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 or derivatives thereof from one or more nucleic acid molecules, the method comprising:

(a) contacting said one or more nucleic acid molecule capture probes on a solid support with said one or more nucleic acid molecules to yield one or more captured nucleic acid molecules, wherein said solid support comprises a plurality of surface primer probes;

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

(c) inserting an adapter at the 3' region of said cDNA molecule or a derivative thereof; and

(d) amplifying said cDNA molecule or a derivative thereof to generate said set of cDNA molecules or derivates thereof, wherein said set of cDNA molecules or derivates thereof 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

nucleic acid molecules, such as messenger RNAs, from a plurality of cells on a surface of a solid support. In some examples, the disclosure is directed to methods for synthesizing cDNAs from nucleic acid molecules from a plurality of cells disposed on a surface of a solid support. In some examples, methods of the disclosure are directed to determining the transcriptomes of cells disposed on the surface of a solid support. In some examples, such methods may comprise (a) providing a solid support, wherein the solid support comprises on a surface one or more nucleic acid molecule capture probes and a plurality of surface primer probes; (b) contacting separate regions of the surface with the one or more nucleic acid molecules of different cells to yield one or more captured nucleic acid molecules in each of the separate regions, wherein captured nucleic acid molecules in different separate regions are from different cells; and (c) synthesizing cDNA molecules from the captured nucleic acid molecules or derivatives thereof, wherein each of the cDNA molecules is coupled to the surface of the solid support and wherein cDNA molecules coupled to different separate regions are from different cells. In some examples, cDNA molecules coupled to the surface comprises cDNA molecules covalently bonded to the surface. In some examples, such method further includes amplifying the cDNA molecules or derivatives thereof to generate a plurality of sets of amplicons of cDNA molecules or derivates thereof, wherein each set of amplicons is from a different cell. In some examples, transcriptomes of the plurality of cells are determined by sequencing the cDNA molecules of the amplicons. In some examples, separate regions of the surface of the solid support are determined by hydrogel chambers enclosing each of the plurality of cells. In some examples, the step of contacting comprises treating the cells with a lysing agent that releases the one or more nucleic acid molecules. In some examples, the one or more nucleic acid molecules are ribonucleic acids (RNAs). In some examples, the surface comprises a diffusivity modifier that reduces or blocks diffusion of the one or more nucleic acids away from the cells. In some examples, a diffusivity modifier comprises a gel barrier. In some examples, a gel barrier modifying diffusivity comprises a hydrogel chamber. In some examples, the surface of the solid support comprises cells disposed thereon. In some examples, the surface of the solid support comprises a diffusivity modifier. In variations of the above examples, the surface of the solid support is a planar surface. In some examples, systems and methods of the disclosure employ an optical system for collecting optical signal from labeled cells and/or molecules on the planar surface.

[006] Examples provided herein are a method for preparing a set of complementary deoxynucleic acid (cDNA) molecules or derivatives thereof from one or more nucleic acid molecules, the method comprising: providing a solid support, wherein the solid support comprises one or more

nucleic acid molecule capture probes and a plurality of surface primer probes; contacting the solid support with the one or more nucleic acid molecules to yield one or more captured nucleic acid molecules; synthesizing a cDNA molecule from the captured nucleic acid molecule or a derivative, wherein the cDNA molecule is coupled to the solid support; inserting an adapter at the 3' region of the cDNA molecule or a derivative thereof; and amplifying the cDNA molecule or a derivative thereof to generate the set of cDNA molecules or derivates thereof, wherein the set of cDNA molecules or derivates thereof is coupled to the solid support. In some examples, synthesizing comprises performing reverse transcription and one or more second strand synthesis reactions. In some examples, the set of cDNA molecules or derivates thereof is coupled to the plurality of surface primer probes. In some examples, the adapter comprises a sequence configured to permit initiation of a sequencing reaction on a cDNA molecule of the set of cDNA molecules or derivatives thereof. In some examples, the set of cDNA molecules or derivatives thereof comprise the adapter. In some examples, the method comprises, following the contacting of the solid support with the one or more nucleic acid molecules, contacting the solid support with a moiety configured to inactivate at least a subset of the one or more nucleic acid molecule capture probes. In some examples, the subset of the one or more nucleic acid molecule capture probes comprises one or more nucleic acid molecule capture probes that did not capture a nucleic acid molecule. In some examples, the moiety configured to inactivate at least the subset of the one or more nucleic acid molecule capture probes comprises an exonuclease. In some examples, the one or more second strand synthesis reactions comprise template switch extension, random priming, or both. In some examples, prior to inserting the adapter at the 3' region of the cDNA molecule, the method comprises amplifying the cDNA molecule or a derivative thereof. In some examples, prior to inserting the adapter at the 3' region of the cDNA molecule, the method comprises in-solution primer sequences. In some examples, the cDNA molecules are amplified using the in-solution primer sequences. In some examples, prior to inserting the adapter at the 3' region of the cDNA molecule, the method comprises fragmentation of the cDNA molecule or a derivative thereof. In some examples, the inserting of the adapter at the 3' region of the cDNA molecule comprises single-strand ligation. In some examples, the inserting of the adapter at the 3' region of the cDNA molecule comprises tagmentation. In some examples, the inserting of the adapter at the 3' region of the cDNA molecule comprises double-stranded ligation. In some examples, at least a subset of the plurality of surface primer probes comprises a blocking agent that blocks an extension reaction on the at least the subset of the plurality of surface primer probes. In some examples, prior to amplifying of the cDNA

