SYSTEMS AND METHODS FOR ANALYZING BIOLOGICAL SAMPLES

CROSS-REFERENCE

[001] This application claims the benefit of U.S. Provisional Application No. 63/209,544, filed June 11, 2021, which is herein entirely incorporated by reference.

INCORPORATION BY REFERENCE

[002] 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.

BACKGROUND

[003] Analysis of a biological sample is one of the cornerstones of modern medicine. While there have been recent developments advancing the analysis of specific deoxynucleic acid (DNA) molecules, analysis of nucleic acid molecules (e.g. DNA, ribonucleic nucleic acid (RNA)) generated from specific cells or tissue samples remains as a hurdle to overcome for the industry. For example, the analysis of the gene expression profile (e.g., a transcriptome) in a cell based on the sequences and abundance of the sequenced nucleic acid in the sample is still inefficient and labor intensive. The sequencing techniques currently available have drawbacks. For example, sequencing RNA samples require sample preparation methods that first convert RNA into a double-stranded cDNA format prior to sequencing. As such, the preparation of the biological sample comprising RNA for sequencing is often labor intensive. Furthermore, current sequencing techniques are less than optimal in preserving strand-specific information of the original single-stranded RNA molecule after being converted into double stranded cDNA. Preserving strand-specific information can be important for annotation for determining gene expression levels.

[004] The field of cellular analysis would be advanced by the availability of multiplex single-cell analysis methods, especially RNA-seq methods, which provided sensitive and convenient measurements of cellular gene expression.

SUMMARY

[004a] In an aspect of the present disclosure, there is provided a method for preparing a set of complementary deoxynucleic acid (cDNA) molecules, the method comprising:

(a) introducing a mixture of cells into a fluidic device comprising a solid support, wherein a cell of said mixture of cells comprises one or more nucleic acid molecules and wherein said solid support comprises a plurality of capture probes and a plurality of surface primer probes;

(b) incubating said plurality of capture probes with said one or more nucleic acid molecules of the cell of the mixture of cells to yield one or more captured nucleic acid molecules;

(c) synthesizing a cDNA molecule from said captured nucleic acid molecules, wherein said cDNA molecule is coupled to a surface primer probe of said plurality of surface primer probes;

(d) inserting an adapter at said 3' region of said cDNA molecule; and

(e) amplifying said cDNA molecule to generate said set of cDNA molecules, wherein said set of cDNA molecules is coupled to a surface primer probe of said plurality of surface primer probes.

[005] The disclosure is directed to methods and systems for generating libraries of cDNAs on surfaces. In some examples, the disclosure is directed to methods and systems for analyzing

CLAIMS

1. A method for preparing a set of complementary deoxynucleic acid (cDNA) molecules, the method comprising:

(a) introducing a mixture of cells into a fluidic device comprising a solid support, wherein a cell of said mixture of cells comprises one or more nucleic acid molecules and wherein said solid support comprises a plurality of capture probes and a plurality of surface primer probes;

(b) incubating said plurality of capture probes with said one or more nucleic acid molecules of the cell of the mixture of cells to yield one or more captured nucleic acid molecules;

(c) synthesizing a cDNA molecule from said captured nucleic acid molecules, wherein said cDNA molecule is coupled to a surface primer probe of said plurality of surface primer probes;

(d) inserting an adapter at said 3' region of said cDNA molecule; and

(e) amplifying said cDNA molecule to generate said set of cDNA molecules, wherein said set of cDNA molecules is coupled to a surface primer probe of said plurality of surface primer probes.

2. The method of claim 1, wherein said adapter comprises a sequence configured to permit initiation of a sequencing reaction on a cDNA molecule of said set of cDNA molecules.

3. The method of claim 1, further comprising, following (b), contacting said solid support with a moiety configured to inactivate at least a subset of said plurality of capture probes.

4. The method of claim 3, wherein said subset of said plurality of capture probes comprise one or more capture probes that did not capture a nucleic acid molecule.

5. The method of claim 3, wherein said moiety configured to inactivate at least said subset of said plurality of capture probes comprises an exonuclease.

6. The method of claim 1, wherein said synthesizing comprises performing one or more second strand synthesis reactions.

7. The method of claim 1, further comprising, prior to (d), amplifying said cDNA molecule, wherein said amplifying comprises using in-solution primer sequences.

8. The method of claim 1, further comprising, prior to (d), fragmenting said cDNA molecule, thereby obtaining one or more cDNA fragments of said cDNA molecule, and wherein said inserting of said adapter in (d) comprises ligating said adapter to a cDNA fragment of said one or more cDNA fragments.

9. The method of claim 1, wherein (d) comprises one of single-strand ligation, tagmentation, and double-strand ligation.

10. The method of claim 1, wherein at least a subset of said plurality of surface primer probes comprises a blocking agent that blocks an extension reaction on said at least said subset of said plurality of surface primer probes, and wherein said method further comprises prior to (e), subjecting said blocking agent to a reaction that unblocks said at least subset of said plurality of surface primer probes to permit said extension reaction.

11. The method of claim 1, further comprising, sequencing said set of cDNA molecules *in situ* on said solid support.

12. The method of claim 1, further comprising, eluting at least a subset of said set of cDNA molecules from said solid support.

13. The method of claim 1, wherein said solid support is not a bead.

14. The method of claim 1, wherein said solid support in (b) comprises a hydrogel chamber disposed thereon, wherein said hydrogel chamber comprises one or more polymer matrix walls, and wherein an interior of said hydrogel chamber comprises said cell of said mixture of cells.

15. The method of claim 14, wherein said one or more polymer matrix walls extend from said solid support to a top surface opposite of said solid support, thereby forming said interior of said hydrogel chamber.

16. The method of claim 15, wherein prior to (b), said one or more nucleic acid molecules are released from said cell.

17. The method of claim 15, wherein any of (b)-(e), or any combination thereof, occur in said interior of said hydrogel chamber.

18. The method of claim 14, further comprising, degrading said hydrogel chamber subsequent to (b) and prior to (c).

19. The method of claim 14, wherein a first hydrogel chamber comprises a first cell of said mixture of cells thereby separating said cell from said mixture of cells.

20. The method of claim 19, wherein said solid support in (b) comprises an additional hydrogel chamber disposed thereon, and wherein said additional hydrogel chamber comprises an additional cell of said mixture of cells thereby separating said additional cell from (i) said mixture of cells and (ii) said cell of said mixture of cells.