

the analyte carrier comprises a template nucleic acid molecule, and wherein the first barcode bead comprises a first nucleic acid barcode molecule comprising a first barcode sequence, and (ii) the second partition comprises a control bead from the plurality of control beads and a second barcode bead from the plurality of barcode beads, wherein the second barcode bead comprises a second nucleic acid barcode molecule comprising a second barcode sequence, wherein the second barcode sequence is different than the first barcode sequence, and wherein the control bead comprises a nucleic acid molecule comprising a known sequence; (c) using (i) the template nucleic acid molecule and the first nucleic acid barcode molecule to generate a barcoded template nucleic acid molecule and (ii) the nucleic acid molecule comprising the known sequence and the second nucleic acid barcode molecule to generate a barcoded nucleic acid molecule comprising the known sequence; and (d) sequencing the barcoded template nucleic acid molecule, or derivative thereof, and the barcoded nucleic acid molecule, or derivative thereof wherein (i) a sequence of the barcoded template nucleic acid molecule identifies the analyte carrier, and (ii) a sequence of the barcoded nucleic acid molecule identifies the control bead.

[0008a] In one aspect, provided is a method for analyzing an assay performed on a single cell ~~process in a cell sample~~ or nucleus, comprising: a) providing a mixed sample comprising i) plurality of cells, a or plurality of nuclei, ii) a plurality of first control beads, and iii) a plurality of second control beads, wherein a ratio between a concentration of the plurality of first control beads and a concentration of the plurality of second control beads is known; and a plurality of barcode beads; b) generating a plurality of partitions comprising a first partition, a second partition, and a third partition, wherein (i) said first partition comprises (A) a cell from said plurality of cells and/or a nucleus from said plurality of nuclei and (B) a first barcode bead from said plurality of barcode beads, wherein said analyte carrier cell comprises a template nucleic acid molecule, and wherein said first barcode bead comprises a first nucleic acid barcode molecule comprising a first barcode sequence, (ii) said second partition comprises a first control bead from said plurality of first control beads and a second barcode bead from said plurality of barcode beads, wherein said second barcode bead comprises a second nucleic acid barcode molecule comprising a second barcode sequence, wherein said second barcode sequence is different than said first barcode sequence, and wherein said first control bead comprises a nucleic acid molecule comprising a first known control sequence, and (iii) said third partition comprises a third barcode bead from said plurality of barcode beads and a second control bead from said plurality of second control beads, wherein said third barcode bead comprises a third nucleic acid barcode molecule comprising a third

barcode sequence, wherein said third barcode sequence is different than said first barcode sequence and said second barcode sequence, and wherein said second control bead comprises a second nucleic acid molecule comprising a second known control sequence, wherein said second known control sequence is different from said [first](#) known control sequence; c) using (i) said template nucleic acid molecule and said first nucleic acid barcode molecule to generate a barcoded template nucleic acid molecule, (ii) said nucleic acid molecule comprising said [first](#) known control sequence and said second nucleic acid barcode molecule to generate a [first](#) barcoded nucleic acid molecule comprising said [first](#) known control sequence, and (iii) said second nucleic acid molecule comprising said second known control sequence and said third nucleic acid barcode molecule to generate a second barcoded nucleic acid molecule comprising said second known control sequence; and d) sequencing said barcoded template nucleic acid molecule, or derivative thereof, and said [first](#) barcoded nucleic acid molecule, or derivative thereof, [and said second barcoded nucleic acid molecule, or derivative thereof,](#) wherein (i) a sequence of said barcoded template nucleic acid molecule identifies said cell [or nucleus,](#) and (ii) a sequence of said [first](#) barcoded nucleic acid molecule identifies said [first](#) control bead, and (iii) a sequence of said second barcoded nucleic acid molecule identifies said second control bead; [and \(e\) using results of said sequencing and said known ratio to analyze an identifier purity or a doublet rate of said assay performed on a single cell or nucleus, wherein said identifier purity is indicative of any change to a set of identifier sequences associated with said first known control sequences of second known control sequences during said assay performed on a single cell or nucleus.](#)

[0009] In some embodiments, the method further comprises identifying the known sequence to analyze the single cell process.

[0010] In some embodiments, the method further comprises providing a plurality of second control beads, wherein the plurality of partitions comprises a third partition, wherein the third partition comprises a third barcode bead from the plurality of barcode beads and a second control bead from the plurality of second control beads, wherein the third barcode bead comprises a third nucleic acid barcode molecule comprising a third barcode sequence, wherein the third barcode sequence is different than the first barcode sequence and the second barcode sequence, and wherein the second control bead comprises a second nucleic acid molecule comprising a second known sequence, wherein the second known sequence is different from the known sequence.