molecule, the plurality of surface primer probes are subjected to the blocking agent to a reaction that unblocks the at least the subset of the plurality of surface primer probes to permit the extension reaction. In some examples, the one or more blocking agents comprise one or more 3' phosphate nucleotides. In some examples, the one or more blocking agents comprise a nucleic acid molecule comprising a sequence complementary to at least the subset of the plurality of surface primer probes. In some examples, the one or more blocking agents comprise a nucleic acid molecule comprising a sequence partially complementary to at least the subset of the plurality of surface primer probes, a reversible terminator nucleotide and a polymerase, or any derivatives thereof. In some examples, the method comprises cleaving or linearizing at least a subset of the set of cDNA molecules or derivatives thereof. In some examples, the method comprises blocking the 3' end of the subset of the set of DNA molecules or derivatives thereof. In some examples, the blocking of the 3' end of the subset of the set of DNA molecules or derivatives thereof comprises contacting the subset of the set of DNA molecules or derivatives thereof with terminal deoxynucleotidyl transferase (TdT). In some examples, the blocking of the 3' end of the subset of the set of DNA molecules or derivatives thereof comprises contacting the subset of the set of DNA molecules or derivatives thereof with an oligonucleotide comprising a sequence complementary to the 3' end of the subset of the set of DNA molecules. In some examples, the blocking of the 3' end of the subset of the set of DNA molecules or derivatives thereof comprises contacting the subset of the set of DNA molecules or derivatives thereof with a cationic-neutral diblock polypeptide copolymer. In some examples, the method comprises sequencing a subset of the cDNA molecules or derivatives thereof *in situ* on the solid support. In some examples, the method comprises eluting at least a subset of the set of cDNA molecules or derivatives thereof from the solid support. In some examples, the one or more nucleic acid molecules comprise DNA or ribonucleic nucleic acid (RNA) molecules. In some examples, the DNA is fragmented and single-stranded DNA. In some examples, the DNA is single-stranded DNA. In some examples, the RNA molecules comprise messenger RNA (mRNA) or microRNA (miRNA). In some examples, the RNA molecules comprise mRNA. In some examples, the one or more nucleic acid molecule capture probes comprise a sequence configured to couple to the one or more nucleic acid molecules. In some examples, the sequence configured to couple to the one or more nucleic acid molecules comprises a poly-T sequence, a randomer, a sequence complementary to at least a subset of the one or more nucleic acid molecules, or any combination thereof. In some examples, the solid support may comprise a well, a bead, a gel matrix or a fluidic channel. In some examples, the one or more nucleic acid molecule capture probes and the plurality of

surface primer probes are attached to a planar surface of the solid support. In some examples, the fluidic channel comprises a flow cell. In some examples, the one or more nucleic acid molecule capture probes comprise one or more tags, wherein a tag comprises a cell-specific or spatial location-specific identifier sequence and optionally a unique molecular identifier (UMI) sequence. In some examples, the amplifying comprises solid-supported amplification. In some examples, the solid-supported amplification is bridge amplification. In some examples, the one or more nucleic acid molecules are derived from a single cell or biological tissue. In some examples, the method occurs in a gel matrix, wherein the gel matrix is adjacent to the solid support.

