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Immobilization in flow cells

Abstract

In an example, a target material is immobilized on two opposed sequencing surfaces of a flow cell using first and second fluids. The first fluid has a density less than a target material density and the second fluid has a density greater than the target material density; or the second fluid has a density less than the target material density and the first fluid has a density greater than the target material density. The first fluid (including the target material) is introduced into the flow cell, whereby at least some of the target material becomes immobilized by capture sites on one of the sequencing surfaces. The first fluid and non-immobilized target material are removed. The second fluid (including target material) is introduced into the flow cell, whereby at least some of the target material becomes immobilized by capture sites on another of the sequencing surfaces.

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Background/Summary

CROSS-REFERENCE TO RELATED APPLICATION (1) This application claims the benefit of U.S. Provisional Application Ser. No. 62/946,717, filed Dec. 11, 2019, the contents of which is incorporated by reference herein in its entirety.

BACKGROUND
(1) Flow cells are used in a variety of methods and applications, such as gene sequencing, genotyping, etc. In some methods and applications, it is desirable to generate a library of fragmented and tagged DNA molecules from double-stranded DNA (dsDNA) target molecules. Often, the purpose is to generate smaller DNA molecules (e.g., DNA fragments) from larger dsDNA molecules for use as templates in DNA sequencing reactions. The templates may enable short read lengths to be obtained. During data analysis, overlapping short sequence reads can be aligned to reconstruct the longer nucleic acid sequences. In some instances, pre-sequencing steps (such as barcoding of particular nucleic acid molecules) can be used to simplify the data analysis.
SUMMARY
(2) Some of the example kits and methods set forth herein are suitable for immobilizing one or more target materials on opposed surfaces of a flow cell. Some examples of the method enable sequential immobilization, and other examples of the method enable simultaneous immobilization.
(3) A first aspect disclosed herein is a method comprising immobilizing a target material at each of two opposed sequencing surfaces of a flow cell, wherein the immobilizing involves: introducing a first fluid, including a first portion of the target material therein, into the flow cell, whereby at least some of the target material becomes immobilized by capture sites on one of the two opposed sequencing surfaces; removing the first fluid and any non-immobilized target material from the flow cell; and introducing a second fluid, including a second portion of the target material therein, into the flow cell, whereby at least some of the target material becomes immobilized by capture sites on another of the two opposed sequencing surfaces; wherein one of: the first fluid has a density less than a density of the target material and the second fluid has a density greater than the density of the target material; or the second fluid has the density less than the density of the target material and the first fluid has the density greater than the density of the target material.
(4) A second aspect disclosed herein is a kit, comprising a preparation fluid including a target material therein; a first introduction fluid having a density less than a density of the target material; and a second introduction fluid having a density greater than the density of the target material.
(5) A third aspect disclosed herein is a method comprising immobilizing a target material at each of two opposed sequencing surfaces of a flow cell by: introducing a fluid, including the target material, into the flow cell, wherein: the target material includes: a magnetic solid support; and sequencing-ready nucleic acid fragments or template strands attached to the magnetic solid support; and the fluid has a density at least approximately equivalent to a density of the magnetic solid support; allowing some of the target material to become immobilized by capture sites on one of the two opposed sequencing surfaces; and applying a magnetic force to another of the two opposed sequencing surfaces, thereby pulling some other of the target material to the other of the two

opposed sequencing surfaces where they become immobilized by capture sites on the other of the two opposed sequencing surfaces.
(6) A fourth aspect disclosed herein is a method comprising simultaneously immobilizing first target materials at a first of two opposed sequencing surfaces of a flow cell and second target materials at a second of the two opposed sequencing surfaces by introducing, into the flow cell, a target fluid including the first target materials and the second target materials, wherein: a carrier fluid of the target fluid has a fluid density; the first target material has a first density less than the fluid density; and the second target material has a second density greater than the fluid density.
(7) A fifth aspect disclosed herein is a target fluid, comprising a carrier fluid having a fluid density; a first target material having a first density less than the fluid density; and a second target material having a second density greater than the fluid density.
(8) A sixth aspect disclosed herein is a method comprising introducing first and second target materials to a flow cell including two opposed sequencing surfaces, wherein the first target material has at least one property that is different from the second target material, wherein the at least one property is selected from the group consisting of density, charge, magnetism, and combinations thereof; and exposing the first and second target materials to at least one condition, thereby causing the first target material to become immobilized by a capture site on a first of the two opposed sequencing surfaces and the second target material to become immobilized by a capture site on a second of the two opposed sequencing surfaces.
(9) It is to be understood that any features of the any one of the aspects may be combined together in any desirable manner. Moreover, it is to be understood that any combination of features of the first aspect and/or of the second aspect and/or of the third aspect and/or of the fourth aspect and/or of the fifth aspect and/or of the sixth aspect may be combined with any of the examples disclosed herein to achieve the benefits as described in this disclosure, including, for example, a more uniform distribution of target material across sequencing surfaces in a flow cell.
(10) Another example set forth herein is suitable for reducing or preventing migration of template strands during on flow cell amplification.
(11) As such, a seventh aspect disclosed herein is a method comprising introducing sequencing-ready nucleic acid fragments to a flow cell, thereby seeding at least some of the sequencing-ready nucleic acid fragments to respective primers on a sequencing surface of the flow cell; removing non-seeded sequencing-ready nucleic acid fragments from the flow cell; introducing an amplification mix including a liquid form of a temperature responsive material to the flow cell; causing the liquid form of the temperature responsive material to gel; initiating amplification of the seeded sequencing-ready nucleic acid fragments to generate template strands, whereby the gel form of the temperature responsive material reduces diffusion of the template strands; causing the gel form of the temperature responsive material to liquify; and removing the liquid form of the temperature responsive material from the flow cell.
(12) It is to be understood that any features of the seventh aspect may be combined together in any desirable manner. Moreover, it is to be understood that any combination of features of the seventh aspect may be combined with any of the other aspects and/or any of the examples disclosed herein to achieve the benefits as described in this disclosure, including, for example, a more uniform distribution of target material across sequencing surfaces in a flow cell and reduced migration of template strands during on flow cell amplification.

Description

BRIEF DESCRIPTION OF THE DRAWINGS
(1) Features of examples of the present disclosure will become apparent by reference to the following detailed description and drawings, in which like reference numerals correspond to

similar, though perhaps not identical, components. For the sake of brevity, reference numerals or features having a previously described function may or may not be described in connection with other drawings in which they appear.

(2) FIGS. **1**A through **1**C are schematic illustrations of different examples of the target materials disclosed herein;

(3) FIG. **2**A is a top view of an example of a flow cell;

(4) FIG. **2**B is an enlarged, cross-sectional view, taken along the **2**B-**2**B line of FIG. **2**A, of an example of a flow channel and non-patterned sequencing surfaces;

(5) FIG. **2**C is an enlarged, cross-sectional view, taken along the **2**C-**2**C line of FIG. **2**A, of an example of a flow channel and patterned sequencing surfaces;

(6) FIG. **2**D is an enlarged, cross-sectional view, taken along the **2**D-**2**D line of FIG. **2**A, of another example of a flow channel and patterned sequencing surfaces;

(7) FIGS. **3**A and **3**B together depict one example of a method disclosed herein;

(8) FIGS. **4**A and **4**B together depict another example of a method disclosed herein;

(9) FIGS. **5**A and **5**B together depict still another example of a method disclosed herein;

(10) FIGS. **6**A and **6**B together depict yet another example of a method disclosed herein;

(11) FIGS. **7**A and **7**B together depict an additional example of a method disclosed herein;

(12) FIGS. **8**A and **8**B together depict still another example of a method disclosed herein;

(13) FIG. **9**A through FIG. **9**C together depict an example of a method for reducing diffusion and convection of template strands during amplification;

(14) FIGS. **10**A and **10**B are brightfield images of complexes immobilized on a top sequencing surface (FIG. **10**A) and a bottom sequencing surface (FIG. **10**B) of a flow cell including patterned sequencing surfaces;

(15) FIG. **11**A is a molecular coverage histogram for the top and bottom sequencing surfaces of one lane of a flow cell after sequencing was performed;

(16) FIG. **11**B is a graph depicting the percentage of Qscores greater than Q30 (Y axis) versus sequencing cycle number (X axis) for the top and bottom sequencing surfaces of the one lane after sequencing was performed;

(17) FIGS. **12**A and **12**B are bar graphs depicting the complex loading (number of beads/mm.sup.2, Y axis) on the bottom surfaces (FIG. **12**A) and top surfaces (FIG. **12**B) of flow cells treated with different concentrations (μM, X axis) of alkyne-biotin, where complex loading was performed using two different introduction liquids;

(18) FIGS. **13**A and **13**B are graphs depicting the target complex loading and the actual the complex loading (number of beads/mm.sup.2, Y axis) on a bottom surface and a top surface along the length (X axis) of two different flow cell channels; and

(19) FIG. **14** is a graph depicting the target/expected complex loading, the actual complex loading (number of beads/mm.sup.2, Y axis) on a bottom surface and a top surface along the length (X axis) of one flow cell channel, and the linear fit for each surface.

DETAILED DESCRIPTION

(20) Some sequencing techniques utilize sequencing-ready nucleic acid fragments. In some examples, each sequencing-ready nucleic acid fragment includes a portion (fragment) of genetic material, as well as adapters at the 3′ and 5′ ends. Sequencing-ready nucleic acid fragments may be bound to a solid support, which forms a complex. In these examples, the use of the solid support may be desirable because it can preserve the contiguity information of the longer genetic material from which the fragments are generated. Other sequencing techniques utilize a clustered solid support, which includes a cluster of template strands attached to the solid support. In these examples, the use of the solid support may be desirable because amplification (formation of the template strands) can be performed off of the flow cell and thus the flow cell chemistry is simplified in that it does not include amplification primers. However, when these target materials (e.g., complexes or clustered solid supports) are used in flow cells having two sequencing surfaces

positioned opposite one another (e.g., an upper/top surface and a lower/bottom surface), it has been found that the target materials have a tendency to sink to the sequencing surface positioned at the bottom of the flow cell. Similar issues may arise when other target materials, such as protein biomarkers, microbiomes, lysates, etc. in flow cells with opposed surfaces.

(21) Some examples of the method disclosed herein provide for more balanced immobilization of a target material across the two opposed sequencing surfaces. In some examples, the same type of target material is immobilized across the two opposed sequencing surfaces. In other examples, two different target materials (having at least one different property) are respectively immobilized on the two opposed sequencing surfaces.

(22) One example of the method disclosed herein utilizes a combination of fluids having different densities. One fluid density enables the target material (e.g., complexes, clustered solid supports) to migrate to and become immobilized at one of the sequencing surfaces, and the other fluid density enables the target material to migrate to and become immobilized at the other of the sequencing surfaces.

(23) Another example of the method utilizes a combination of a fluid, a substantially uniform magnetic force, and a magnetically responsive target material (e.g., a solid support). In this example, the fluid is selected to have a density that is approximately the same as the magnetically responsive target material. In this fluid, some of the target material sinks (and becomes immobilized at one of the sequencing surfaces), while some other of the target material floats. When the substantially uniform magnetic force is applied to the other of the sequencing surfaces, the floating target material migrates to and becomes immobilized at the other of the sequencing surfaces.

(24) Still another example of the method disclosed herein utilizes two different target materials having different densities. Both target materials are contained in the same fluid. The density of one of the target materials (with respect to the fluid) enables that target material (e.g., complexes, clustered solid supports) to migrate to and become immobilized at one of the sequencing surfaces, and the density of the other of the target materials (with respect to the fluid) enables that target material to migrate to and become immobilized at the other of the sequencing surfaces.

(25) Yet another example of the method disclosed herein utilizes two different target materials having at least one different property, such as density, charge, magnetism, or combinations thereof. Exposure to at least one condition causes the different target materials to migrate to a respective one of the opposed sequencing surfaces.

(26) Immobilization of the target material(s) (e.g., complexes, clustered solid supports) on both sequencing surfaces improves the overall utilization of the flow cell.

(27) A more balanced distribution of the immobilized target material(s) across the two sequencing surfaces may lead to improved downstream metrics obtained using the flow cell. In one example, the more balanced distribution of the immobilized target material across the two sequencing surfaces may lead to improved sequencing metrics. In one example, the target material may include complexes, and when the complexes are more evenly distributed across the two sequencing surfaces of the flow cell, the library fragments released from the complexes also seed more evenly across the respective sequencing surfaces. This leads to the formation of individual clusters that are relatively localized with respect to the position of the complexes from which the clusters are formed. In another example, the target material may include clustered solid supports. When the clustered solid supports are more evenly distributed across the two sequencing surfaces of the flow cell, the clustered template strands are also more evenly distributed. During sequencing, individual clusters generate “spatial clouds” of fluorescence signals as nucleotides are incorporated into respective template strands of the clusters. The even distribution can improve the readability of the spatial clouds.

