

droplet. The droplet may contain a gel bead which may contain a tag (such as a barcode) that may be used to barcode macromolecular constituents (e.g., nucleic acid molecules) of a single biological particle.

[0005] Methods and systems of the present disclosure may be used to generate target sequence or sequencing reads (“reads”) specific to macromolecular constituents of interest at a higher rate than non-target specific reads. For instance, the methods and systems are characterized by their suppression of no template control (NTC) effects.

[0005a] In one aspect, the present disclosure refers to a method for processing or analyzing one or more components from a sample, comprising:

(a) providing a plurality of cell beads and a plurality of barcode beads, wherein (i) a cell bead of said plurality of cell beads is a particulate material that comprises a cell, wherein said cell comprises a nucleic acid molecule in an interior region of said cell, and (ii) a barcode bead of said plurality of barcode beads comprises a plurality of nucleic acid barcode molecules for barcoding said nucleic acid molecule;

(b) subjecting said plurality of cell beads to bulk processing, which bulk processing comprises (i) subjecting said cell bead to conditions sufficient to lyse said cell, thereby rendering said nucleic acid molecule accessible to a species external to said interior region and (ii) denaturing said nucleic acid molecule to generate a denatured nucleic acid molecule in said cell bead; and

(c) partitioning said plurality of cell beads and said plurality of barcode beads into a plurality of partitions, wherein upon partitioning, a partition of said plurality of partitions comprises said cell bead and said barcode bead.

[0006] In an example, the present disclosure provides a method for analysis of a single biological particle, comprising (a) providing a first liquid phase comprising a plurality of biological particles; (b) providing a second liquid phase comprising a plurality of beads each including a tag to barcode one or more macromolecular constituents of each of the plurality of biological particles; (c) bringing the plurality of biological particles from the first liquid phase and the plurality of beads from the second liquid phase in contact with a third liquid phase that is immiscible with the first or second liquid phase, to partition each of the plurality of biological particles and the plurality of beads into a plurality of partitions (e.g., droplets), wherein upon partitioning, a given partition of the plurality of partitions includes a single biological particle from the plurality of biological particles and a single bead from the plurality of beads; (d) in the given partition (e.g., droplet), using the tag from the single bead to barcode the one or more macromolecular constituents of the single biological particle, forming one or more barcoded macromolecules; and (e) subjecting the barcoded macromolecules to sequencing to generate reads characterized by a specific target read(s) to non-target specific read(s) ratio greater than 1,

CLAIMS

WHAT IS CLAIMED IS:

1. A method for processing or analyzing one or more components from a sample, comprising:
 - (a) providing a plurality of cell beads and a plurality of barcode beads, wherein (i) a cell bead of said plurality of cell beads is a particulate material that comprises a cell, wherein said cell comprises a nucleic acid molecule in an interior region of said cell, and (ii) a barcode bead of said plurality of barcode beads comprises a plurality of nucleic acid barcode molecules for barcoding said nucleic acid molecule;
 - (b) subjecting said plurality of cell beads to bulk processing, which bulk processing comprises (i) subjecting said cell bead to conditions sufficient to lyse said cell, thereby rendering said nucleic acid molecule accessible to a species external to said interior region and (ii) denaturing said nucleic acid molecule to generate a denatured nucleic acid molecule in said cell bead; and
 - (c) partitioning said plurality of cell beads and said plurality of barcode beads into a plurality of partitions, wherein upon partitioning, a partition of said plurality of partitions comprises said cell bead and said barcode bead.
2. The method of claim 1, further comprising using a nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules and said denatured nucleic acid molecule or a derivative thereof to generate a barcoded nucleic acid molecule comprising a first sequence corresponding to said denatured nucleic acid molecule and a second sequence corresponding to a barcode sequence of said nucleic acid barcode molecule.
3. The method of claim 2, further comprising subjecting said barcoded nucleic acid molecule or derivative thereof to sequencing.
4. The method of claim 1, wherein the plurality of partitions is a plurality of droplets or a plurality of wells.
5. The method of claim 1, wherein said barcode bead or said cell bead is degradable upon application of a stimulus selected from the group consisting of a chemical stimulus, a biological stimulus, a temperature change, exposure to light, and a pH change.
6. The method of claim 1, further comprising: prior to (a), partitioning a plurality of cells comprising said cell and polymer or gel precursors into a plurality of first partitions; and subjecting said plurality of first partitions to conditions sufficient to polymerize or crosslink said polymer or gel precursors in said plurality of first partitions to generate said plurality of cell beads.