[007] Examples provide herein are a method for preparing a set of complementary deoxynucleic acid (cDNA) molecules or derivatives thereof from one or more nucleic acid molecules, the method comprising: providing a solid support, wherein the solid support comprises one or more nucleic acid molecule capture probes; contacting the solid support with the one or more nucleic acid molecules to yield one or more captured nucleic acid molecules; synthesizing a cDNA molecule from the captured nucleic acid molecule or a derivative; first amplifying the cDNA molecule or a derivative thereof to generate an amplified cDNA population; inserting an adapter at the 3' region of the amplified cDNA molecule or a derivative thereof, thereby generating a tagged amplified cDNA population; and performing solid-supported amplification on the tagged amplified cDNA population to generate the set of cDNA molecules or derivatives thereof. In some examples, synthesizing comprises performing reverse transcription of a captured RNA template. In some examples, the solid support comprises a plurality of surface primer probes. In some examples, the cDNA molecule or the derivative thereof, the amplified cDNA population, the tagged amplified cDNA population, the set of cDNA molecules or derivates thereof, or any combination thereof is coupled to the plurality of surface primer probes. In some examples, the adapter comprises a sequence configured to permit initiation of a sequencing reaction on a cDNA molecule of the set of cDNA molecules or derivatives thereof. In some examples, the set of cDNA molecules or derivatives thereof comprise the adapter. In some examples, the method comprises contacting the solid support with a moiety configured to inactivate at least a subset of the one or more nucleic acid molecule capture probes. In some examples, the subset of the one or more nucleic acid molecule capture probes comprise one or more nucleic acid molecule capture probes that did not capture a nucleic acid molecule. In some examples, the moiety configured to inactivate at least the subset of the one or more nucleic acid molecule capture probes comprises an exonuclease. In some examples, the synthesizing comprises performing one or more second strand synthesis reactions comprising the

cDNA molecule or a derivative thereof. In some examples, the one or more second strand synthesis reactions comprise template switch extension. In some examples, the one or more second strand synthesis reactions comprise random priming. In some examples, the method comprises fragmentation of the amplified cDNA molecule. In some examples, the inserting of the adapter comprises single-strand ligation. In some examples, the inserting of the adapter comprises tagmentation. In some examples, the inserting of the adapter comprises double-strand ligation. In some examples, at least a subset of the plurality of surface primer probes comprises a blocking agent that blocks an extension reaction on the at least the subset of the plurality of surface primer probes. In some examples, the method comprises subjecting the blocking agent to a reaction that unblocks at least a subset of the plurality of surface primed probes to permit the extension reaction. In some examples, the one or more blocking agents comprise one or more 3' phosphate nucleotides. In some examples, the one or more blocking agents comprise a nucleic acid molecule comprising a sequence complementary to at least the subset of the plurality of surface primer probes. In some examples, the one or more blocking agents comprise a nucleic acid molecule comprising a sequence partially complementary to at least the subset of the plurality of surface primer probes, a reversible terminator nucleotide and a polymerase, or any derivatives thereof. In some examples, the method comprises cleaving or linearizing at least a subset of the set of cDNA molecules or derivatives thereof. In some examples, the method comprises blocking the 3' end of the subset of the set of DNA molecules or derivatives thereof. In some examples, the blocking of the 3' end of the subset of the set of DNA molecules or derivatives thereof comprises contacting the subset of the set of DNA molecules or derivatives thereof with terminal deoxynucleotidyl transferase (TdT). In some examples, the blocking of the 3' end of the subset of the set of DNA molecules or derivatives thereof comprises contacting the subset of the set of DNA molecules or derivatives thereof with an oligonucleotide comprising a sequence complementary to the 3' end of the subset of the set of DNA molecules. In some examples, the blocking of the 3' end of the subset of the set of DNA molecules or derivatives thereof comprises contacting the subset of the set of DNA molecules or derivatives thereof with a cationic-neutral diblock polypeptide copolymer. In some examples, the method comprises sequencing the at least the subset of the cDNA molecules or derivatives thereof *in situ* on the solid support. In some examples, the method comprises eluting at least a subset of the set of cDNA molecules or derivatives thereof from the solid support. In some examples, the one or more nucleic acid molecules comprise DNA or ribonucleic nucleic acid (RNA) molecules. In some examples, the DNA is fragmented and single-stranded DNA. In some examples, the DNA is single-stranded DNA. In some