(28) Moreover, loading both sequencing surfaces generates more area for generating these spatial clouds.

Definitions

(29) Terms used herein will be understood to take on their ordinary meaning in the relevant art unless specified otherwise. Several terms used herein and their meanings are set forth below.

(30) As used herein, the singular forms “a,” “an,” and “the” refer to both the singular as well as plural, unless the context clearly indicates otherwise. The term “comprising” as used herein is synonymous with “including,” “containing,” or “characterized by,” and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps.

(31) Reference throughout the specification to “one example,” “another example,” “an example,” and so forth, means that a particular element (e.g., feature, structure, composition, configuration, and/or characteristic) described in connection with the example is included in at least one example described herein, and may or may not be present in other examples. In addition, it is to be understood that the described elements for any example may be combined in any suitable manner in the various examples unless the context clearly dictates otherwise.

(32) The terms “substantially” and “about” used throughout this disclosure, including the claims, are used to describe and account for small fluctuations, such as due to variations in processing. For example, these terms can refer to less than or equal to ±10% from a stated value, such as less than or equal to ±5% from a stated value, such as less than or equal to ±2% from a stated value, such as less than or equal to ±1% from a stated value, such as less than or equal to ±0.5% from a stated value, such as less than or equal to ±0.2% from a stated value, such as less than or equal to ±0.1% from a stated value, such as less than or equal to ±0.05% from a stated value.

(33) Adapter. A linear oligonucleotide sequence that can be fused to a nucleic acid molecule, for example, by ligation or tagmentation. Suitable adapter lengths may range from about 10 nucleotides to about 100 nucleotides, or from about 12 nucleotides to about 60 nucleotides, or from about 15 nucleotides to about 50 nucleotides. The adapter may include any combination of nucleotides and/or nucleic acids. In some examples, the adapter can include a sequence that is complementary to at least a portion of a primer, for example, a primer including a universal nucleotide sequence (such as a P5 or P7 sequence). As an example, the adapter at one end of a fragment includes a sequence that is complementary to at least a portion of a first flow cell or solid support primer, and the adapter at the other end of the fragment includes a sequence that is identical to at least a portion of a second flow cell or solid support primer. The complementary adapter can hybridize to the first flow cell or solid support primer, and the identical adapter is a template for its complementary copy, which can hybridize to the second flow cell or solid support primer during clustering. In some examples, the adapter can include a sequencing primer sequence or sequencing binding site. Combinations of different adapters may be incorporated into a nucleic acid molecule, such as a DNA fragment.

(34) Approximately Equivalent: At least approximately equivalent means that the density of one component (e.g., fluid) is within 0.08 g/cm.sup.3 of the density of another component (e.g., a solid support). In some instances the densities of two components are equivalent.

(35) Capture site or Chemical capture site: A portion of a flow cell surface having been modified with a chemical property that allows for localization of a target material (e.g., complexes, clustered solid supports, protein biomarkers, etc.). In an example, the capture site may include a chemical capture agent (i.e., a material, molecule or moiety that is capable of attaching, retaining, or binding to a target molecule (e.g., a complex, a clustered solid support, a protein biomarker, etc.). One example chemical capture agent includes a member of a receptor-ligand binding pair (e.g., avidin, streptavidin, biotin, lectin, carbohydrate, nucleic acid binding protein, epitope, antibody, etc.) that is capable of binding to the target material (or to a linking moiety attached to the target material). Yet another example of the chemical capture agent is a chemical reagent capable of forming an electrostatic interaction, a hydrogen bond, or a covalent bond (e.g., thiol-disulfide exchange, click chemistry, Diels-Alder, etc.) with the target material.

(36) Complex: A carrier, such as a solid support, and sequencing-ready nucleic acid fragments

attached to the carrier. The carrier may also include one member of a binding pair whose other member is part of the capture site.
(37) Clustered solid support: A carrier, such as a solid support, having a plurality of amplified template strands attached thereto. The plurality of amplified template strands may be referred to as a “cluster.”
(38) Depositing: Any suitable application technique, which may be manual or automated, and, in some instances, results in modification of the surface properties. Generally, depositing may be performed using vapor deposition techniques, coating techniques, grafting techniques, or the like. Some specific examples include chemical vapor deposition (CVD), spray coating (e.g., ultrasonic spray coating), spin coating, dunk or dip coating, doctor blade coating, puddle dispensing, flow through coating, aerosol printing, screen printing, microcontact printing, inkjet printing, or the like.
(39) Depression: A discrete concave feature in a substrate or a patterned resin having a surface opening that is at least partially surrounded by interstitial region(s) of the substrate or the patterned resin. Depressions can have any of a variety of shapes at their opening in a surface including, as examples, round, elliptical, square, polygonal, star shaped (with any number of vertices), etc. The cross-section of a depression taken orthogonally with the surface can be curved, square, polygonal, hyperbolic, conical, angular, etc. As examples, the depression can be a well or two interconnected wells. The depression may also have more complex architectures, such as ridges, step features, etc.
(40) Each: When used in reference to a collection of items, each identifies an individual item in the collection, but does not necessarily refer to every item in the collection. Exceptions can occur if explicit disclosure or context clearly dictates otherwise.
(41) External immobilizing agent: A gaseous, liquid or viscous medium that is not miscible with a complex that has been introduced to the flow cell. The gaseous external immobilizing agent may be used to create a droplet around a complex or sample. An example of a gaseous external immobilizing agent is air that is directed at a suitable flow rate through the flow cell. For example, air may be used to aspirate a fluid from the flow cell, which forms droplets of the liquid around complexes immobilized within the flow cell. The formed droplet acts as a diffusion barrier. The liquid or viscous medium is used to minimize diffusion of a sequencing library released from a complex. The external immobilizing agent can form a diffusion barrier, as the sequencing libraries or any other polynucleotide have little to no solvation in the external immobilizing agent. Example external immobilizing agents in liquid form include hydrophobic oils, such as mineral oil, silicone oil, perfluorinated oil, a fluorinated carbon oil (e.g., FC40), or a combination thereof. Example external immobilizing agents in viscous medium form include buffers containing polymers (e.g., polyethylene glycol, polyvinylpyrrolidone, etc.), dextran, sucrose, glycerol, and the like. In some examples, the viscous medium is a temperature responsive gel. The temperature responsive gel is non-viscous at non-seeding temperatures, and turns into a viscous medium at seeding temperatures. Examples of temperature responsive gels include poly(N-isopropylacrylamide) and polyethylene oxide-polypropylene oxide-polyethylene oxide (PEO-PPO-PEO)/laponite nanoparticle composites.
(42) Flow Cell: A vessel having a chamber (e.g., a flow channel) where a reaction can be carried out, an inlet for delivering reagent(s) to the chamber, and an outlet for removing reagent(s) from the chamber. In some examples, the chamber enables the detection of the reaction that occurs in the chamber. For example, the chamber can include one or more transparent surfaces allowing for the optical detection of arrays, optically labeled molecules, or the like.
(43) Flow channel: An area defined between two bonded or otherwise attached components, which can selectively receive a liquid sample. In some examples, the flow channel may be defined between two patterned or non-patterned sequencing surfaces, and thus may be in fluid communication with one or more components of the sequencing surfaces.
(44) Fragment: A portion or piece of genetic material (e.g., DNA, RNA, etc.). Contiguity preserved library fragments are smaller pieces of the longer nucleic acid sample that has been fragmented, where the contiguity information of the longer nucleic acid sample has been preserved in the

fragments.
(45) Nucleic acid molecule or sample: A polymeric form of nucleotides of any length, and may include ribonucleotides, deoxyribonucleotides, analogs thereof, or mixtures thereof. The term may refer to single stranded or double stranded polynucleotides.
(46) A “template” nucleic acid molecule (or strand) may refer to a sequence that is to be analyzed. A cluster of template strands includes amplicons of a library fragment.
(47) The nucleotides in a nucleic acid sample may include naturally occurring nucleic acids and functional analogs thereof. Examples of functional analogs are capable of hybridizing to a nucleic acid in a sequence specific fashion or capable of being used as a template for replication of a particular nucleotide sequence. Naturally occurring nucleotides generally have a backbone containing phosphodiester bonds. An analog structure can have an alternate backbone linkage including any of a variety known in the art. Naturally occurring nucleotides generally have a deoxyribose sugar (e.g., found in DNA) or a ribose sugar (e.g., found in RNA). An analog structure can have an alternate sugar moiety including any of a variety known in the art. Nucleotides can include native or non-native bases. A native DNA can include one or more of adenine, thymine, cytosine and/or guanine, and a native RNA can include one or more of adenine, uracil, cytosine and/or guanine. Any non-native base may be used, such as a locked nucleic acid (LNA) and a bridged nucleic acid (BNA).
(48) Primer. A nucleic acid molecule that can hybridize to a target sequence, such as an adapter attached to a library fragment. As one example, an amplification primer can serve as a starting point for template amplification and cluster generation. As another example, a synthesized nucleic acid (template) strand may include a site to which a primer (e.g., a sequencing primer) can hybridize in order to prime synthesis of a new strand that is complementary to the synthesized nucleic acid strand. Any primer can include any combination of nucleotides or analogs thereof. In some examples, the primer is a single-stranded oligonucleotide or polynucleotide. The primer length can be any number of bases long and can include a variety of non-natural nucleotides. In an example, the sequencing primer is a short strand, ranging from 10 to 60 bases, or from 20 to 40 bases.
(49) Sequencing-ready nucleic acid fragments: A portion of genetic material having adapters at the 3′ and 5′ ends. In the sequencing-ready nucleic acid fragment, each adapter includes a known universal sequence (e.g., which is complementary to or identical to at least a portion of a primer on a flow cell) and a sequencing primer sequence. Both of the adapters may also include an index (barcode or tag) sequence. In an example, one side (e.g., including a P5′ or P5 sequence) may contain a bead index and the other side (including a P7 or P7′ sequence) may contain a sample index. A sequencing-ready nucleic acid fragment may be bound to a solid support via insertion of transposons, where inserted DNA molecules are immobilized to the surface of a solid support (e.g., bead); or directly immobilized through a binding pair or other cleavable linker; or bound via hybridization, where complementary adapter sequences are present on the surface of the solid support.
(50) Sequencing surface: A surface of a flow cell where sequencing can take place. In some examples, the sequencing surface includes a polymeric hydrogel having one or more types of amplification primers grafted thereto. In these examples, the sequencing surface may also include a capture site to immobilize complexes at or near the amplification primers. In other examples, the sequencing surface includes capture sites to immobilize clustered solid supports.
(51) Solid support: A small body made of a rigid or semi-rigid material having a shape characterized, for example, as a sphere, oval, microsphere, or other recognized particle shape whether having regular or irregular dimensions. In some examples, the solid support can have a sequencing library attached thereto. In other examples, the solid support can have a cluster of template strands attached thereto.
(52) Target Material: Any substance that is to be immobilized on a flow cell surface.