[0011] In some embodiments, the method further comprises generating a second barcoded nucleic acid molecule comprising the second known sequence, wherein a sequence of the second barcoded nucleic acid molecule identifies the second known sequence. In some embodiments, the method further comprises processing the known sequence and the second known sequence to analyze the single cell process. In some embodiments, the method further comprises processing comprises comparing a frequency of the known sequence and a frequency of the second known sequence to analyze the single cell process. In some embodiments, the processing further comprises determining a doublet rate.

[0012] In some embodiments, the second control bead has a size within about 70% deviation from an average size of the analyte carrier. In some embodiments, the second control bead has a

CLAIMS

WHAT IS CLAIMED IS:

1. A method for analyzing an assay performed on a single cell ~~process in a cell sample~~or nucleus, comprising:
 - (a) providing a mixed sample comprising i) plurality of cells, ~~a~~ or plurality of nuclei, ii) a plurality of first control beads, and iii) a plurality of second control beads, wherein a ratio between a concentration of the plurality of first control beads and a concentration of the plurality of second control beads is known; and a plurality of barcode beads;
 - (b) generating a plurality of partitions comprising a first partition, a second partition, and a third partition, wherein (i) said first partition comprises (A) a cell from said plurality of cells ~~and~~ or a nucleus from said plurality of nuclei and (B) a first barcode bead from said plurality of barcode beads, wherein said ~~analyte-carrier~~cell comprises a template nucleic acid molecule, and wherein said first barcode bead comprises a first nucleic acid barcode molecule comprising a first barcode sequence, (ii) said second partition comprises a first control bead from said plurality of first control beads and a second barcode bead from said plurality of barcode beads, wherein said second barcode bead comprises a second nucleic acid barcode molecule comprising a second barcode sequence, wherein said second barcode sequence is different than said first barcode sequence, and wherein said first control bead comprises a nucleic acid molecule comprising a first known control sequence, and (iii) said third partition comprises a third barcode bead from said plurality of barcode beads and a second control bead from said plurality of second control beads, wherein said third barcode bead comprises a third nucleic acid barcode molecule comprising a third barcode sequence, wherein said third barcode sequence is different than said first barcode sequence and said second barcode sequence, and wherein said second control bead comprises a second nucleic acid molecule comprising a second known control sequence, wherein said second known control sequence is different from said first known control sequence;

- (c) using (i) said template nucleic acid molecule and said first nucleic acid barcode molecule to generate a barcoded template nucleic acid molecule, (ii) said nucleic acid molecule comprising said first known control sequence and said second nucleic acid barcode molecule to generate a first barcoded nucleic acid molecule comprising said first known control sequence, and (iii) said second nucleic acid molecule comprising said second known control sequence and said third nucleic acid barcode molecule to generate a second barcoded nucleic acid molecule comprising said second known control sequence; and
- (d) sequencing said barcoded template nucleic acid molecule, or derivative thereof, and said first barcoded nucleic acid molecule, or derivative thereof, and said second barcoded nucleic acid molecule, or derivative thereof, wherein (i) a sequence of said barcoded template nucleic acid molecule identifies said cell or nucleus, and (ii) a sequence of said first barcoded nucleic acid molecule identifies said first control bead, and (iii) a sequence of said second barcoded nucleic acid molecule identifies said second control bead-; and
- (e) using results of said sequencing and said known ratio to analyze an identifier purity or a doublet rate of said assay performed on a single cell or nucleus, wherein said identifier purity is indicative of any change to a set of identifier sequences associated with said first known control sequences of second known control sequences during said assay performed on a single cell or nucleus.
2. The method of claim 1, further comprising identifying said first known control sequence or said second known control sequence to analyze said assay performed on a single cell ~~processor~~ nucleus.
3. The method of any one of claims 1-2, further comprising processing said first known control sequence and said second known control sequence to analyze said assay performed on a single cell ~~processor~~ nucleus.
4. The method of ~~any one of claims 1-claim~~ claim 3, wherein said processing comprises comparing a frequency of said first known control sequence and a frequency of said second known control sequence to analyze said assay performed on a single cell ~~processor~~ nucleus.