examples, the RNA molecules comprise messenger RNA (mRNA) or microRNA (miRNA). In some examples, the RNA molecules comprise mRNA. In some examples, the one or more nucleic acid molecule capture probes comprise a sequence configured to couple to the one or more nucleic acid molecules. In some examples, the sequence configured to couple to the one or more nucleic acid molecules comprises a poly-T sequence, a randomer, a sequence complementary to at least a subset of the one or more nucleic acid molecules, or any combination thereof. In some examples, the solid support comprises a well, a bead, a gel matrix or a fluidic channel. In some examples, the fluidic channel is a flow cell. In some examples, the solid support is not a bead. In some examples, the one or more nucleic acid molecule capture probes comprise one or more tags, wherein a tag comprises a cell-specific or spatial location-specific identifier sequence and optionally a unique molecular identifier (UMI) sequence. In some examples, the first amplifying comprises solid-supported amplification. In some examples, the first amplifying comprises in-solution primer sequences. In some examples, the solid-supported amplification is bridge amplification. In some examples, the one or more nucleic acid molecules are derived from a single cell or biological tissue. In some examples, the method occurs in a gel matrix, wherein the gel matrix is adjacent to the solid support.

[008] Examples provided herein are a method for preparing a set of complementary deoxynucleic acid (cDNA) molecules or derivatives thereof from one or more nucleic acid molecules, the method comprising: providing a solid support, wherein the solid support comprises one or more nucleic acid molecule capture probes and a plurality of surface primer probes, wherein at least a subset of the plurality of surface primer probes comprise a template switch moiety; contacting the solid support with the one or more nucleic acid molecules to yield one or more captured nucleic acid molecule; synthesizing a cDNA molecule from the captured nucleic acid molecule or a derivative, wherein the synthesizing comprises performing reverse transcription; inserting an adapter at the 3’ end of the cDNA molecule or a derivative thereof; and amplifying the cDNA molecule or a derivative thereof to generate the set of cDNA molecules or derivates thereof. In some examples, the synthesizing comprises performing one or more second strand synthesis reactions comprising the cDNA molecule or a derivative thereof. In some examples, the one or more second strand synthesis reactions are mediated by the subset of the plurality of surface primer probes comprising the template switch moiety. In some examples, the one or more second strand synthesis reactions comprise template switch extension. In some examples, the cDNA molecule or the derivative thereof, the set of cDNA molecules or derivates thereof, or both is coupled to the plurality of surface primer probes. In some examples, the adapter

comprises a sequence configured to permit initiation of a sequencing reaction on a cDNA molecule of the set of cDNA molecules or derivatives thereof. In some examples, the set of cDNA molecules or derivatives thereof comprise the adapter. In some examples, the method comprises contacting the solid support with a moiety configured to inactivate at least a subset of the one or more nucleic acid molecule capture probes. In some examples, the subset of the one or more nucleic acid molecule capture probes comprise one or more nucleic acid molecule capture probes that did not capture a nucleic acid molecule. In some examples, the moiety configured to inactivate at least the subset of the one or more nucleic acid molecule capture probes comprises an exonuclease. In some examples, the method comprises amplifying the cDNA molecule or a derivative thereof. In some examples, the amplifying comprises in-solution primer sequences. In some examples, the inserting of the adapter comprises fragmentation of the cDNA molecule. In some examples, the inserting of the adapter comprises single-strand ligation. In some examples, the inserting of the adapter comprises tagmentation. In some examples, the inserting of the adapter comprises ligation. In some examples, at least a subset of the plurality of surface primer probes comprises a blocking agent that blocks an extension reaction on the at least the subset of the plurality of surface primer probes. In some examples, the method comprises subjecting the blocking agent to a reaction that unblocks the at least the subset of the plurality of surface primed probes to permit the extension reaction. In some examples, the one or more blocking agents comprise one or more 3’ phosphate nucleotides. In some examples, the one or more blocking agents comprise a nucleic acid molecule comprising a sequence complementary to at least the subset of the plurality of surface primer probes. In some examples, the one or more blocking agents comprise a nucleic acid molecule comprising a sequence partially complementary to at least the subset of the plurality of surface primer probes, a reversible terminator nucleotide and a polymerase, or any derivatives thereof. In some examples, the method comprises cleaving or linearizing at least a subset of the set of cDNA molecules or derivatives thereof. In some examples, the method comprises blocking the 3’ end of the subset of the set of DNA molecules or derivatives thereof. In some examples, the blocking of the 3’ end of the subset of the set of DNA molecules or derivatives thereof comprises contacting the subset of the set of DNA molecules or derivatives thereof with terminal deoxynucleotidyl transferase (TdT). In some examples, the blocking of the 3’ end of the subset of the set of DNA molecules or derivatives thereof comprises contacting the subset of the set of DNA molecules or derivatives thereof with an oligonucleotide comprising a sequence complementary to the 3’ end of the subset of the set of DNA molecules. In some examples, the blocking of the 3’ end of the subset of the set of