(53) Transposome: A complex formed between an integration enzyme (e.g., an integrase or a transposase) and a nucleic acid including an integration recognition site (e.g., a transposase recognition site).
(54) In the examples disclosed herein, target materials are introduced to a flow cell that includes two opposed sequencing surfaces. The target materials and flow cell will now be described, followed by different examples of the methods for immobilizing the target materials on each of the two opposed sequencing surfaces.
(55) Target Materials
(56) Example target materials **11** are shown in FIG. **1**A through FIG. **1**C. In the examples disclosed herein, any target material **11** that is to be immobilized on a surface of a flow cell may be utilized. As examples, the target material **11** may be a complex **10**A, **10**B as defined herein (see FIG. **1**A and FIG. **1**B), a clustered solid support **13** as defined herein (see FIG. **1**C), other DNA libraries from a specific sample, cells, oligonucleotide conjugated proteins bound to solid supports, a protein biomarker, a microbiome, or the like. The following description provides some examples of the complexes **10**A, **10**B and of the clustered solid support **13**.
(57) Complexes
(58) Some example complexes **10**A and **10**B are shown, respectively, in FIG. **1**A and FIG. **1**B. In the examples of the method disclosed herein, the complexes **10**A, **10**B include a solid support **12**, **12′** and sequencing-ready nucleic acid fragments **14**, **14′**, **14″** attached to the solid support **12**, **12′**.
(59) In examples of the method that utilize the combination of fluids having different densities, or target materials **11** with different densities, or non-charged target materials **11**, the solid support **12** may be, without limitation, hydrogels; glass (e.g., controlled pore glass beads); plastic, such as acrylic, polystyrene or a copolymer of styrene and another material, polypropylene, polyethylene, polybutylene, polyurethane or polytetrafluoroethylene (TEFLON® from The Chemours Co); polysaccharides or cross-linked polysaccharides such as agarose, SEPHAROSE® beads (cross-linked beaded form of agarose, available from Cytivia), or SEPHADEX® beads (cross-linked beaded form of dextran, available from Cytivia); nylon; nitrocellulose; resin; silica or silica-based materials including silicon and modified silicon; carbon-fiber; metal; inorganic glass; an optical fiber bundle; or a variety of other polymers. Some examples of the solid support **12** may have the form of solids beads, porous beads, or hollow beads.
(60) In examples of the method that utilize the combination of the fluid and the magnetic force, the solid support **12′** is a magnetically responsive material. A “magnetically responsive” material is responsive to a magnetic field. Examples of magnetically responsive solid supports include or are composed of magnetically responsive materials. Examples of magnetically responsive materials include paramagnetic materials, ferromagnetic materials, ferrimagnetic materials, and metamagnetic materials. Examples of suitable paramagnetic materials include iron, nickel, and cobalt, as well as metal oxides, such as Fe.sub.3O.sub.4, BaFe.sub.12O.sub.19, CoO, NiO, Mn.sub.2O.sub.3, Cr.sub.2O.sub.3, and CoMnP. One commercially available example includes DYNABEADS™ M-280 Streptavidin (superparamagnetic beads coated with streptavidin) from ThermoFisher Scientific. In some examples, the magnetically responsive material is embedded in the shell of a polymer bead. In other examples, the magnetically responsive material is in bead form and is coated with a passivating material, such as silicon oxide or silicon nitrite. In example methods utilizing two different target materials **11**, one of the target materials **11** may include any of the magnetically responsive solid supports **12′** disclosed herein.
(61) In examples of the method that utilize an electric field for immobilization, the solid support **12** of the target material **11** may be positively charged or negatively charged. In these examples, any of the examples set forth for the solid support **12** may be used, and may be coated or functionalized to impart the desired charge. Either small molecules or polymers may be used to impart charge to the solid support **12**. For example, any of the solid supports **12** (e.g., polystyrene, silica, etc.) may be functionalized with amines to render them positively charged. Any primary, secondary, or tertiary

amine may be used. Examples of suitable amines include amino-silane, polylysine, or chitosan. For another example, any of the solid supports **12** (e.g., polystyrene, silica, SEPHADEX®, etc.) may be functionalized with carboxyl groups or sulfate groups to render them negatively charged. For still another example, any of the solid supports **12** (e.g., polystyrene, silica, SEPHADEX®, etc.) may be coated with polyglutamic acid to render them negatively charged.

(62) While not shown in FIG. **1**A and FIG. **1**B, the solid support **12**, **12′** may be functionalized with one member of a binding pair. A "binding pair" refers to two agents (e.g., materials, molecules, moieties) that are capable of attaching to one another. In this example, the member on the solid support **12**, **12′** is a binding pair with another member that is located on the sequencing surface of the flow cell. In other examples, the solid support **12**, **12′** may be capable of being chemically conjugated to the sequencing surface of the flow cell.

(63) Functionalization of the solid support **12**, **12′** may involve coating the solid support **12**, **12′** with the binding pair member, or forming a bond between the binding pair member and a functional group at the surface of the solid support **12**, **12′**. One example binding pair member includes a member of a receptor-ligand binding pair (e.g., avidin, streptavidin, biotin, lectin, carbohydrate, nucleic acid binding protein, epitope, antibody, etc.) that is capable of binding to the other binding pair member that is located on the sequencing surface of the flow cell. The binding pair members may also be chemical reagents that are capable of forming an electrostatic interaction, a hydrogen bond, or a covalent bond (e.g., thiol-disulfide exchange, click chemistry, Diels-Alder, etc.). Any form of chemical coupling may also attach the solid support **12**, **12′** to the sequencing surface of the flow cell. In many instances, a reversible or cleavable interaction is desirable so that the solid support **12**, **12′** may be removed prior to sequencing.

(64) In examples of the complex **10**A, **10**B, the sequencing-ready nucleic acid fragments **14**, **14′**, **14″** are attached to the solid support **12**, **12′**. Each sequencing-ready nucleic acid fragment **14**, **14′**, **14″** includes a portion (e.g., fragment **16**, **16′**, **16″**) of a longer piece of genetic material that has adapters (e.g., **18**, **18′**, **18″**, **22**, **22′**, **22″**) at the 3′ and 5′ ends. The sequencing-ready fragments **14**, **14′**, **14″** may be prepared using any library preparation technique that fragments a longer piece of genetic material and incorporates the desired adapters **18**, **18′**, **18″**, **22**, **22′**, **22″** to the ends of the fragments **16**, **16′**, **16″**. Some suitable library preparation techniques are described in reference to FIG. **1**A and FIG. **1**B. It is to be understood, however, that other library preparation techniques may also be used.

(65) FIG. **1**A depicts an example of a complex **10**A including sequencing-ready nucleic acid fragments **14**, **14′** which include fragments **16**, **16′** from the larger nucleic acid sample, whose contiguity is preserved on the solid support **12**, **12′**. An example method for making the complex **10**A is described herein, but it is to be understood that other methods may be used as long as sequencing-ready nucleic acid fragments **14**, **14′** are attached to the solid support **12**, **12′**.

(66) In one example method to form the complex **10**A shown in FIG. **1**A, an adapter sequence **18**, **18′** is bound to the solid support **12**, **12′** through one member **20** of a binding pair. In an example, this adapter sequence **18**, **18′** may include a first sequencing primer sequence (e.g., a read 1 sequencing primer sequence) and a first sequence (P5′) that is complementary to at least a portion of one of the amplification primers (e.g., P5) on the flow cell (shown in FIG. **2**A, FIG. **2**B and FIG. **2**C). The adapter sequence **18**, **18′** may also include an index or barcode sequence. The adapter sequence **18**, **18′** is bound to the one member **20** (e.g., biotin) of the binding pair so that it can be bound to the surface of the solid support **12**, **12′**, which includes the other member (e.g., avidin, streptavidin, etc.) of the binding pair. In this example, the member of the binding pair on the solid support **12**, **12′** may be multi-functional in that it can i) bind to the member **20** used to attach the sequencing-ready nucleic acid fragments **14**, **14′** and ii) bind to the sequencing surface of the flow cell. In other examples, the solid support **12**, **12′** may be functionalized with two different binding pair members, e.g., i) one of which can bind to the member **20** used to attach the sequencing-ready nucleic acid fragments **14**, **14′** and ii) another of which can bind to the sequencing surface of the

flow cell.
(67) In this example, a transposome complex (not shown) may also be bound to the solid support **12**, **12′** at the outset of the library preparation method. Prior to loading the transposome complex on the solid support **12**, **12′**, a partial Y-adapter may be mixed with a transposase enzyme (e.g., two Tn5 molecules) to form a transposome complex. The partial Y-adapter may include two mosaic end sequences that are hybridized to each other. One of the mosaic end sequences is referred to as a free mosaic end sequence because it has two free ends, e.g., one that is able to attach to the adapter **18**, **18′** and another that is able to attach to fragmented DNA strands **16**, **16′** during tagmentation. The other of the mosaic end sequences may be attached to another adapter (e.g., **22**, **22′**), which includes a second sequencing primer sequence (e.g., a read 2 sequencing primer sequence) and a second sequence (P7) that is identical to the at least a portion of another of the amplification primers (P7) on the flow cell. During amplification, the identical sequence enables the formation of a copy that is complementary to at least a portion of the other of the amplification primers (P7) on the flow cell. The adapter sequences **22**, **22′** are not attached to the fragmented DNA strands **16**, **16′** during tagmentation.
(68) Loading the transposome complex on the solid support **12**, **12′** may involve mixing the transposome complex with the solid support **12**, **12′**, and exposing the mixture to suitable conditions for ligating one of free ends of the free mosaic end to the 3′-end of the adapter sequence **18**, **18′**. Individual transposome complexes may be attached to each of the adapter sequences **18**, **18′** on the solid support **12**, **12′**.
(69) In this example method to form the complex **10**A, a tagmentation process may then be performed. A fluid (e.g., a tagmentation buffer) including the longer nucleic acid sample (e.g., DNA) may be added to the solid support **12**, **12′** having the adapter sequence **18**, **18′** and the transposome complexes bound thereto. As the sample contacts the transposome complexes, the longer nucleic acid sample is tagmented. The longer nucleic acid sample is fragmented into fragments **16**, **16′**, and each is tagged, at its 5′ end, to the partial Y-adapter (e.g., through ligation of the other free end of the free mosaic end sequence). Successive tagmentation of the longer nucleic acid sample results in a plurality of bridged molecules between the transposome complexes. The bridged molecules wrap around the solid support **12**, **12′**. The transposome complexes maintain the contiguity of the longer nucleic acid sample as bridged molecules.
(70) The transposase enzyme may then be removed via sodium dodecyl sulfate (SDS) treatment or heat or proteinase K digestion. Removal of the transposase enzymes leaves the contiguity preserved fragments **16**, **16′** attached to the solid support **12**, **12′**.
(71) To complete the sequencing ready fragments **14**, **14′**, further extension and ligation is undertaken to ensure sample fragments **16**, **16′** are attached to sequences **22** and **22′**. The resulting complex **10**A is shown in FIG. **1**A.
(72) Each sequencing-ready nucleic acid fragment **14**, **14′** includes a contiguity preserved library fragment **16**, **16′** having respective adapter sequences **18** and **22** or **18′** and **22′** attached at either end. The adapter sequences **18**, **18′** are those initially bound to the solid support **12**, **12′**, and include the first sequencing primer sequence and the first sequence complementary to one of the flow cell primers. The adapter sequences **18**, **18′** are attached to the one member **20** of a binding pair. The adapter sequences **22**, **22′** are from the partial Y-adapter, and include the second sequence identical to another flow cell primer and the second sequencing primer sequence. Because each sequencing-ready nucleic acid fragment **14**, **14′** includes suitable adapters for amplification (e.g., bridge amplification) and sequencing, PCR amplification is not performed. These fragments **14**, **14′** are thus sequencing-ready. Moreover, because the contiguity preserved library fragments **16**, **16′** are from the same longer nucleic acid sample, the contiguity of the original sample is preserved and the library fragments **14**, **14′** may be suitable for linked long read applications.
(73) FIG. **1**B illustrates another complex **10**B that includes a solid support **12**, **12′** and sequencing-ready nucleic acid fragments **14″** attached to the solid support **12**, **12′**. In one example, a PCR-free

nucleotide library is created in a tube, and then the library is hybridized on the solid support **12**, **12′** in the tube. In the example shown in FIG. **1**B, adapters **18″**, **22″** are added to the library fragments **16″** in the tube, primers having one member **20** of a binding pair are hybridized to the adapters **18″** in the tube, and then the sequencing-ready nucleic acid fragments **14″** are bound to the solid support **12**, **12′** through the member **20** of a binding pair. In another example, the solid support **12**, **12′** may have primers attached thereto via a binding pair (e.g., avidin on the support **12**, **12′** and biotin attached to the primer). These primers hybridize to adapters **18″** attached to the library fragments **16″** (and thus the primer and binding pair member are at one end of the fragments and not at the other). In still other example, extension may be performed using a strand displacing enzyme. This will result in an entirely double stranded library (e.g., no fork or Y-adapter, as shown in FIG. **1**B).

(74) As mentioned, other library preparation techniques may also be used. For example, ligation based library preparation techniques may be used where the complementary adapter sequence is immobilized on the flow cell. For another example, mRNA may be immobilized to the solid support **12**, **12′** via polyA tail hybridization.

(75) Clustered Solid Supports

(76) An example clustered solid support **13** is shown in FIG. **1**C. The clustered solid support **13** includes a solid support **12**, **12′** and template strands **64** attached to the solid support **12**, **12′** through a primer **42** or **42′**.

(77) Any example of the solid support **12**, **12′** may be used as the core of the clustered solid support **13**. The type of solid support **12**, **12′**, and its property/properties (e.g., density, charge, magnetism, etc.), may depend upon the immobilization method that is to be used.

(78) While not shown in FIG. **1**C and similar to the complexes **10**A and **10**B shown in FIG. **1**A and FIG. **1**B, the solid support **12**, **12′** may be functionalized with one member of a binding pair for attachment to a capture site of a flow cell.

(79) As shown in FIG. **1**C, this example of the solid support **12**, **12′** is functionalized with primers **42**, **42′**. The primers **42**, **42′** may be amplification primers **42**, **42′** that can be immobilized to the solid support **12**, **12′** by single point covalent attachment or a strong non-covalent interaction at or near the 5′ end of the primers **42**, **42′**. The attachment leaves i) an adapter-specific portion of the primers **42**, **42′** free to anneal to its cognate sequencing-ready nucleic acid fragment and ii) the 3′ hydroxyl group free for primer extension. At or near the 5′ end, the primer **42**, **42′** includes a chemically modifiable functional group that is capable of covalent attachment or strong non-covalent interaction. Examples of chemically modifiable functional groups include thiol, azido, alkyne, amino, biotin, etc.

