capable of hybridizing to the target nucleic acid is complementary to at least a portion of the adapter sequence. In some examples, the adapter sequence is added to the template nucleic acid molecule by a transposase. In some examples, the adapter sequence comprises a sequencing primer sequence and wherein the sequence capable of hybridizing to the target nucleic acid is complementary to the sequencing primer sequence.

[0010] In another example, described herein is a partially double-stranded nucleic acid barcode molecule comprising: (a) a first strand comprising a (i) a complement of a primer annealing sequence and (ii) a barcode sequence; and (b) a second strand comprising a (i) a 5' overhang and (ii) an abasic site, wherein the first strand and the second strand are annealed to each other.

[0011] In some examples, the abasic site is in the 5' overhang. In some examples, the abasic site is positioned at least 3 base positions 5' of a base of the second strand annealed to a base at a 3' end of the first strand. In some examples, the abasic site is positioned 3, 4, 5, 6, 7, or 8 base positions 5' of a base of the second strand annealed to a base at the 3' end of the first strand. In some examples, the abasic site is at a position in the second strand where a base at a 3' end of the first strand would anneal if a base were present at the position. In some examples, the second strand comprises the primer annealing sequence. In some examples, the partially double-stranded nucleic acid barcode molecule is attached to a bead. In some examples, the bead is a gel bead.

[0012] In another example, disclosed herein is a partially double-stranded nucleic acid barcode molecule comprising (a) a first strand comprising a (i) a complement of a primer annealing sequence and (ii) a barcode sequence; and (b) a second strand comprising (i) DNA, (ii) a 5' overhang, and (iii) a noncanonical base, wherein the first strand and the second strand are annealed to each other.

[0013] In some examples, the noncanonical base is in the 5' overhang. In some examples, the noncanonical base is positioned at least 3 bases 5' of a base of the second strand annealed to a base at a 3' end of the first strand. In some examples, the noncanonical base is positioned 3, 4, 5, 6, 7, or 8 bases 5' of a base of the second strand annealed to a base at the 3' end of the first strand. In some examples, the noncanonical base is annealed to a base at the 3' end of the first

strand. In some examples, the noncanonical base comprises uracil. In some examples, the second strand comprises the primer annealing sequence. In some examples, the partially double-stranded nucleic acid barcode molecule is attached to a bead. In some examples, the bead is a gel bead.

[0014] In another example, provided herein is a partially double-stranded nucleic acid barcode molecule comprising (a) a first strand comprising (i) a complement of a primer annealing sequence and (ii) a barcode sequence; and (b) a second strand comprising a 5' overhang, wherein the first strand and the second strand are annealed to each other, and wherein the second strand is configured to prevent a polymerase from synthesizing a strand using all of the 5' overhang as a template.

[0015] In some examples, the partially double-stranded nucleic acid barcode molecule is attached to a bead. In some examples, the bead is a gel bead.

[0016] In another example, provided herein is a partially double-stranded nucleic acid barcode molecule comprising (a) a first strand comprising (i) a complement of a primer annealing sequence and (ii) a barcode sequence; and (b) a second strand comprising a 5' overhang and a 3' end comprising a block that prevents extension of the 3' end of the second strand by an enzyme, wherein the first strand and the second strand are annealed to each other.

[0017] In some examples, the block comprises a C3 spacer, 2'-3'-dideoxycytosine (ddC), an inverted dT, an amino group, or a phosphate. In some examples, the partially double-stranded nucleic acid barcode molecule is attached to a bead. In some examples, the bead is a gel bead.

[0018] In an example, provided herein is a method of generating a partially double-stranded nucleic acid barcode molecule comprising an abasic site, the method comprising (a) providing a partially double-stranded nucleic acid barcode molecule comprising (i) a first strand comprising (A) a complement of a primer annealing sequence and (B) a barcode sequence; and (ii) a second strand comprising (A) DNA, (B) a 5' overhang, and (C) a noncanonical base, wherein the first strand and the second strand are annealed to each other; and (b) treating the partially double-stranded DNA with an agent, thereby generating an abasic site at a position of the noncanonical base.

[0019] In some examples, the noncanonical base is uracil. In some examples, the agent is uracil-DNA-glycosylase (UDG). In some examples, the second strand comprises the primer annealing sequence.