DNA molecules or derivatives thereof comprises contacting the subset of the set of DNA molecules or derivatives thereof with a cationic-neutral diblock polypeptide copolymer. In some examples, the method comprises sequencing the at least the subset of the cDNA molecules or derivatives thereof *in situ* on the solid support. In some examples, the method comprises eluting at least a subset of the set of cDNA molecules or derivatives thereof from the solid support. In some examples, the one or more nucleic acid molecules comprise DNA or ribonucleic nucleic acid (RNA) molecules. In some examples, the DNA is fragmented and single-stranded DNA. In some examples, the DNA is single-stranded DNA. In some examples, the RNA molecules comprise messenger RNA (mRNA) or microRNA (miRNA). In some examples, the RNA molecules comprise mRNA. In some examples, the one or more nucleic acid molecule capture probes comprise a sequence configured to couple to the one or more nucleic acid molecules. In some examples, the sequence configured to couple to the one or more nucleic acid molecules comprises a poly-T sequence, a randomer, a sequence complementary to at least a subset of the one or more nucleic acid molecules, or any combination thereof. In some examples, the solid support comprises a well, a bead, a gel matrix or a fluidic channel. In some examples, the fluidic channel is a flow cell. In some examples, the one or more nucleic acid molecule capture probes comprise one or more tags, wherein a tag comprises a cell-specific or spatial location-specific identifier sequence and optionally a unique molecular identifier (UMI) sequence. In some examples, the amplifying comprises solid-supported amplification. In some examples, the solid-supported amplification is bridge amplification. In some examples, the one or more nucleic acid molecules are derived from a single cell or biological tissue. In some examples, the method occurs in or adjacent to a gel matrix, wherein the gel matrix is adjacent to the solid support.

[009] Examples provided herein is a solid support comprising one or more nucleic acid molecule capture probes and a plurality of surface primer probes, wherein at least a subset of the plurality of surface primer probes comprise a template switch moiety. In some examples, the one or more nucleic acid molecule capture probes comprise a sequence configured to couple to one or more nucleic acid molecules. In some examples, the sequence configured to couple to the one or more nucleic acid molecules comprises a poly-T sequence, a randomer, a sequence complementary to at least a subset of the one or more nucleic acid molecules, or any combination thereof. In some examples, the solid support comprises a well, a bead, a gel matrix or a fluidic channel. In some examples, the fluidic channel is a flow cell. In some examples, the one or more nucleic acid molecule capture probes comprise one or more tags, wherein a tag comprises a cell-specific or spatial location-specific identifier sequence and

optionally a unique molecular identifier (UMI) sequence. In some examples, the one or more nucleic acid molecules comprise DNA or ribonucleic nucleic acid (RNA) molecules. In some examples, the DNA is fragmented. In some examples, the DNA is single-stranded DNA. In some examples, the RNA molecules comprise messenger RNA (mRNA) or microRNA (miRNA). In some examples, the RNA molecules comprise mRNA. In some examples, the solid support comprises a gel matrix, wherein the gel matrix is adjacent to the solid support.