(80) Specific examples of suitable primers **42**, **42′** include P5 and P7 primers used on the surface of commercial flow cells sold by Illumina Inc. for sequencing on HISEQ™, HISEQX™, MISEQ™, MISEQDX™, MINISEQ™, NEXTSEQ™, NEXTSEQDX™, NOVASEQ™, GENOME ANALYZER™, ISEQ™, and other instrument platforms. Both P5 and P7 primers may be grafted to each of the solid supports **12**, **12′**.

(81) In an example, grafting of the primers **42**, **42′** to the solid support **12**, **12′** may involve dunk coating, which involves immersing the solid support **12**, **12′** in a primer solution or mixture, which may include the primers **42**, **42′**, water, a buffer, and a catalyst. Other grafting techniques may involve spray coating, puddle dispensing, or another suitable method that will attach the primer(s) **42**, **42′** to the solid support **12**, **12′**. With any of the grafting methods, the primers **42**, **42′** react with reactive groups of the solid support **12**, **12′**.

(82) During grafting, the chemically modifiable functional group of the primer **42**, **42′** reacts or interacts with the reactive groups of the solid support **12**, **12′**. The following are examples of reactions or interactions that may take place during grafting: reacting an azido (e.g., succinimidyl (NHS) ester) terminated primer with a hydrazine on the surface of the solid support **12**, **12′**, or reacting an alkyne terminated primer with an azide on the surface of the solid support **12**, **12′**, or

reacting an amino terminated primer to an activated carboxylate group or NHS ester on the surface of the solid support **12**, **12′**, or reacting a thiol terminated primer with an alkylating reactant (e.g., iodoacetamine or maleimide) on the surface of the solid support **12**, **12′**, or reacting a phosphoramidite terminated primer with a thioether on the surface of the solid support **12**, **12′**, or interacting a biotin-modified primer with streptavidin on the surface of the solid support **12**, **12′**. Some nucleic acid primers **42**, **42′** can be captured onto silica beads in the presence of a chaotropic agent (KI, NI, or NaSCN). As one specific example, a dibenzocyclooctyne (DBCO, which includes an alkyne) terminated primer may be used for copper free click grafting.

(83) To generate the template strands **64** on the solid support **12**, **12′**, library templates may first be prepared from any nucleic acid sample (e.g., a DNA sample or an RNA sample). When an RNA sample is used, it is first converted to a complementary deoxyribonucleic acid (cDNA) sample. This may be done using reverse transcription, which utilizes a reverse transcriptase enzyme. In some examples, a kit for reverse transcription and second strand synthesis is used. In these examples, the high capacity cDNA reverse transcription kit, from ThermoFisher Scientific, may be used. In other examples, a kit for reverse transcription and template switch (for the second strand) is used. In these examples, the template switching RT enzyme mix, from New England Biolabs, may be used.

(84) The DNA or cDNA sample may then be fragmented into single-stranded, similarly sized (e.g., <1000 bp) fragments. During preparation, adapters may be added to the ends of these fragments. Through reduced cycle amplification, different motifs may be introduced in the adapters, such as sequencing binding sites, indices, and regions that are complementary or identical to the primers **42**, **42′** on the solid support **12**, **12′**. The final library templates include the DNA or cDNA fragment and adapters at both ends. In some examples, the fragments from a single nucleic acid sample have the same adapters added thereto.

(85) A plurality of library templates may be introduced to a plurality of the solid supports **12**, **12′**. A library template hybridizes to one of two types of primers **42**, **42′** immobilized on a respective solid support **12**, **12′**. Cluster generation may then be performed. In one example of cluster generation, the library template on the solid support **12**, **12′** is copied from the hybridized primer by 3′ extension using a high-fidelity DNA polymerase. The original library template is denatured, leaving the copy (e.g., template strand **64**) immobilized on the solid support **12**, **12′**, e.g., through the primer **42** as shown in FIG. **1**C. Isothermal bridge amplification or some other form of amplification may be used to amplify the immobilized copies. For example, the copied template loops over to hybridize to an adjacent, complementary primer (e.g., primer **42′**), and a polymerase copies the copied template to form a double stranded bridge, which is denatured to form two single stranded strands. These two strands loop over and hybridize to adjacent, complementary primers **42**, **42′** and are extended again to form two new double stranded loops. The process is repeated on each template copy by cycles of isothermal denaturation and amplification to create dense clonal clusters. Each cluster of double stranded bridges is denatured. In an example, the reverse strand is removed by specific base cleavage, leaving forward template polynucleotide strands. Clustering results in the formation of several template polynucleotide strands **64** on the solid support **12**, **12′**. This example of clustering is bridge amplification, and is one example of the amplification that may be performed.

(86) Flow Cell

(87) A top view of an example of the flow cell **24** is shown in FIG. **2**A. As mentioned herein, the flow cell **24** includes two sequencing opposed sequencing surfaces. An example of non-patterned sequencing surfaces **30**, **30′** are shown in FIG. **2**B, an example of patterned sequencing surfaces **32**, **32′** are shown in FIG. **2**C, and another example of patterned sequencing surfaces **31**, **31′** are shown in FIG. **2**D. The non-patterned sequencing surfaces **30**, **30′** and patterned sequencing surfaces **32**, **32′** include primers **42**, **42′**, and thus may be utilized with target materials **11** that introduce library fragments that are to be amplified on the flow cell **24**. Other sequencing surfaces, such as patterned

sequencing surfaces **31**, **31′**, do not include primers **42**, **42′**, and thus may be utilized with clustered solid supports **13**.

(88) Each sequencing surface **30**, **30′** or **32**, **32′** or **31**, **31′** is supported by a substrate (generally shown as **26** in FIG. **2**A), and a flow channel (generally shown as **28** in FIG. **2**A) is defined between the sequencing surfaces **30**, **30′** or **32**, **32′** or **31**, **31′**.

(89) The substrate **26** may be a single layer/material. Examples of the single layer substrate are shown at reference numeral **26**A and **26**A′ in FIG. **2**B. Examples of suitable single layer substrates **26**A, **26**A′ include epoxy siloxane, glass, modified or functionalized glass, plastics (including acrylics, polystyrene and copolymers of styrene and other materials, polypropylene, polyethylene, polybutylene, polyurethanes, polytetrafluoroethylene (such as TEFLON® from Chemours), cyclic olefins/cyclo-olefin polymers (COP) (such as ZEONOR® from Zeon), polyimides, etc.), nylon (polyamides), ceramics/ceramic oxides, silica, fused silica, or silica-based materials, aluminum silicate, silicon and modified silicon (e.g., boron doped p+ silicon), silicon nitride (Si.sub.3N.sub.4), silicon oxide (SiO.sub.2), tantalum pentoxide (Ta.sub.2O.sub.5) or other tantalum oxide(s) (TaO.sub.x), hafnium oxide (HfO.sub.2), carbon, metals, inorganic glasses, or the like.

(90) The substrate **26** may also be a multi-layered structure. Examples of the multi-layered substrate are shown at reference numeral **26**B and **26**B′ in FIG. **2**C and in FIG. **2**D. Some examples of the multi-layered structure **26**B, **26**B′ include glass or silicon, with a coating layer of tantalum oxide or another ceramic oxide at the surface. With specific reference to FIG. **2**C and FIG. **2**D, other examples of the multi-layered structure **26**B, **26**B′ include an underlying support **34**, **34′** having a patterned resin **36**, **36′** thereon. Still other examples of the multi-layered substrate **26**B, **26**B′ may include a silicon-on-insulator (SOI) substrate.

(91) In an example, the substrate **26** (whether single or multi-layered) may have a diameter ranging from about 2 mm to about 300 mm, or a rectangular sheet or panel having its largest dimension up to about 10 feet (˜3 meters). In an example, the substrate **26** is a wafer having a diameter ranging from about 200 mm to about 300 mm. In another example, the substrate **26** is a die having a width ranging from about 0.1 mm to about 10 mm. While example dimensions have been provided, it is to be understood that a substrate **26** with any suitable dimensions may be used. For another example, a panel may be used that is a rectangular support, which has a greater surface area than a 300 mm round wafer.

(92) In the example shown in FIG. **2**A, the flow cell **24** includes flow channels **28**. While several flow channels **28** are shown, it is to be understood that any number of channels **28** may be included in the flow cell **24** (e.g., a single channel **28**, four channels **28**, etc.). In the examples disclosed herein, each flow channel **28** is an area defined between two sequencing surfaces (e.g., **30** and **30′** or **32** and **32′** or **31** and **31′**) and by two attached substrates (e.g., **26**A and **26**A′ or **26**B and **26**B′). The fluids described herein can be introduced into and removed from the flow channel(s) **28** via inlet(s) and outlet(s), respectively. Each flow channel **28** may be isolated from each other flow channel **28** in a flow cell **24** so that fluid introduced into any particular flow channel **28** does not flow into any adjacent flow channel **28**.

(93) A portion of the flow channel **28** may be defined in the substrate **26** using any suitable technique that depends, in part, upon the material(s) of the substrate **26**. In one example, a portion of the flow channel **28** is etched into a glass substrate **26**. In another example, a portion of the flow channel **28** may be patterned into a resin **36**, **36′** of a multi-layered substrate **26**B, **26**B′ using photolithography, nanoimprint lithography, etc. In still another example, a separate material (e.g., material **50** in FIG. **2**B and FIG. **2**C and FIG. **2**D) may be applied to the substrate **26** so that the separate material defines at least a portion of the walls of the flow channel **28**.

(94) In an example, the flow channel **28** has a rectangular configuration. The length and width of the flow channel **28** may be smaller, respectively, than the length and width of the substrate **26** so that portion of the substrate surface surrounding the flow channel **28** is available for attachment to

another substrate **26**. In some instances, the width of each flow channel **28** can be at least about 1 mm, at least about 2.5 mm, at least about 5 mm, at least about 7 mm, at least about 10 mm, or more. In some instances, the length of each flow channel **28** can be at least about 10 mm, at least about 25 mm, at least about 50 mm, at least about 100 mm, or more. The width and/or length of each flow channel **28** can be greater than, less than or between the values specified above. In another example, the flow channel **28** is square (e.g., 10 mm×10 mm).

(95) The depth of each flow channel **28** can be as small as a few monolayers thick, for example, when microcontact, aerosol, or inkjet printing is used to deposit a separate material (e.g., material **50**) that defines the flow channel walls. For other examples, the depth of each flow channel **28** can be about 1 μm, about 10 μm, about 50 μm, about 100 μm, or more. In an example, the depth may range from about 10 μm to about 100 μm. In another example, the depth is about 5 μm or less. It is to be understood that the depth of each flow channel **28** be greater than, less than or between the values specified above. The depth of the flow channel **28** may also vary along the length and width of the flow cell **24**, e.g., when a patterned sequencing surface **32**, **32′** or **31**, **31′** is used.

(96) FIG. **2**B illustrates a cross-sectional view of the flow cell **24** including non-patterned opposed sequencing surfaces **30**, **30′**. In an example, each of these surfaces **30**, **30′** may be prepared on the substrate **26**A, **26**A′, and then the substrates **26**A, **26**A′ may be attached to one another to form an example of the flow cell **24**. Any suitable bonding material **50**, such as an adhesive, a radiation-absorbing material that aids in bonding, etc., may be used to bond the substrates **26**A, **26**B together.

(97) In the example shown in FIG. **2**B, a portion of the flow channel **28** is defined in each of the single layer substrates **26**A, **26**A′. For example, each substrate **26**A, **26**A′ may have a concave region **38**, **38′** defined therein where the components of the sequencing surface **30**, **30′** may be introduced. It is to be understood that any space within the concave region **38**, **38′** not occupied by the components of the sequencing surface **30**, **30′** may be considered to be part of the flow channel **28**.

(98) The sequencing surfaces **30**, **30′** include a polymeric hydrogel **40**, **40′**, amplification primers **42**, **42′** attached to the polymeric hydrogel **40**, **40′**, and chemical capture sites **44**, **44′**.

(99) An example of the polymeric hydrogel **40**, **40′** includes an acrylamide copolymer, such as poly(N-(5-azidoacetamidylpentyl)acrylamide-co-acrylamide, PAZAM. PAZAM and some other forms of the acrylamide copolymer are represented by the following structure (I):

(100) ##STR00001##

wherein: R.sup.A is selected from the group consisting of azido, optionally substituted amino, optionally substituted alkenyl, optionally substituted alkyne, halogen, optionally substituted hydrazone, optionally substituted hydrazine, carboxyl, hydroxy, optionally substituted tetrazole, optionally substituted tetrazine, nitrile oxide, nitrone, sulfate, and thiol; R.sup.B is H or optionally substituted alkyl; R.sup.C, R.sup.D, and R.sup.E are each independently selected from the group consisting of H and optionally substituted alkyl; each of the —(CH.sub.2).sub.p— can be optionally substituted; p is an integer in the range of 1 to 50; n is an integer in the range of 1 to 50,000; and m is an integer in the range of 1 to 100,000.