[0020] In another example, provided herein is a method for nucleic acid processing, comprising, in a plurality of partitions, generating a plurality of barcoded nucleic acid molecules using nucleic acid molecules of a plurality of nucleic acid molecules and a partially double-stranded nucleic acid barcode molecule.

[0021] In some examples, the generating comprises ligating a nucleic acid molecule of the plurality of nucleic acid molecules to the partially double-stranded nucleic acid barcode molecule. In some examples, the ligating comprises ligating the nucleic acid molecule of the plurality of nucleic acid molecules to a 3' end of the first strand of the partially double-stranded nucleic acid barcode molecule. In some examples, the plurality of partitions is a plurality of droplets. In some examples, the plurality of partitions is a plurality of wells. In some examples, the nucleic acid molecules are tagged nucleic acid molecules.

[0022] In some examples, in each partition of the plurality of partitions, the barcode sequence of the partially double-stranded nucleic acid barcode molecule is unique, and the complement of the primer annealing sequence is common.

[0023] In another example, provided herein is a method of nucleic acid processing comprising, (a) in a plurality of partitions (i) annealing a 5' overhang of a second strand of a partially double-stranded nucleic acid barcode molecule to a nucleic acid molecule, wherein a first strand of the partially double-stranded nucleic acid barcode molecule comprises (A) a complement of a common primer annealing sequence and (B) a unique barcode sequence and; and (i) ligating the partially double-stranded nucleic acid barcode molecule to the nucleic acid molecule, thereby generating a barcoded nucleic acid molecule; (a) pooling the barcoded nucleic acid molecules from the plurality of partitions; (b) amplifying the barcoded nucleic acid molecules using a primer that anneals to the common primer annealing sequence, wherein a rate of barcode exchange of the barcoded nucleic acid molecules following the amplifying is less than 50%.

[0024] In some examples, the rate of barcode exchange is less than 10%. In some examples, the rate of barcode exchange is less than 1%. In some examples, the partially double-stranded nucleic acid barcode molecule is coupled to a bead. In some examples, the bead is a gel bead. In some examples, the plurality of partitions is a plurality of droplets. In some examples, the plurality of partitions is a plurality of wells. In some examples, the nucleic acid molecule is a tagged nucleic acid.

[0025] Disclosed herein, in some examples, are methods for nucleic acid processing, comprising: (a) in a plurality of partitions, using nucleic acid barcode molecules from a plurality of nucleic acid barcode molecules and a plurality of nucleic acid molecules to generate a plurality of barcoded nucleic acid molecules; (b) recovering the plurality of barcoded nucleic acid molecules from the plurality of partitions, to yield a mixture comprising the plurality of nucleic acid barcode molecules and a remainder of the plurality of nucleic acid barcode molecules; (c) subsequent to (b), subjecting the mixture to conditions sufficient to couple a blocking oligonucleotide to the remainder of the plurality of nucleic acid barcode molecules,

wherein the blocking oligonucleotide comprises: (i) a complementary region comprising a sequence at least 75% complementary to a sequence in the remainder of the plurality of nucleic acid barcode molecules; and (ii) a 3' blocking group.

[0026] In some examples, the 3' blocking group is configured to prevent ligation of the remainder to a nucleic acid molecule. In some examples, the 3' blocking group is configured to prevent extension of the primer sequence when hybridized to a target nucleic acid molecule. In some examples, the plurality of partitions is a plurality of droplets. In some examples, the plurality of partitions is a plurality of wells.

[0027] In some examples, the blocking oligonucleotide comprises, from 5' to 3', a first stem sequence, a loop sequence, a second stem sequence at least 75% complementary to the first stem sequence, and the complementary region. In some examples, the 3' blocking group is at a 3' terminus of the blocking oligonucleotide. In some examples, the 3' blocking group is a: 2'-3'-dideoxycytosine (ddC), a 2'-5' linked nucleoside, a 3' C3 spacer, an amino-modified C6, an inverted deoxythymidine (dT), a 3' amino modified nucleotide, or a 3' phosphate group. In some examples, the 2'-5' linked nucleoside is a 3'-deoxythymidine (3'-dT), 3'-deoxycytidine (3'-dC), 3'-deoxyadenosine (3'-dA), or a 3'-deoxyguanosine (3'-dG). In some examples, the blocking oligonucleotide is ligated to the remainder. In some examples, the blocking oligonucleotide comprises a 5' phosphate group.