[0010] Examples provided herein are a method for preparing a set of complementary deoxynucleic acid (cDNA) molecules or derivatives thereof from one or more nucleic acid molecules, the method comprising: providing a solid support, wherein the solid support comprises one or more nucleic acid molecule capture probes and a plurality of surface primer probes; contacting the solid support with the one or more nucleic acid molecules to yield one or more captured nucleic acid molecules; synthesizing a cDNA molecule from the captured nucleic acid molecule or a derivative, wherein the synthesizing comprises performing reverse transcription, and wherein the cDNA molecule is coupled to the solid support; inserting an adapter at the 3’ end of the cDNA molecule or a derivative thereof; and amplifying the cDNA molecule or a derivative thereof to generate the set of cDNA molecules or derivates thereof, wherein the set of cDNA molecules or derivates thereof is coupled to the solid support. In some examples, the set of cDNA molecules or derivates thereof is coupled to the plurality of surface primer probes. In some examples, the adapter comprises a sequence configured to permit initiation of a sequencing reaction on a cDNA molecule of the set of cDNA molecules or derivatives thereof. In some examples, the set of cDNA molecules or derivatives thereof comprise the adapter. In some examples, the method comprises contacting the solid support with a moiety configured to inactivate at least a subset of the one or more nucleic acid molecule capture probes. In some examples, the subset of the one or more nucleic acid molecule capture probes comprise one or more nucleic acid molecule capture probes that did not capture a nucleic acid molecule. In some examples, the moiety configured to inactivate at least the subset of the one or more nucleic acid molecule capture probes comprises an exonuclease. In some examples, the synthesizing comprises performing one or more second strand synthesis reactions comprising the cDNA molecule or a derivative thereof. In some examples, the one or more second strand synthesis reactions comprise template switch extension. In some examples, the one or more second strand synthesis reactions comprise random priming. In some examples, the method comprises amplifying the cDNA molecule or a derivative thereof. In some examples, the amplifying comprises in-solution primer sequences. In some examples, the inserting of the adapter comprises fragmentation of the cDNA molecule. In some examples, the inserting of

the adapter comprises single-strand ligation. In some examples, the inserting of the adapter comprises tagmentation. In some examples, the inserting of the adapter comprises ligation. In some examples, at least a subset of the plurality of surface primer probes comprises a blocking agent that blocks an extension reaction on the at least the subset of the plurality of surface primer probes. In some examples, the method comprises subjecting the blocking agent to a reaction that unblocks the at least the subset of the plurality of surface primed probes to permit the extension reaction. In some examples, the one or more blocking agents comprise one or more 3' phosphate nucleotides. In some examples, the one or more blocking agents comprise a nucleic acid molecule comprising a sequence complementary to at least the subset of the plurality of surface primer probes. In some examples, the one or more blocking agents comprise a nucleic acid molecule comprising a sequence partially complementary to at least the subset of the plurality of surface primer probes, a reversible terminator nucleotide and a polymerase, or any derivatives thereof. In some examples, the method comprises cleaving or linearizing at least a subset of the set of cDNA molecules or derivatives thereof. In some examples, the method comprises blocking the 3' end of the subset of the set of DNA molecules or derivatives thereof. In some examples, the blocking of the 3' end of the subset of the set of DNA molecules or derivatives thereof comprises contacting the subset of the set of DNA molecules or derivatives thereof with terminal deoxynucleotidyl transferase (TdT). In some examples, the blocking of the 3' end of the subset of the set of DNA molecules or derivatives thereof comprises contacting the subset of the set of DNA molecules or derivatives thereof with an oligonucleotide comprising a sequence complementary to the 3' end of the subset of the set of DNA molecules. In some examples, the blocking of the 3' end of the subset of the set of DNA molecules or derivatives thereof comprises contacting the subset of the set of DNA molecules or derivatives thereof with a cationic-neutral diblock polypeptide copolymer. In some examples, the method comprises sequencing the at least the subset of the cDNA molecules or derivatives thereof *in situ* on the solid support. In some examples, the method comprises eluting at least a subset of the set of cDNA molecules or derivatives thereof from the solid support. In some examples, the one or more nucleic acid molecules comprise DNA or ribonucleic nucleic acid (RNA) molecules. In some examples, the DNA is fragmented and single-stranded DNA. In some examples, the DNA is single-stranded DNA. In some examples, the RNA molecules comprise messenger RNA (mRNA) or microRNA (miRNA). In some examples, the RNA molecules comprise mRNA. In some examples, the one or more nucleic acid molecule capture probes comprise a sequence configured to couple to the one or more nucleic acid molecules. In some examples, the sequence configured to couple to the

one or more nucleic acid molecules comprises a poly-T sequence, a randomer, a sequence complementary to at least a subset of the one or more nucleic acid molecules, or any combination thereof. In some examples, the solid support comprises a well, a bead, a gel matrix or a fluidic channel. In some examples, the fluidic channel is a flow cell. In some examples, the solid support is not a bead. In some examples, the one or more nucleic acid molecule capture probes comprise one or more tags, wherein a tag comprises a cell-specific or spatial location-specific identifier sequence and optionally a unique molecular identifier (UMI) sequence. In some examples, the amplifying comprises solid-supported amplification. In some examples, the solid-supported amplification is bridge amplification. In some examples, the one or more nucleic acid molecules are derived from a single cell or biological tissue. In some examples, the method occurs in a gel matrix, wherein the gel matrix is adjacent to the solid support.