(101) One of ordinary skill in the art will recognize that the arrangement of the recurring “n” and “m” features in structure (I) are representative, and the monomeric subunits may be present in any order in the polymer structure (e.g., random, block, patterned, or a combination thereof).

(102) The molecular weight of PAZAM and other forms of the acrylamide copolymer may range from about 5 kDa to about 1500 kDa or from about 10 kDa to about 1000 kDa, or may be, in a specific example, about 312 kDa.

(103) In some examples, PAZAM and other forms of the acrylamide copolymer are linear polymers. In some other examples, PAZAM and other forms of the acrylamide copolymer are a lightly cross-linked polymers.

(104) In other examples, the polymeric hydrogel **40**, **40′** may be a variation of the structure (I). In one example, the acrylamide unit may be replaced with N,N-dimethylacrylamide

(105) ##STR00002##
In this example, the acrylamide unit in structure (I) may be replaced with
(106) ##STR00003##
where R.sup.D, R.sup.E, and R.sup.F are each H or a C1-C6 alkyl, and R.sup.G and R.sup.H are each a C1-C6 alkyl (instead of H as is the case with the acrylamide). In this example, q may be an integer in the range of 1 to 100,000. In another example, the N,N-dimethylacrylamide may be used in addition to the acrylamide unit. In this example, structure (I) may include
(107) ##STR00004##
in addition to the recurring "n" and "m" features, where R.sup.D, R.sup.E, and R.sup.F are each H or a C1-C6 alkyl, and R.sup.G and R.sup.H are each a C1-C6 alkyl. In this example, q may be an integer in the range of 1 to 100,000.
(108) As another example of the polymeric hydrogel **40**, **40**′, the recurring "n" feature in structure (I) may be replaced with a monomer including a heterocyclic azido group having structure (II):
(109) ##STR00005##
wherein R.sup.1 is H or a C1-C6 alkyl; R.sub.2 is H or a C1-C6 alkyl; L is a linker including a linear chain with 2 to 20 atoms selected from the group consisting of carbon, oxygen, and nitrogen and 10 optional substituents on the carbon and any nitrogen atoms in the chain; E is a linear chain including 1 to 4 atoms selected from the group consisting of carbon, oxygen and nitrogen, and optional substituents on the carbon and any nitrogen atoms in the chain; A is an N substituted amide with an H or a C1-C4 alkyl attached to the N; and Z is a nitrogen containing heterocycle. Examples of Z include 5 to 10 ring members present as a single cyclic structure or a fused structure. Some specific examples of Z include pyrrolidinyl, pyridinyl, or pyrimidinyl.
(110) As still another example, the polymeric hydrogel **40**, **40**′ may include a recurring unit of each of structure (III) and (IV):
(111) ##STR00006##
wherein each of R.sub.1a, R.sup.2a, R.sup.1b and R.sup.2b is independently selected from hydrogen, an optionally substituted alkyl or optionally substituted phenyl; each of R.sup.3a and R.sup.3b is independently selected from hydrogen, an optionally substituted alkyl, an optionally substituted phenyl, or an optionally substituted C7-C14 aralkyl; and each L.sup.1 and L.sup.2 is independently selected from an optionally substituted alkylene linker or an optionally substituted heteroalkylene linker.
(112) It is to be understood that other molecules may be used to form the polymeric hydrogel **40**, **40**′, as long as they are functionalized to graft oligonucleotide primers **42**, **42**′ thereto. Other examples of suitable polymer layers include those having a colloidal structure, such as agarose; or a polymer mesh structure, such as gelatin; or a cross-linked polymer structure, such as polyacrylamide polymers and copolymers, silane free acrylamide (SFA), or an azidolyzed version of SFA. Examples of suitable polyacrylamide polymers may be synthesized from acrylamide and an acrylic acid or an acrylic acid containing a vinyl group, or from monomers that form [2+2] photo-cycloaddition reactions. Still other examples of suitable polymeric hydrogels **42** include mixed copolymers of acrylamides and acrylates. A variety of polymer architectures containing acrylic monomers (e.g., acrylamides, acrylates etc.) may be utilized in the examples disclosed herein, such as branched polymers, including star polymers, star-shaped or star-block polymers, dendrimers, and the like. For example, the monomers (e.g., acrylamide, etc.) may be incorporated, either randomly or in block, into the branches (arms) of a star-shaped polymer.
(113) To introduce the polymeric hydrogel **40**, **40**′ into the concave regions **38**, **38**′, a mixture of the polymeric hydrogel **40**, **40**′ may be generated and then applied to the respective substrates **26**A, **26**A′ (having the concave regions **38**, **38**′ defined therein). In one example, the polymeric hydrogel **40**, **40**′ may be present in a mixture (e.g., with water or with ethanol and water). The mixture may then be applied to the respective substrate surfaces (including in the concave regions **38**, **38**′) using spin coating, or dipping or dip coating, or flow of the material under positive or negative pressure,

or another suitable technique. These types of techniques blanketly deposit the polymeric hydrogel **40**, **40**′ on the substrate respective substrates **26**A, **26**A′ (e.g., in the concave regions **38**, **38**′ and on interstitial regions **46**, **46**′ adjacent thereto). Other selective deposition techniques (e.g. involving a mask, controlled printing techniques, etc.) may be used to specifically deposit the polymeric hydrogel in the concave regions **38**, **38**′ and not on the interstitial regions **46**, **46**′.

(114) In some examples, the substrate surface (including the concave regions **38**, **38**′) may be activated, and then the mixture (including the polymeric hydrogel **40**, **40**′ may be applied thereto. In one example, a silane or silane derivative (e.g., norbornene silane) may be deposited on the substrate surface using vapor deposition, spin coating, or other deposition methods. In another example, the substrate surface may be exposed to plasma ashing to generate surface-activating agent(s) (e.g., —OH groups) that can adhere to the polymeric hydrogel **40**, **40**′.

(115) Depending upon the chemistry of the polymeric hydrogel **40**, **40**′, the applied mixture may be exposed to a curing process. In an example, curing may take place at a temperature ranging from room temperature (e.g., about 25° C.) to about 95° C. for a time ranging from about 1 millisecond to about several days.

(116) Polishing may then be performed in order to remove the polymeric hydrogel **40**, **40**′ from the interstitial regions **46**, **46**′ at the perimeter of the concave regions **38**, **38**′, while leaving the polymeric hydrogel **40**, **40**′ on the surface in the concave regions **38**, **38**′ at least substantially intact.

(117) The sequencing surfaces **30**, **30**′ also include amplification primers **42**, **42**′ attached to the polymeric hydrogel **40**, **40**′.

(118) A grafting process may be performed to graft the amplification primers **42**, **42**′ to the polymeric hydrogel **40**, **40**′ in the concave regions **38**, **38**′. In an example, the amplification primers **42**, **42**′ can be immobilized to the polymeric hydrogel **40**, **40**′ by single point covalent attachment or strong non-covalent interaction at or near the 5′ end of the primers **42**, **42**′. The attachment leaves i) an adapter-specific portion of the primers **42**, **42**′ free to anneal to its cognate sequencing-ready nucleic acid fragment and ii) the 3′ hydroxyl group free for primer extension. Any suitable covalent attachment or strong non-covalent interaction may be used for this purpose. Examples of terminated primers that may be used include alkyne terminated primers (e.g., which may attach to an azide surface moiety of the polymeric hydrogel **40**, **40**′), or azide terminated primers (e.g., which may attach to an alkyne surface moiety of the polymeric hydrogel **40**, **40**′), or any of the other terminated primers described in reference to the clustered solid support **13**.

(119) Specific examples of suitable primers **42**, **42**′ include P5 and P7 primers. Both P5 and P7 primers may be grafted to each of the polymeric hydrogels **40**, **40**′.

(120) In an example, grafting may involve flow through deposition (e.g., using a temporarily bound lid), dunk coating, spray coating, puddle dispensing, or by another suitable method that will attach the primer(s) **42**, **42**′ to the polymeric hydrogel **40**, **40**′. Each of these example techniques may utilize a primer solution or mixture, which may include the primer(s) **42**, **42**′, water, a buffer, and a catalyst. With any of the grafting methods, the primers **42**, **42**′ react with reactive groups of the polymeric hydrogel **40**, **40**′ in the concave region **38**, **38**′ and have no affinity for the surrounding substrate **26**A, **26**A′. As such, the primers **42**, **42**′ selectively graft to the polymeric hydrogel **40**, **40**′.

(121) In the example shown in FIG. **2**B, the chemical capture site **44**, **44**′ includes a chemical capture agent that is attached to or applied on at least a portion of the polymeric hydrogel **40**, **40**′. Any examples of the chemical capture agent disclosed herein may be used. For example, the chemical capture agent may be a member of a binding pair, where the other member of the binding pair is attached to the solid support **12**, **12**′.

(122) In some examples, free functional groups (e.g., those not attached to primers **42**, **42**′) of the polymeric hydrogel **40**, **40**′ may be functionalized with the chemical capture agent so that several chemical capture sites **44**, **44**′ are formed across the surface of the polymeric hydrogel **40**, **40**′. In an

example, alkyne-PEG-biotin linkers or alkyne-biotin free azide groups may be covalently attached to free azides on the polymeric hydrogel **40**, **40′** using click chemistry. In another example, primers that are complementary to the amplification primers **42**, **42′** may have the chemical capture agent attached thereto. These complementary primers may be hybridized to some of the amplification primers **42**, **42′** to form the chemical capture site **44**, **44′**.

(123) In another example, the chemical capture agent may be deposited in a desirable location using microcontact printing, aerosol printing, etc. to form the chemical capture site(s) **44**, **44′**. In still another example, a mask (e.g., a photoresist) may be used to define the space/location where the chemical capture agent will be deposited, and thus where the chemical capture site **44**, **44′** will be formed. The chemical capture agent may then be deposited, and the mask removed (e.g., via lift-off, dissolution, or another suitable technique). In this example, the chemical capture site **44**, **44′** may include a monolayer or thin layer of the chemical capture agent.

(124) FIG. **2**C illustrates a cross-sectional view of the flow cell **24** including patterned opposed sequencing surfaces **32**, **32′**. In an example, each of these surfaces **32**, **32′** may be prepared on the substrate **26**B, **26**B′, and then the substrates **26**B, **26**B′ may be attached to one another (e.g., via material **50**) to form an example of the flow cell **24**.

(125) In the example shown in FIG. **2**C, the flow cell **24** includes the multi-layer substrate **26**B, **26**B′, each of which includes the support **34**, **34′** and the patterned material **36**, **36′** positioned on the support **34**, **34′**. The patterned material **36**, **36′** defines depressions **48**, **48′** separated by interstitial regions **46**, **46′**.

(126) In the example shown in FIG. **2**C, the patterned material **36**, **36′** is respectively positioned on the support **34**, **34′**. It is to be understood that any material that can be selectively deposited, or deposited and patterned to form the depressions **48**, **48′** and the interstitial regions **46**, **46′** may be used for the patterned material **36**, **36′**.

(127) As one example, an inorganic oxide may be selectively applied to the support **34**, **34′** via vapor deposition, aerosol printing, or inkjet printing. Examples of suitable inorganic oxides include tantalum oxide (e.g., Ta.sub.2O.sub.5), aluminum oxide (e.g., Al.sub.2O.sub.3), silicon oxide (e.g., SiO.sub.2), hafnium oxide (e.g., HfO.sub.2), etc.

(128) As another example, a resin may be applied to the support **34**, **34′** and then patterned. Suitable deposition techniques include chemical vapor deposition, dip coating, dunk coating, spin coating, spray coating, puddle dispensing, ultrasonic spray coating, doctor blade coating, aerosol printing, screen printing, microcontact printing, etc. Suitable patterning techniques include photolithography, nanoimprint lithography (NIL), stamping techniques, embossing techniques, molding techniques, microetching techniques, printing techniques, etc. Some examples of suitable resins include a polyhedral oligomeric silsesquioxane resin (POSS)-based resin, a non-POSS epoxy resin, a poly(ethylene glycol) resin, a polyether resin (e.g., ring opened epoxies), an acrylic resin, an acrylate resin, a methacrylate resin, an amorphous fluoropolymer resin (e.g., CYTOP® from Bellex), and combinations thereof.