7. The method of claim 1, wherein said plurality of nucleic acid barcode molecules is a plurality of double-stranded nucleic acid barcode molecules.
8. The method of claim 7, further comprising (i) performing one or more nucleic acid extension reactions on said denatured nucleic acid molecule to generate a double-stranded nucleic acid molecule comprising a sequence of said denatured nucleic acid molecule and (ii) ligating said nucleic acid barcode molecule to said double-stranded nucleic acid molecule to generate a barcoded nucleic acid molecule.
9. The method of claim 8, wherein said one or more nucleic acid extension reactions comprise:
 - (i) annealing a first primer to said denatured nucleic acid molecule and performing a first nucleic acid extension reaction in the presence of uracil to generate a first nucleic acid extension product comprising a uracil-containing moiety;
 - (ii) excising said uracil-containing moiety to generate a nick in said first nucleic acid extension product;
 - (iii) performing a second nucleic acid extension reaction on said first nucleic acid extension product comprising said nick to generate a plurality of single-stranded nucleic acid fragments;
 - (iv) annealing a second primer to a single-stranded nucleic acid fragment of said plurality of single-stranded nucleic acid fragments; and
 - (v) performing a third nucleic acid extension reaction to generate a second nucleic acid extension product.
10. The method of claim 9, wherein said first primer or said second primer comprises a random primer sequence or a targeted primer sequence.
11. The method of claim 9, wherein said first primer and said second primer comprises a random primer sequence.
12. The method of claim 9, wherein said second nucleic acid extension reaction is completed with use of a polymerase having strand displacement activity, wherein the polymerase engages the first nucleic acid extension product at the nick, and wherein said plurality of single stranded nucleic acid fragments are displaced from the first nucleic acid extension product.
13. The method of claim 1, wherein said bulk processing comprises contacting said plurality of cell beads with a chemical denaturant.
14. The method of claim 13, wherein said chemical denaturant is sodium hydroxide.

15. The method of claim 1, wherein said plurality of cell beads comprise a plurality of magnetic particles.
16. The method of claim 15, further comprising, prior to (c), using a magnetic source to purify said plurality of cell beads subsequent to said bulk processing.
17. The method of claim 1, wherein said plurality of nucleic acid barcode molecules are releasable from said barcode bead upon application of a stimulus selected from the group consisting of a chemical stimulus, a biological stimulus, a temperature change, exposure to light, and a pH change.
18. The method of claim 1, wherein said bulk processing comprises subjecting said cell bead to conditions sufficient to lyse said cell.
19. The method of claim 18, wherein said conditions sufficient to lyse said cell comprise exposing said plurality of cell beads to sodium hydroxide, potassium hydroxide, sodium dodecyl sulfate, a non-ionic surfactant, a saponin, a proteinase, a lytic enzyme, freeze thawing, ultraviolet light, heat, or any combination thereof.
20. The method of claim 1, wherein in (a), said cell bead comprises said cell enclosed within a gel or polymer matrix comprising one or more members selected from the group consisting of disulfide crosslinked polyacrylamide, agarose, alginate, polyvinyl alcohol, PEG-diacrylate, PEG-acrylate/thiol, PEG-azide/alkyne, other acrylates, chitosan, hyaluronic acid, collagen, fibrin, gelatin, and elastin.
21. The method of claim 1, wherein said cell bead comprises a cell encapsulated within a polymer or gel matrix.