[0011] Examples provided herein include a method for preparing sets of complementary deoxynucleic acid (cDNA) molecules or derivatives thereof from one or more nucleic acid molecules from a plurality of cells, the method comprising: providing a solid support comprising a surface comprising one or more nucleic acid molecule capture probes and a plurality of surface primer probes attached thereto and comprising a plurality of cells disposed thereon; contacting separate regions of the surface with the one or more nucleic acid molecules of different cells of the plurality of cells to yield one or more captured nucleic acid molecules in each of the separate regions, wherein nucleic acid molecules in different separate regions are from different cells of the plurality of cells; and synthesizing cDNA molecules from the captured nucleic acid molecules or derivatives thereof, wherein each of the cDNA molecules is coupled to a surface primer probe of the plurality of surface primer probes and cDNAs coupled to different separate areas are from different cells of the plurality of cells. In some examples, the contacting comprises treating the different cells of the plurality of cells with a lysing reagent to release the one or more nucleic acid molecules from the different cells of the plurality of cells. In some examples, the method further comprises amplifying the cDNA molecules or derivatives thereof to generate a plurality of sets of amplicons of cDNA molecules or derivates thereof. In some examples, transcriptomes of the plurality of cells are determined by sequencing the cDNA molecules of the amplicons. In some examples, the cDNA molecules attached to the surface comprise spatial barcodes that encode positions on the surface and further comprising eluting the cDNA molecules from the surface prior to the sequencing. In some examples, the contacting the one or more nucleic acid molecules is carried out in the presence of a diffusivity modifier that reduces diffusivities of the one or more nucleic acid molecules. In some

examples, the surface comprises a gel layer encapsulating the cells disposed thereon or wherein each of the cells disposed on the surface are encapsulated by a separate gel body. In some examples, each of the cells disposed on the surface are enclosed by a hydrogel chamber. In some examples, the hydrogel chamber comprises an interior area and wherein the contacting further comprises incubating the released one or more nucleic acid molecules at a predetermined temperature so that captured one or more nucleic acid molecules are released and re-captured by capture probes within the interior area. In some examples, the interior area of the hydrogel chamber is selected so that the coupled cDNA molecules have an expected nearest neighbor distance of at least 0.25 μm. In some examples, the interior area of the hydrogel chamber is selected so that the coupled cDNA molecules have an expected nearest neighbor distance of at least 1 μm. In some examples, the interior area of the hydrogel chamber is selected so that the coupled cDNA molecules have an expected nearest neighbor distance of at least 2 μm. In some examples, the coupled cDNA molecules have an expected nearest neighbor distance in the range of from 0.5 μm and 5 μm.

[0012] Examples provided herein include a hydrogel chamber disposed on a surface wherein the hydrogel chamber comprises an interior area comprising a substantially uniform distribution of nucleic acid molecules from a single cell. In some examples, the nucleic acid molecules are mRNA molecules. In some examples, the substantially uniform distribution is a Poisson distribution having an expected nearest neighbor distance between the nucleic acid molecules of 1 μm or greater. In some examples, the uniform distribution of the nucleic acid molecules is substantially a Poisson distribution.

[0013] Examples provide herein include a method for preparing a set of complementary deoxynucleic acid (cDNA) molecules or derivatives thereof from one or more nucleic acid molecules, the method comprising: providing a solid support comprising a surface, wherein the surface is not partitioned, and wherein the solid support comprises one or more nucleic acid molecule capture probes and a plurality of surface primer probes; generating a discrete region on the solid support, wherein the discrete region comprises one or more cells unique to the discrete region; extracting one or more ribonucleic acid ("RNA") molecules from the one or more cells, wherein the one or more RNA molecules are captured by one or more nucleic acid molecule capture probes located in the discrete region, thereby generating one or more captured RNA molecules unique to the discrete region; synthesizing a cDNA molecule from the one or more captured RNA molecules or a derivative thereof, wherein the cDNA molecule is coupled to a surface primer probe of the plurality of surface primer probes located in the discrete region; inserting an adapter at the 3' region of the cDNA molecule or a derivative thereof; and