(129) As used herein, the term “polyhedral oligomeric silsesquioxane” (commercially available as POSS® from Hybrid Plastics) refers to a chemical composition that is a hybrid intermediate (e.g., RSiO.sub.1.5) between that of silica (SiO.sub.2) and silicone (R.sub.2SiO). An example of polyhedral oligomeric silsesquioxane can be that described in Kehagias et al., Microelectronic Engineering 86 (2009), pp. 776-778, which is incorporated by reference in its entirety. In an example, the composition is an organosilicon compound with the chemical formula [RSiO.sub.3/2].sub.n, where the R groups can be the same or different. Example R groups for polyhedral oligomeric silsesquioxane include epoxy, azide/azido, a thiol, a poly(ethylene glycol), a norbornene, a tetrazine, acrylates, and/or methacrylates, or further, for example, alkyl, aryl, alkoxy, and/or haloalkyl groups. The resin composition disclosed herein may comprise one or more different cage or core structures as monomeric units. The polyhedral structure may be a T.sub.8 structure, such as:

(130) ##STR00007##
and represented by:
(131) ##STR00008##
This monomeric unit typically has eight arms of functional groups R.sub.1 through R.sub.8.
(132) The monomeric unit may have a cage structure with 10 silicon atoms and 10 R groups, referred to as T.sub.10, such as:
(133) ##STR00009##
or may have a cage structure with 12 silicon atoms and 12 R groups, referred to as T.sub.12, such as:
(134) ##STR00010##
The polyhedral oligomeric silsesquioxane-based material may alternatively include T.sub.6, T.sub.14, or T.sub.16 cage structures. The average cage content can be adjusted during the synthesis, and/or controlled by purification methods, and a distribution of cage sizes of the monomeric unit(s) may be used in the examples disclosed herein.
(135) In some of the polyhedral oligomeric silsesquioxane examples disclosed herein, at least one of R.sub.1 through R.sub.8 or R.sub.10 or R.sub.12 comprises an epoxy. R.sub.1 through R.sub.8 or R.sub.10 or R.sub.12 may or may not be the same, and in some examples at least one of R.sub.1 through R.sub.8 or R.sub.10 or R.sub.12 comprises epoxy and at least one other of R.sub.1 through R.sub.8 or R.sub.10 or R.sub.12 is a non-epoxy functional group. The non-epoxy functional group may be (a) a reactive group that is orthogonally reactive to an epoxy group (i.e., reacts under different conditions than an epoxy group), that serves as a handle for coupling the resin to an amplification primer, a polymer, or a polymerization agent; or (b) a group that adjusts the mechanical or functional properties of the resin, e.g., surface energy adjustments. In some examples, the non-epoxy functional group is selected from the group consisting of an azide/azido, a thiol, a poly(ethylene glycol), a norbornene, a tetrazine, an amino, a hydroxyl, an alkynyl, a ketone, an aldehyde, an ester group, an alkyl, an aryl, an alkoxy, and a haloalkyl.
(136) As shown in FIG. **2**C, the patterned material **36**, **36**′ includes the depressions **48**, **48**′ respectively defined therein, and interstitial regions **46**, **46**′ separating adjacent depressions **48**, **48**′. Many different layouts of the depressions **48**, **48**′ may be envisaged, including regular, repeating, and non-regular patterns. In an example, the depressions **48**, **48**′ are disposed in a hexagonal grid for close packing and improved density. Other layouts may include, for example, rectilinear (rectangular) layouts, triangular layouts, and so forth. In some examples, the layout or pattern can be an x-y format of depressions **48**, **48**′ that are in rows and columns. In some other examples, the layout or pattern can be a repeating arrangement of depressions **48**, **48**′ and/or interstitial regions **46**, **46**′. In still other examples, the layout or pattern can be a random arrangement of depressions **48**, **48**′ and/or interstitial regions **46**, **46**′. The pattern may include spots, stripes, swirls, lines, triangles, rectangles, circles, arcs, checks, plaids, diagonals, arrows, squares, and/or cross-hatches.
(137) The layout or pattern of the depressions **48**, **48**′ may be characterized with respect to the density of the depressions **48**, **48**′ (e.g., number of depressions **48**, **48**′) in a defined area. For example, the depressions **48**, **48**′ may be present at a density of approximately 2 million per mm.sup.2. The density may be tuned to different densities including, for example, a density of about 100 per mm.sup.2, about 1,000 per mm.sup.2, about 0.1 million per mm.sup.2, about 1 million per mm.sup.2, about 2 million per mm.sup.2, about 5 million per mm.sup.2, about 10 million per mm.sup.2, about 50 million per mm.sup.2, or more, or less. It is to be further understood that the density of depressions **48**, **48**′ in the patterned material **36**, **36**′ can be between one of the lower values and one of the upper values selected from the ranges above. As examples, a high density array may be characterized as having depressions **48**, **48**′ separated by less than about 100 nm, a medium density array may be characterized as having depressions **48**, **48**′ separated by about 400 nm to about 1 μm, and a low density array may be characterized as having depressions **48**, **48**′ separated by greater than about 1 μm. While example densities have been provided, it is to

be understood that any suitable densities may be used. The density of the depressions **48**, **48′** may depend, in part, on the depth of the depressions **48**, **48′**. In some instances, it may be desirable for the spacing between depressions **48**, **48′** to be even greater than the examples listed herein.
(138) The layout or pattern of the depressions **48**, **48′** may also or alternatively be characterized in terms of the average pitch, or the spacing from the center of the depression **48**, **48′** to the center of an adjacent depression **48**, **48′** (center-to-center spacing) or from the left edge of one depression **48**, **48′** to the right edge of an adjacent depression **48**, **48′** (edge-to-edge spacing). The pattern can be regular, such that the coefficient of variation around the average pitch is small, or the pattern can be non-regular in which case the coefficient of variation can be relatively large. In either case, the average pitch can be, for example, about 50 nm, about 0.1 μm, about 0.5 μm, about 1 μm, about 5 μm, about 10 μm, about 100 μm, or more or less. The average pitch for a particular pattern of depressions **48**, **48′** can be between one of the lower values and one of the upper values selected from the ranges above. In an example, the depressions **48**, **48′** have a pitch (center-to-center spacing) of about 1.5 μm. While example average pitch values have been provided, it is to be understood that other average pitch values may be used.
(139) The size of each depression **48**, **48′** may be characterized by its volume, opening area, depth, and/or diameter.
(140) Each depression **48**, **48′** can have any volume that is capable of confining at least some fluid that is introduced into the flow cell **24**. The minimum or maximum volume can be selected, for example, to accommodate the throughput (e.g., multiplexity), resolution, nucleotides, or analyte reactivity expected for downstream uses of the flow cell **24**. For example, the volume can be at least about 1×10.sup.−3 μm.sup.3, at least about 1×10.sup.−2 μm.sup.3, at least about 0.1 μm.sup.3, at least about 1 μm.sup.3, at least about 10 μm.sup.3, at least about 100 μm.sup.3, or more. Alternatively or additionally, the volume can be at most about 1×10.sup.4 μm.sup.3, at most about 1×10.sup.3 μm.sup.3, at most about 100 μm.sup.3, at most about 10 μm.sup.3, at most about 1 μm.sup.3, at most about 0.1 μm.sup.3, or less.
(141) The area occupied by each depression opening can be selected based upon similar criteria as those set forth above for the volume. For example, the area for each depression opening can be at least about 1×10.sup.−3 μm.sup.2, at least about 1×10.sup.−2 μm.sup.2, at least about 0.1 μm.sup.2, at least about 1 μm.sup.2, at least about 10 μm.sup.2, at least about 100 μm.sup.2, or more. Alternatively or additionally, the area can be at most about 1×10.sup.3 μm.sup.2, at most about 100 μm.sup.2, at most about 10 μm.sup.2, at most about 1 μm.sup.2, at most about 0.1 μm.sup.2, at most about 1×10.sup.−2 μm.sup.t, or less. The area occupied by each depression opening can be greater than, less than or between the values specified above.
(142) The depth of each depression **48**, **48′** can be large enough to house some of the polymeric hydrogel **40**, **40′**. In an example, the depth may be at least about 0.1 μm, at least about 0.5 μm, at least about 1 μm, at least about 10 μm, at least about 100 μm, or more. Alternatively or additionally, the depth can be at most about 1×10.sup.3 μm, at most about 100 μm, at most about 10 μm, or less. In some examples, the depth is about 0.4 μm. The depth of each depression **48**, **48′** can be greater than, less than or between the values specified above.
(143) In some instances, the diameter or length and width of each depression **48**, **48′** can be at least about 50 nm, at least about 0.1 μm, at least about 0.5 μm, at least about 1 μm, at least about 10 μm, at least about 100 μm, or more. Alternatively or additionally, the diameter or length and width can be at most about 1×10.sup.3 μm, at most about 100 μm, at most about 10 μm, at most about 1 μm, at most about 0.5 μm, at most about 0.1 μm, or less (e.g., about 50 nm). In some examples, the diameter or length and width is about 0.4 μm. The diameter or length and width of each depression **48**, **48′** can be greater than, less than or between the values specified above.
(144) In this example, at least some of components of the sequencing surface **32**, **32′** may be introduced into the depressions **48**, **48′**. It is to be understood that any space within the depressions **48**, **48′** not occupied by the components of the sequencing surface **32**, **32′** may be considered to be

part of the flow channel **28**.
(145) In the example shown in FIG. **2**C, the polymeric hydrogel **40**, **40**′ is positioned within each of the depressions **48**, **48**′. The polymeric hydrogel **40**, **40**′ may be applied as described in reference to FIG. **2**B, so that the polymeric hydrogel **40**, **40**′ is present in the depressions **48**, **48**′ and not present on the surrounding interstitial regions **46**, **46**′.
(146) In the example shown in FIG. **2**C, the primers **42**, **42**′ may be grafted to the polymeric hydrogel **40**, **40**′ within each of the depressions **48**, **48**′. The primers **42**, **42**′ may be applied as described in reference to FIG. **2**B, and thus will graft to the polymeric hydrogel **40**, **40**′ and not to the surrounding interstitial regions **46**, **46**′.
(147) In the example shown in FIG. **2**C, the chemical capture site **44**, **44**′ includes a chemical capture agent that is applied on at least some of the interstitial regions **46**, **46**′. For example, the chemical capture agent may be deposited on at least some of the interstitial regions **46**, **46**′ using microcontact printing, aerosol printing, etc. to form the chemical capture site(s) **44**, **44**′. In still another example, a mask (e.g., a photoresist) may be used to define the space/location where the chemical capture agent will be deposited, and thus where the chemical capture site **44**, **44**′ will be formed. The chemical capture agent may then be deposited, and the mask removed (e.g., via lift-off, dissolution, or another suitable technique).
(148) In other examples, the chemical capture site **44**, **44**′ includes a chemical capture agent that is attached to free functional groups (e.g., those not attached to primers **42**, **42**′) of the polymeric hydrogel **40**, **40**′. In still other examples, the chemical capture site **44**, **44**′ includes a chemical capture agent that is attached to primers that are hybridized to some of the amplification primers **42**, **42**′. In these examples, the chemical capture site **44**, **44**′ will be present in the depressions **48**, **48**′ and not on the interstitial regions **46**, **46**′.
(149) Any examples of the chemical capture agent disclosed herein may be used in the example shown in FIG. **2**C.
(150) FIG. **2**D illustrates a cross-sectional view of the flow cell **24** including patterned opposed sequencing surfaces **31**, **31**′. In an example, each of these surfaces **31**, **31**′ may be prepared on the substrate **26**B, **26**B′, and then the substrates **26**B, **26**B′ may be attached to one another (e.g., via material **50**) to form an example of the flow cell **24**. Each of the multi-layer substrates **26**B, **26**B′ includes the support **34**, **34**′ and the patterned material **36**, **36**′ positioned on the support **34**, **34**′. The patterned material **36**, **36**′ defines depressions **48**, **48**′ separated by interstitial regions **46**, **46**′.
(151) The opposed sequencing surfaces **31**, **31**′ do not include the polymeric hydrogel **40**, **40**′ or the primers **42**, **42**′. Rather, the opposed sequencing surfaces **31**, **31**′ include the chemical capture site **44**, **44**′ positioned in each of the depressions **48**, **48**′. The respective chemical capture sites **44**, **44**′ are able to immobilize respective clustered solid supports **13**. Each of the clustered solid supports introduces a respective cluster of template strands **64** into each of the depressions **48**, **48**′.
(152) The chemical capture site **44**, **44**′ in FIG. **2**D includes any example of the chemical capture agent set forth herein. In this example, the chemical capture agent may be deposited in the depressions **48**, **48**′ using microcontact printing, aerosol printing, etc. to form the chemical capture site(s) **44**, **44**′. In still another example, a mask (e.g., a photoresist) may be used to block the interstitial regions **46**, **46**′, so that the chemical capture agent is deposited into the depressions **48**, **48**′ and not on the interstitial regions **46**, **46**′. In this example, the chemical capture agent may then be deposited, and the mask removed (e.g., via lift-off, dissolution, or another suitable technique).
(153) While not shown, another example of the flow cell **24** combines the non-patterned surface of FIG. **2**B with the capture site **44**, **44**′ of FIG. **2**D. In this example, the concave regions **38**, **38**′ (similar to those shown in FIG. **2**B) may be coated with the chemical capture agent rather than with the polymeric hydrogel **40**, **40**′ and primers **42**, **42**′. As such, the chemical capture sites **44**, **44**′ may be formed along the entire channel **28** in the concave regions **38**, **38**′. In this example, the respective chemical capture sites **44**, **44**′ are able to immobilize clustered solid supports **13** in a random distribution along the opposed sequencing surfaces.