5. The method of any one of claims ~~1~~3-4, wherein said processing further ~~comprising~~comprises determining a doublet rate.
6. The method of any one of claims 1-5, wherein said second control bead has a size within 25% deviation from an average size of said plurality of cells, an average size of said plurality of first control beads, said plurality of second control beads, or ~~both~~any combination thereof.
7. The method of ~~claim~~any one of claims 1-6, wherein said first known control sequence is derived from a first species and said second known control sequence is derived from a second species.
8. The method of any one of claims 1-7, wherein said first control bead or said second control bead has a size within 25% deviation from an average size of said plurality of cells.
9. The method of any one of claims 1-8, wherein said first control bead or said second control bead has a size between from about 15 micrometers to about 60 micrometers.
10. The method of any one of claims 1-9, wherein a given bead from said plurality of barcode beads comprises a plurality of nucleic acid barcode molecules comprising a common barcode sequence, and wherein said common barcode sequence is different from common barcode sequences of other beads of said plurality of beads.
11. The method of any one of claims 1-10, further comprising, prior to (b), mixing cells from said plurality of cells with first control beads of said plurality of first control beads and second control beads of said second plurality of control beads.
12. The method of claim 11, wherein said first control beads from said plurality of first control beads is present in said mixture at a first concentration, wherein said second control beads from said second plurality of control beads is present in said mixture at a second concentration, and wherein said first concentration and said second concentration are known.
13. The method of claim 12, wherein a ratio of a concentration of said cells in said mixture to said first concentration is known.
14. The method of any one of claims 1-13, wherein said first control bead or said second control bead comprises a plurality of unique nucleic acid molecules with known nucleic acid sequences that vary in length, concentration, GC content, or a combination thereof.

15. The method of any one of claims 1-14, wherein (i) said [first](#) control bead comprises a first plurality of unique nucleic acid molecules with known nucleic acid sequences that vary in length, concentration, GC content, or a combination thereof, and wherein (ii) said second control bead comprises a second plurality of unique nucleic acid molecules with known nucleic acid sequences that vary in length, concentration, ~~-~~GC content, or a combination thereof, wherein said first plurality of unique nucleic acid molecules and said second plurality of unique nucleic acid molecules are different.
16. The method of claim 15, wherein a given bead from said plurality of barcode beads comprises a plurality of nucleic acid barcode molecules, wherein each of said plurality of nucleic acid barcode molecules comprises an identifier sequence associated with said each of said plurality of nucleic acid barcode molecules.
17. The method of any one of claims 15-16, further comprising identifying a first set of identifiers associated with said first plurality of unique nucleic acid molecules and a second set of identifiers associated with said second plurality of unique nucleic acid molecules and processing said first set of identifiers and said second set of identifiers to determine an identifier purity for said single cell process.
18. The method of any one of claims 1-17, wherein a given bead from said plurality of barcode beads comprises a plurality of nucleic acid barcode molecules releasably attached thereto, and wherein said first nucleic acid barcode molecule and said second nucleic acid barcode molecule ~~is~~[are](#) released from said first barcode bead and said second barcode bead.
19. The method of any one of claims 1-18, wherein said plurality of barcode beads is a plurality of gel beads.
20. The method of any one of claims 1-19, wherein said [first control bead or said second](#) control bead is a gel bead.
21. The method of any one of claims 1-20, wherein said [first](#) control bead comprises a first functional group, and said nucleic acid molecule comprising ~~the~~[said first](#) known control sequence comprises a second functional group and wherein said nucleic acid molecule comprising ~~the~~[said first](#)

known control sequence is attached to said first control bead by reacting said first functional group with said second functional group.

22. The method of claim 21, wherein (i) said first functional group is an alkyne, a *trans*-cyclooctene, an avidin, or any combination thereof, and (ii) said second functional group is an azide, a tetrazine, a biotin, or any combination thereof.

23. The method of claim 21, wherein said first functional group reacts with said second functional group in a click reaction, wherein said click reaction is a copper-catalyzed azide-alkyne cycloaddition reaction, an inverse-electron demand Diels-Alder reaction, or an avidin-biotin interaction.

24. The method of any one of claims 1-23, wherein said nucleic acid molecule comprising ~~the~~said first known control sequence or said second known control sequence is releasably attached to said control bead.

25. The method of any one of claims 1-24, wherein said nucleic acid molecule comprising ~~the~~said first known control sequence or said second known control sequence is within said control bead.

26. The method of any one of claims 1-25, wherein said first known control sequence or said second known control sequence is synthetic.

27. The method of any one of claims 1-25, wherein said first known control sequence or said second known control sequence is derived from a biological sample.

28. The method of any one of claims 1-27, wherein said first control bead or said second control bead comprises a protein-DNA complex and said nucleic acid molecule comprising ~~the~~said first known control sequence or said second known control sequence is part of said protein-DNA complex, wherein said first known control sequence or said second known control sequence comprises defined protein binding sites.

~~29.~~—The method of any one of claims 1-28, wherein said plurality of partitions is a plurality of droplets, a plurality of wells, or both.

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