amplifying the cDNA molecule or a derivative thereof to generate the set of cDNA molecules or derivates thereof, wherein the set of cDNA molecules or derivates thereof is coupled to the solid support. In some examples, the discrete region is encompassed by a polymer matrix. In some examples, each discrete region comprises a single cell. In some examples, the method occurs within the polymer matrix. In some examples, the polymer matrix forms a hydrogel. In some examples, the polymer matrix is formed from one or more polymer precursors. In some examples, the polymer matrix comprises pores that are sized to allow diffusion of a reagent through the polymer matrix, wherein the RNA molecule cannot diffuse through the pores of the polymer matrix. In some examples, the solid support comprises one or more discrete regions, and wherein the one or more discrete regions are not in fluidic communication with another discrete region. In some examples, the solid support is not a bead. In some examples, a surface that is not partitioned comprises a planar surface wherein each point or location on the planar surface is in fluid communication with every other point or location on the planar surface.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] This patent application contains at least one drawing executed in color. Copies of this patent or patent application with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0015] FIG. 1A illustrates a diagram for current techniques for generating a transcriptome, in accordance with some embodiments.

[0016] FIG. 1B illustrates a workflow for current techniques for generating a transcriptome, in accordance with some embodiments.

[0017] FIG. 2A illustrates a workflow for obtaining an improved transcriptome from *in situ* and direct generation of sequence-able library from captured mRNA. A surface coating of first and second primers (such as, P5 and P7 primers) is used to generate the library directly on the surface. The amplification step can potentially be done using bridge amplification but could be done through in solution primers. The surface clusters thus generated can either be directly sequenced *in situ* or the library can be eluted off of surface for sequencing separately.

[0018] FIG. 2B illustrates a problem addressed by one embodiment of the invention which relates to analyzing nucleic acid molecules released by adjacent cells on a planar surface.

[0019] FIG. 2C illustrates an embodiment for addressing the problem of FIG. 2B using a diffusivity modifier.

[0020] FIG. 2D illustrates an embodiment wherein a diffusivity modifier is a gel layer encapsulating cells disposed on a planar surface.

CLAIMS

1. A method for preparing a set of complementary deoxynucleic acid (cDNA) molecules or derivatives thereof from one or more nucleic acid molecules, the method comprising:

(a) contacting said one or more nucleic acid molecule capture probes on a solid support with said one or more nucleic acid molecules to yield one or more captured nucleic acid molecules, wherein said solid support comprises a plurality of surface primer probes;

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

(c) inserting an adapter at the 3' region of said cDNA molecule or a derivative thereof; and

(d) amplifying said cDNA molecule or a derivative thereof to generate said set of cDNA molecules or derivates thereof, wherein said set of cDNA molecules or derivates thereof 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 or derivatives thereof.

3. The method of claim 1, comprising, following (a), contacting said solid support with a moiety configured to inactivate at least a subset of said one or more nucleic acid molecule capture probes.

4. The method of claim 3, wherein said subset of said one or more nucleic acid molecule capture probes comprise one or more nucleic acid molecule 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 one or more nucleic acid molecule capture probes comprises an exonuclease.

6. The method of claim 1, wherein said synthesizing comprises performing one or more second strand synthesis reactions comprising said cDNA molecule or a derivative thereof.

7. The method of claim 1, comprising, prior to (c), amplifying said cDNA molecule or a derivative thereof, wherein said amplifying comprises in-solution primer sequences.

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

9. The method of claim 1, wherein said inserting of said adapter at said 3’ region of said cDNA molecule or a derivative thereof in (c) 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 the method further comprises prior to (d), subjecting said blocking agent to a reaction that unblocks said at least said subset of said plurality of surface primed probes to permit said extension reaction.

11. The method of claim 1, comprising sequencing said at least said subset of said cDNA molecules or derivatives thereof *in situ* on said solid support.

12. The method of claim 1, comprising eluting at least a subset of said set of cDNA molecules or derivatives thereof 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 comprises a hydrogel chamber disposed thereon, wherein the hydrogel chamber comprises one or more polymer matrix walls.

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 an interior of said hydrogel chamber.

16. The method of claim 15, wherein said one or more nucleic acids are derived from a single cell, and wherein prior to (a), said interior of said hydrogel chamber comprises said single cell.

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

18. The method of claim of claim 16, wherein prior to (a), a lysis reagent is introduced to said fluidic device, thereby causing said one or more nucleic acid molecules to be released from said single cell.

19. The method of claim 15, wherein any of (a)-(d), or any combination thereof, occur in said interior of said hydrogel chamber.

20. The method of claim 14, further comprising degrading the hydrogel chamber subsequent to (a) and prior to (b).