(154) As shown in FIG. **2**B through FIG. **2**D, the substrates **26**A and **26**A′ or **26**B and **26**B′ are attached to one another so that the sequencing surfaces **30** and **30′** or **32** and **32′** or **31** and **31′** face each other with the flow channel **28** defined therebetween.
(155) The substrates **26**A and **26**A′ or **26**B and **26**B′ may be bonded to each other at some or all of the interstitial regions **46**, **46′**. The bond that is formed between the substrates **26**A and **26**A′ or **26**B and **26**B′ may be a chemical bond, or a mechanical bond (e.g., using a fastener, etc.).
(156) Any suitable technique, such as laser bonding, diffusion bonding, anodic bonding, eutectic bonding, plasma activation bonding, glass frit bonding, or other methods known in the art may be used to bond the substrates **26**A and **26**A′ or **26**B and **26**B′ together. In an example, a spacer layer (e.g., material **50**) may be used to bond the substrates **26**A and **26**A′ or **26**B and **26**B′. The spacer layer may be any material **50** that will seal at least some portion of the substrates **26**A and **26**A′ or **26**B and **26**B′ together. In some examples, the spacer layer can be a radiation-absorbing material that aids in bonding.
(157) Method and Kit with Multiple Fluids
(158) An example of the method that utilizes a combination of fluids having different densities is shown in FIG. **3**A and FIG. **3**B.
(159) The method generally includes immobilizing a target material **11** (such as complexes **10**A, **10**B, clustered solid supports **13**) at each of two opposed sequencing surfaces **30**, **30′** or **32**, **32′** or **31**, **31′** of a flow cell **24** by introducing a first fluid **52** (FIG. **3**A), including a first portion of the target material **11** therein, into the flow cell **24**, whereby at least some of the target material **11** become immobilized by capture sites **44**, **44′** on one **30** or **30′**, or **32** or **32′**, or **31** or **31′** of the two opposed sequencing surfaces **30**, **32** or **30′**, **32′**, or **31**, **31′**; removing the first fluid and any non-immobilized target material from the flow cell **24**; and introducing a second fluid **54** (FIG. **3**B), including a second portion of the target material **11** therein, into the flow cell **24**, whereby at least some of the target material **11** become immobilized by capture sites **44**, **44′** on another **30′** or **30**, or **32′** or **32**, or **31′** or **31** of the two opposed sequencing surfaces **30**, **32** or **30′**, **32′**, or **31**, **31′**; wherein one of: the first fluid **52** has a density less than a density of the target material **11** and the second fluid **54** has a density greater than the density of the target material **11**; or the second fluid **54** has the density less than the density of the target material **11** and the first fluid **52** has the density greater than the density of the target material **11**.
(160) Prior to performing the method shown in FIG. **3**A and FIG. **3**B, the target material **11** may be prepared or obtained.
(161) In one example, the complexes **10**A or **10**B may be prepared using a nucleic acid sample and a library preparation fluid including a plurality of solid supports **12**, **12′** therein. In some examples, each of the solid supports **12**, **12′** in the library preparation fluid may have, for example, adapters (such as adapters **18**) and transposome complexes attached thereto, as described in reference to FIG. **1**A. Tagmentation and library preparation may be performed as defined in FIG. **1**A to form the complexes **10**A. The nucleic acid sample, the solid supports **12**, **12′**, the partial Y-adapters, and the transposase enzyme may be contained in separate fluids until it is desirable to form the complexes **10**A. In other examples, each of the solid supports **12**, **12′** in the library preparation fluid may have, for example, oligonucleotides attached thereto. In some examples, PCR-free nucleotide library preparation may take place separately from the solid supports **12**, **12′**, and then the prepared library fragments can be hybridized to the oligonucleotides at the surface of the solid supports **12**, **12′**, as described in reference to FIG. **1**B. Other examples of library preparation may be used (e.g., including PCR), as long as the fragments are denatured into single stranded fragments before being hybridized to the oligos on the solid supports **12**, **12′**.
(162) In another example, the clustered solid supports **13** may be prepared by amplifying a library fragment in the presence of a plurality of solid supports **12**, **12′** functionalized with primers **42**, **42′**.
(163) The target material **11** (e.g., complexes **10**A or **10**B, or any other solid support **12**, **12′** having sequencing-ready fragments **14**, **14′** attached thereto, or clustered solid supports **13**) may be divided

into first and second portions. The first portion of the target material **11** may be incorporated into the first fluid **52** and the second portion of the target material **11** may be introduced into the second fluid **54**.

(164) The first and second fluids **52**, **54** have different densities. In one example, the first fluid **52** has a density less than a density of the target material **11** and the second fluid **54** has a density greater than the density of the target material **11**. In one specific example, the first fluid **52** has a density less than a density of the solid support **12**, **12′** of the complexes **10**A or **10**B or clustered solid support **13** and the second fluid **54** has a density greater than the density of the solid support **12**, **12′** of the complexes **10**A or **10**B or clustered solid support **13**. In another example, the second fluid **54** has the density less than the density of the target material **11** and the first fluid **52** has the density greater than the density of the target material **11**. In another specific example, the second fluid **54** has the density less than the density of the solid support **12**, **12′** of the complexes **10**A or **10**B or clustered solid support **13** and the first fluid **52** has the density greater than the density of the solid support **12**, **12′** of the complexes **10**A or **10**B or clustered solid support **13**. As such, the density of each of the fluids **52**, **54** depends upon the target material **11** that is used. In some examples, the density of the complexes **10**A or **10**B or clustered solid support **13** is approximately equal to the density of the solid support **12**, **12′** used in the complex **10**A or **10**B or the clustered solid support **13**, and thus in the specific examples that are provided, the density of each of the fluids **52**, **54** depends upon the solid support **12**, **12′** that is used in the target material **11**.

(165) The densities of the fluid **52**, **54** may be measured at a capture temperature of the target material **11** (e.g., complex **10**A, **10**B or clustered solid support **13**) that is introduced into the flow cell **24**. In an example, the capture temperature ranges from about 18° C. to about 40° C.

(166) In one example, the density of one of the fluids **52** or **54** at the capture temperature is at least 0.1 g/cm.sup.3 less than the density of the target material **11** (e.g., the solid support **12**, **12′** of the complexes **10**A or **10**B or clustered solid support **13**) at the capture temperature, and the density of the other of the fluids **54** or **52** at the capture temperature is at least 0.1 g/cm.sup.3 greater than the density of the target material **11** (e.g., the solid support **12**, **12′** of the complexes **10**A or **10**B or clustered solid support **13**) at the capture temperature. In one specific example, when the density of the target material (e.g., solid support **12**, **12′**) is X g/cm.sup.3, the density of one of the fluids **52** or **54** at the capture temperature is X g/cm.sup.3−0.1 g/cm.sup.3, and the density of the other of the fluids **54** or **52** at the capture temperature is X g/cm.sup.3+0.1 g/cm.sup.3.

(167) In an addition to having the respective densities, the fluids **52**, **54** should also be compatible with the target material **11**. When complexes **10**A, **10**B are used, the fluids **52**, **54** should be compatible the complexes **10**A, **10**B and the sequencing surfaces **30**, **30′** or **32**, **32′** or **31**, **31′** so that the fragments **14**, **14′**, **14″** and the primers **42**, **42′** are not deleteriously affected. When clustered solid supports **13** are used, the fluids **52**, **54** should be compatible the clustered solid support **13** so that the template strands **64** are not deleteriously affected.

(168) The lower density fluid **52** or **54** may be any aqueous buffer solution (e.g., a weak acid and one of its salts (conjugate base) or a weak base and one of its salts (conjugate acid). The salt concentration in the aqueous buffer solution may be adjusted so that the density of the lower density fluid **52** or **54** is less than the density of the target material **11** (e.g., the density of the solid support **12**, **12′** of the complexes **10**A, **10**B or clustered solid supports **13**). The greater the density difference is between the target material **11** and the lower density fluid **52** or **54**, the faster the settling time is of the target material **11** (e.g., complexes **10**A, **10**B or clustered solid supports **13**) in the lower density fluid **52** or **54**. As examples, the lower density fluid **52** or **54** may be a Tris-HCl buffer or 0.5× saline sodium citrate (SSC) buffer. In an example, the lower density fluid **52** or **54** is an aqueous buffer solution having a density of about 1 g/cm.sup.3. This lower density fluid **52** or **54** may be particular suitable for use with a target material **11** having a density of about 1.18 g/cm.sup.3.

(169) The higher density fluid **54** or **52** may be an aqueous salt solution. The salt selected should

render the fluid **52** or **54** as “heavy” and should also not deleteriously affect the target material. When complexes **10**A, **10**B are used, the salt should not deleteriously affect the complexes **10**A, **10**B or the primers **42**, **42**′. When clustered solid supports **13** are used, the salt should not deleteriously affect the template strands **64**. The salt concentration in the aqueous buffer solution may be adjusted so that the density of the higher density fluid **54** or **52** is greater than the density of the target material **11**. Examples of the higher density fluid **54** or **52** include sodium polytungstate solutions and sodium chloride solutions. In an example, the higher density fluid **54** or **52** is a sodium polytungstate solution having a density ranging from about 2 g/cm.sup.3 to about 3 g/cm.sup.3. These higher density fluids **54** or **52** may be particular suitable for use with a target material **11** having a density of about 1.18 g/cm.sup.3. In these examples, the sodium polytungstate solution has a concentration ranging from about 1 gram of sodium polytungstate per 1 milliliter of water to about 2.52 grams of sodium polytungstate per 1 milliliter of water. In another example, a 25% (w/v) sodium chloride solution has a density of about 1.2 g/cm.sup.3.

(170) In one example, the first or second fluid **52** or **54** having the density less than the density of the target material is an aqueous buffer solution, and the second or first fluid **54** or **52** having the density greater than the density of the target material is a sodium polytungstate solution or a sodium chloride solution. In another example, the density of the first or second fluid **52** or **54** that is less than the density of the target material is about 1 g/cm.sup.3 at a capture temperature, and wherein the density of the second or first fluid **54** or **52** that is greater than the density of the target material is about 2 g/cm.sup.3 at the capture temperature.

(171) As shown in FIG. **3**A, one example of the method involves introducing the first fluid **52** including some of the target material **11** (e.g., complexes **10**A in FIG. **3**A) into the flow cell **24**. In this example, the first fluid **52** has a lower density than the density of the solid support **12**, **12**′ of the complexes **10**A, and thus the complexes **10**A migrate to or settle at the bottom sequencing surface **30**′. The capture sites **44**′ (not shown in FIG. **3**A) immobilize at least some of the complexes **10**A at the bottom sequencing surface **30**′.

(172) It is to be understood that some complexes **10**A (or other target material **11**) in the first fluid **52** may not settle, and these complexes **10**A (or other target material) will be removed from the flow cell **24** before further processing. A predetermined time may be allowed to pass before removing the first fluid **52** and any non-immobilized target material (e.g., complexes **10**A) from the flow cell **24**. In an example, the predetermined time may range from about 5 minutes to about 30 minutes in order to obtain a desirable number of immobilized complexes **10**A or other target material **11**. Longer incubation times may also be used.

(173) This example method then includes washing away the first fluid **52** and non-immobilized target material **11** (e.g., complexes **10**A) from the flow cell **24**. Washing may involve introducing a washing fluid into the flow cell **24**. The flow may push any complexes **10**A (or other target materials **11**) that have not settled and become immobilized at the sequencing surface **30**′ out through an exit port of the flow cell **24**. The immobilization mechanism (e.g., binding pair, hybridization, covalent bonding, etc.) between the complexes **10**A (or other target materials **11**) and the capture sites **44**′ of the sequencing surface **30**′ may prevent any settled and immobilized complexes **10**A (or other immobilized target materials **11**) from becoming part of the exit flow. Moreover, the target material **11** (e.g., complexes **10**A in FIG. **3**A) immobilized on one of the two opposed sequencing surfaces (e.g., sequencing surface **30**′ in FIG. **3**A) remains immobilized on that sequencing surface when the second fluid **54** is introduced.