[0028] Also disclosed herein, in some examples, is a composition, comprising, from 5' to 3': (a) a first region comprising a barcode sequence and a primer sequence; and (b) a second region comprising (i) a sequence complementary to the primer sequence; and (ii) 3' a blocking group. In some examples, the 3' blocking group is at a 3' terminus of the blocking oligonucleotide. In some examples, the 3' blocking group is a 2'-3'-dideoxycytosine (ddC), a 2'-5' linked nucleoside, a 3' C3 spacer, an amino-modified C6, an inverted deoxythymidine (dT), a 3' amino modified nucleotide, or a 3' phosphate group. In some examples, the second region comprises, from 5' to 3': a first stem sequence, a loop sequence, a second stem sequence at least 75% complementary to the first stem sequence, the sequence complementary to the primer sequence, and the 3' blocking group.

[0029] Also disclosed herein, in some examples, are methods for nucleic acid processing, comprising: (a) providing a reaction mixture comprising: (i) a template nucleic acid molecule; (ii) an RNase enzyme; and (iii) a plurality of nucleic acid barcode molecules, wherein each nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules comprises: (A) a common barcode sequence; (B) a sequence capable of hybridizing to a target nucleic acid; (C) at least one ribonucleotide; and (D) a 3' blocking group; (b) subjecting the reaction mixture to conditions sufficient for the template nucleic acid molecule to hybridize to a nucleic acid

barcode molecule of the plurality of nucleic acid barcode molecules; (c) subjecting the reaction mixture to conditions sufficient for the RNase enzyme to degrade the ribonucleotide, thereby releasing the 3' blocking group; and (d) using the template nucleic acid molecule and the nucleic acid barcode molecule to generate a barcoded template nucleic acid molecule.

[0030] In some examples, the 3' blocking group is configured to prevent ligation of the remainder to a nucleic acid molecule. In some examples, the 3' blocking group is configured to prevent extension of the primer sequence when hybridized to a target nucleic acid molecule. In some examples, the barcode sequence is 5' to the at least one ribonucleotide and the 3' blocking group.

[0031] In some examples, the 3' blocking group is at a 3' terminus of the plurality of nucleic acid barcode molecules. In some examples, the 3' blocking group is a 2'-3'-dideoxycytosine (ddC), a 2'-5' linked nucleoside, a 3' C3 spacer, an amino-modified C6, an inverted deoxythymidine (dT), a 3' amino modified nucleotide, or a 3' phosphate group. In some examples, the 2'-5' linked nucleoside is a 3'-deoxythymidine (3'-dT) 3'-deoxycytidine (3'-dC), 3'-deoxyadenosine (3'-dA), or a 3'-deoxyguanosine (3'-dG).

[0032] In some examples, the method for nucleic acid processing further comprises co-partitioning the reaction mixture into a partition. In some examples, the partition is a well. In some examples, the partition is an aqueous droplet in an emulsion.

[0033] In some examples, the plurality of nucleic acid barcode molecules is attached to a bead. In some examples, the plurality of nucleic acid barcode molecules is releasably attached to the bead. In some examples, the bead is a gel

bead. In some examples, the gel bead is a degradable gel bead. In some examples, the degradable gel bead is degradable by a chemical stimulus. In some examples, the chemical stimulus is a reducing agent. In some examples, the reaction mixture comprises the reducing agent.

[0034] In some examples, the RNase enzyme is an RNase H2 enzyme. In some examples, the barcoded template nucleic acid molecule is generated by primer extension. In some examples, the barcoded template nucleic acid is generated by nucleic acid amplification, extension, or synthesis. In some examples, the barcoded template nucleic acid molecule is generated by ligation.

[0035] In some examples, the method for nucleic acid processing further comprises adding an adapter sequence to the template nucleic acid molecule prior to (b), wherein the adapter sequence is capable of hybridizing to the target nucleic acid is complementary to at least a portion of the adapter sequence. In some examples, the adapter sequence is added to the template nucleic acid molecule by a transposase. In some examples, the adapter sequence comprises a sequencing primer sequence, wherein the sequence capable of hybridizing to the target nucleic acid is complementary to the sequencing primer sequence.

[0036] 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

[0037] 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

1. A method for nucleic acid processing, comprising:

(a) contacting a plurality of biological particles comprising template nucleic acid molecules with a plurality of transposase complexes comprising adapter sequences to generate, from said template nucleic acid molecules, a plurality of adapter-flanked fragments, wherein said plurality of biological particles is a plurality of cells or a plurality of cell nuclei;

(b) partitioning said plurality of biological particles into a plurality of partitions, wherein a partition of said plurality of partitions comprises:

(i) a biological particle of said plurality of biological particles, wherein said biological particle comprises an adapter-flanked fragment of said plurality of adapter-flanked fragments;

(ii) a plurality of nucleic acid barcode molecules comprising a common barcode sequence; and

(iii) a nucleic acid splint molecule comprising a first sequence complementary to said adapter sequence and a second sequence complementary to a sequence of said nucleic acid barcode molecule, wherein said nucleic acid splint molecule comprises a non-canonical base; and

(c) using said adapter-flanked fragment, said nucleic acid barcode molecule, and said nucleic acid splint molecule to generate a barcoded nucleic acid molecule comprising a sequence of said adapter-flanked fragment and said common barcode sequence.

2. The method of claim 1, wherein said nucleic acid splint molecule further comprises a blocking group.

3. The method of claim 2, wherein said blocking group is a 3' blocking group.

4. The method of claim 3, wherein said 3' blocking group is a 2'-3'-dideoxycytosine (ddC), a 2'-5' linked nucleoside, a 3' C3 spacer, an amino-modified C6, an inverted deoxythymidine (dT), a 3' amino modified nucleotide, or a 3' phosphate group.

5. The method of claim 4, wherein said 2'-5' linked nucleoside is a 3'-deoxythymidine (3'-dT) 3'-deoxycytidine (3'-dC), 3'-deoxyadenosine (3'-dA), or a 3'-deoxyguanosine (3'-dG).

6. The method of claim 1, wherein said noncanonical base is uracil.

7. The method of claim 1, further comprising, subsequent to (c), generating an abasic site in said nucleic acid splint molecule at a position of said noncanonical base.
8. The method of claim 7, wherein said noncanonical base is uracil and wherein said abasic site is generated using a uracil-DNA-glycosylase (UDG).
9. The method of claim 1, further comprising, subsequent to (c), pooling the contents of said plurality of partitions, processing said pooled contents of said plurality of partitions to generate an abasic site at a position of said non-canonical base, and performing one or more nucleic acid reactions on said barcoded nucleic acid molecule or a derivative thereof to generate a reaction product, wherein said processing reduces barcode exchange in said reaction product.
10. The method of claim 9, wherein said noncanonical base is uracil and wherein said processing comprises using an enzyme to generate said abasic site.
11. The method of claim 10, wherein said enzyme is uracil-DNA-glycosylase (UDG).
12. The method of claim 9, wherein said one or more nucleic acid reactions comprises one or more nucleic acid amplification reactions.
13. The method of claim 1, wherein (c) comprises ligating said adapter-flanked fragment to said nucleic acid barcode molecule to generate said barcoded nucleic acid molecule.
14. The method of claim 1, wherein said adapter-flanked fragment comprises a first adapter sequence and a second adapter sequence and wherein said plurality of transposase complexes comprises a plurality of transposase molecules, and wherein said adapter sequences comprise said first adapter sequence; and said second adapter sequence.
15. The method of claim 1, wherein said plurality of partitions is a plurality of droplets in an emulsion.
16. The method of claim 1, wherein said plurality of partitions is a plurality of microwells in an array, wherein said array comprises 1,000 microwells.
17. The method of claim 1, wherein said plurality of nucleic acid barcode molecules are attached to a bead.
18. The method of claim 17, wherein said plurality of nucleic acid barcode molecules is releasable from said bead upon application of a stimulus.
19. The method of claim 17, wherein said bead is a gel bead.
20. The method of claim 19, wherein said gel bead is degradable upon application of a stimulus.