(174) As shown in FIG. **3**B, this example of the method involves introducing the second fluid **54** including some other of the target material **11** (e.g., complexes **10**A) into the flow cell **24**. In this example, the second fluid **54** has a higher density than the density of the solid support **12**, **12**′ of the complexes **10**A (or other target material **11**), and thus the complexes **10**A migrate to the top sequencing surface **30**. The capture sites **44** (not shown in FIG. **3**B) immobilize at least some of the complexes **10**A at the sequencing surface **30**.

(175) Prior to performing seeding, amplification, and sequencing or sequencing (as described below), this example method may further include removing the second liquid **54** and non-immobilized target material **11** from the flow cell **24**. As such, this example method may then include washing away the second fluid **54** and non-trapped target material **11** (e.g., non-immobilized complexes **10**A) from the flow cell **24**. Washing may be performed as described herein. The flow may push any complexes **10**A (or other target materials **11**) that have not become immobilized at the upper sequencing surface **30** out through an exit port of the flow cell **24**. It is to be understood that he immobilization mechanism (e.g., binding pair, hybridization, covalent bonding, etc.) between the complexes **10**A (or other target materials **11**) and the respective capture sites **44**, **44′** of the sequencing surfaces **30**, **30′** may prevent any immobilized complexes **10**A (or other immobilized target materials **11**) from becoming part of the exit flow.
(176) When complexes **10**A or **10**B are used, this washing step may be followed by library fragment release and amplification (e.g., an example of which is described in reference to FIG. **9**A through FIG. **9**C). When clustered solid supports **13** are used, this washing step may be followed by sequencing.
(177) While the example shown in FIG. **3**A and FIG. **3**B illustrates the introduction of the lower density fluid and then the higher density fluid, it is to be understood that the higher density fluid may be introduced first to immobilize target material **11** on the upper sequencing surface **30**, and then the lower density fluid may be introduced to immobilize target material **11** on the lower/bottom sequencing surface **30′**. Moreover, it is to be understood that this method may be performed with any example of the flow cell **24** disclosed herein, including those with the patterned surfaces **32**, **32′**. When the clustered solid supports **13** are used, a flow cell **24** without amplification primers **42**, **42′**, such as that shown and described in reference to FIG. **2**D, may be used.
(178) A kit to perform the method described in reference to FIGS. **3**A and **3**B may include a preparation fluid including a target material **11** therein; a first introduction fluid (e.g., fluid **52** or **54**) having a density less than a density of the target material **11**; and a second introduction fluid (fluid **54** or **52**) having a density greater than the density of the target material **11**. In one example kit, the first introduction fluid is an aqueous buffer solution, and the second introduction fluid is a sodium polytungstate solution or a sodium chloride solution. In one example when the second introduction fluid is the sodium polytungstate solution, the sodium polytungstate solution has a concentration of about 1 gram of sodium polytungstate per 1 milliliter of water. In another example kit, the density of the first introduction fluid at a capture temperature is at least 0.1 g/cm.sup.3 less than the density of the target material **11** at the capture temperature, and the density of the second introduction fluid at the capture temperature is at least 0.1 g/cm.sup.3 greater than the density of the target material **11** at the capture temperature. In still another example, the density of the first introduction fluid is about 1 g/cm.sup.3 at a capture temperature, and wherein the density of the second introduction fluid is about 2 g/cm.sup.3 at the capture temperature.
(179) In some examples, the preparation fluid including the target material **11** includes the solid supports **12**, **12′**, and the kit may also include other library preparation components, such as a nucleic acid sample, partial Y-adapters, transposase enzymes, etc.; each of which may be contained in a separate fluid until it is desirable to form the target material **11**, such as the complex **10**A, **10**B, the clustered solid support **13**, etc. Some examples of the kit may also include the flow cell **24**. Other examples of the kit may include preparation fluids that include any examples of the target material **11** disclosed herein.
(180) Methods and Kits with One Fluid
(181) Other examples of the method disclosed herein utilize one fluid during the immobilization of the target material **11**. Some methods utilize one target material **11** and different modalities to achieve immobilization across the opposed sequencing surfaces **30**, **30′** or **32**, **32′** or **31**, **31′**. Other methods utilize two different target materials **11** (each having at least one property that is different

from each other), and the same or different modalities to achieve immobilization across the opposed sequencing surfaces **30**, **30′** or **32**, **32′** or **31**, **31′**. Different examples are described herein in reference to FIG. **4**A and FIG. **4**B through FIG. **8**A and FIG. **8**B.

(182) Prior to performing any of the methods shown in FIG. **4**A and FIG. **4**B through FIG. **8**A and FIG. **8**B, the complexes **10**A or **10**B or clustered solid supports **13** may be prepared as described herein.

(183) The complexes **10**A or **10**B may be prepared using a nucleic acid sample and a library preparation fluid including a plurality of magnetic solid supports **12′** therein. In some examples, each of the magnetic solid supports **12′** in the library preparation fluid may have, for example, adapters (such as adapters **18**) and transposome complexes attached thereto, as described in reference to FIG. **1**A. Tagmentation and library preparation may be performed as defined in FIG. **1**A to form the complexes **10**A. The nucleic acid sample, the magnetic solid supports **12′**, the partial Y-adapters, and the transposase enzyme may be contained in separate fluids until it is desirable to form the complexes **10**A. In other examples, each of the magnetic solid supports **12′** in the library preparation fluid may have, for example, oligonucleotides attached thereto. In some examples, PCR-free nucleotide library preparation may take place separately from the magnetic solid supports **12′**, and then the prepared library fragments can be hybridized to the oligonucleotides at the surface of the magnetic solid supports **12′**, as described in reference to FIG. **1**B. Other examples of library preparation may be used (e.g., including PCR), as long as the fragments are denatured into single stranded fragments before being hybridized to the oligos on the magnetic solid supports **12′**.

(184) The clustered solid supports **13** may be prepared by amplifying a library fragment in the presence of a plurality of solid supports **12**, **12′** funcionalized with primers **42**, **42′**.

(185) An example of the method that utilizes a fluid, a substantially uniform magnetic force, and a magnetically responsive target material, such as the solid support **12′** is shown in FIG. **4**A and FIG. **4**B. The method generally includes immobilizing a target material **11** at each of two opposed sequencing surfaces **30**, **30′** or **32**, **32′** of a flow cell **24** by introducing a fluid **56**, including the target material **11**, into the flow cell **24**, wherein the fluid **56** has a density approximately equivalent to a density of the magnetic solid support **12′**; allowing some of the target material **11** to become immobilized by capture sites **44** or **44′** (not shown in FIG. **4**A) on one **30** or **30′**, or **32** or **32′**, or **31** or **31′**, of the two opposed sequencing surfaces **30**, **30′** or **32**, **32′** or **31**, **31′**; and applying a magnetic force to another **30′** or **30**, or **32′** or **32**, or **31′** or **31** of the two opposed sequencing surfaces **30**, **30′** or **32**, **32′** or **31**, **31′**, thereby pulling some other of the target material **11** to the other **30′** or **30**, or **32′** or **32**, or **31′** or **31** of the two opposed sequencing surfaces **30**, **30′** or **32**, **32′** or **31**, **31′** where they become immobilized by capture sites **44′** or **44** (not shown in FIG. **4**B) on the other of the two opposed sequencing surfaces **30**, **30′** or **32**, **32′** or **31**, **31′**. When complexes **10**A, **10**B are used and prior to performing seeding and amplification (as described below), this example method may further include ceasing the application of the magnetic force and removing the fluid and non-immobilized target material from the flow cell **24**. These steps may be followed by library fragment release and amplification (e.g., as described in reference to FIG. **9**A through FIG. **9**C).

(186) The target material **11** (e.g., complexes **10**A, **10**B, or any other magnetic solid support **12′** having sequencing-ready fragments **14**, **14′**, **14″**, or clustered solid supports **13**) attached thereto, may be incorporated into the fluid **56**. As one example, from about 25,000 target materials **11** (e.g., complexes **10**A, **10**B or clustered solid supports **13**) to about 500,000 target materials **11** may be included in a microliter of fluid. As another example, from about 100,000 target materials **11** to about 500,000 target materials **11** may be included in a microliter of fluid. Other concentrations may be used depending upon the size of the flow cell **24**.

(187) The density of the fluid **56** may be measured at a capture temperature of the target materials **11** that is introduced into the flow cell **24**. In an example, the capture temperature ranges from about 18° C. to about 40° C.

(188) The fluid **56** is selected to have a density that is at least approximately equivalent to the density of the magnetic solid support **12′** of the target material **11**. In these examples, "at least approximately equivalent," means that the density of the fluid **56** is within 0.08 g/cm.sup.3 of the density of the magnetic solid support **12′**. In some instances, the densities of the fluid **56** and the magnetic solid supports **12′** are the same. By having an at least approximately equivalent density with the magnetic solid support **12′**, the fluid **56** functions as a mild floating agent. As used herein, the term "mild floating agent" refers to a fluid in which the target material **11** (e.g., complexes **10**A, **10**B, clustered solid supports **13**, etc.) are able to float for at least some time period before sinking or settling. In the fluid **56**, some of the target material **11** begins to sink and become immobilized to the lower/bottom sequencing surface **30′**, **32′**, **31′** in the flow cell **24**, while other target material **11** remains afloat (at least for some period of time).
(189) The fluid **56** may be any aqueous buffer solution. The salt concentration in the aqueous buffer solution may be adjusted so that the density of the fluid **56** is at least approximately equal to the density of the magnetic solid support **12′**. In other words, the salt concentration in the aqueous buffer solution may be adjusted so that the density of the fluid **56** is within +/−0.08 g/cm.sup.3 of the density of the magnetic solid support **12′**. As examples, the fluid **56** may be a Tris-HCl buffer or 0.5× saline sodium citrate (SSC) buffer or a 75 mM sodium citrate solution (pH=7) containing about 750 mM NaCl. In an example, the density of each of the magnetic solid support **12′** and the fluid **56** is about 1.1 g/cm.sup.3.
(190) After the fluid **56** and the target material **11** are introduced into the flow cell **24**, the target material **11** initially floats in the fluid **56**. As time passes, some of the target material **11** will settle to the lower/bottom sequencing surface **30′**, **32′**, **31′** where it becomes immobilized at the capture site(s) **44′**. An example is shown in FIG. **4**A, where some of the complexes **10**A have settled on the lower/bottom sequencing surface **30′**. The fluid **56** helps to prevent settling of all of the target material **11** on the lower/bottom sequencing surface **30′**, **32′**, **31′** too fast.
(191) As such, after introduction of the fluid **56** and immobilization of some of the target material **11**, there is time for an externally applied magnetic force to be applied to the other sequencing surfaces **30**, **32**, **31** in the flow cell **24**. The magnetic force attracts the floating target material **11** to the upper/top sequencing surface **30**, **32**, **31** in the flow cell **24**. An example is shown in FIG. **4**B, where some of the complexes **10**A have migrated to the upper/top sequencing surface **30**.
(192) In this example method, a predetermined time period may be allowed to pass between the introduction of the fluid **56** and the application of the magnetic force. This may be desirable so that some of the target material **11** settles and becomes immobilized at the one sequencing surface **30′**, **32′**, **31′** while the remaining target material **11** stays afloat in the fluid **56**. In an example, this predetermined time ranges from about 5 minutes to about 30 minutes. In some examples, the predetermined time period passes between the introduction of the fluid **56** and the application of the magnetic force, and the predetermined time ranges from about 5 seconds to about 2 minutes.
(193) As shown in FIG. **4**B, the magnetic force is then applied by placing a magnet **58** on an exterior surface **60** of the flow cell **24** that is adjacent to the sequencing surface **30**, **32**. The magnet **58** should have a magnetic field strength that is sufficient to attract the floating target material **11** (e.g., complexes **10**A, **10**B, clustered solid supports **13**, etc.) without attracting the target material **11** that is already immobilized on the lower/bottom sequencing surface **30′**, **32′**, **31′**. The magnetic field strength is relatively weak, but is at least substantially uniformly applied across the entire length and width of the flow channel **28**. A relatively weak magnetic field strength may range from about 1 mT (milliTesla) to about 100 mT. In some examples, the strength of the relatively weak magnetic field ranges from about 1 mT to about 10 mT, or from about 10 mT to about 100 mT. This enables floating target material **11** to become immobilized to capture sites **44** across the upper/top sequencing surface **30**, **32**, **31**. Stronger magnets, such as neodymium magnets, may be used in some instances, and these magnets have a field strength of about 1 T (Tesla).
(194) In an example, the magnet **58** has the same length and width as the flow channel **28** and/or

the flow cell **24**. In an example, the magnet **58** is similar to a refrigerator magnet and has a magnetic field strength of about 5 mT. In another example, the magnet **58** is an elastomeric strip that has small magnetic particles embedded therein. These types of flexible magnets are commercially available, for example, from Uline, Arnold Magnetic Technologies (FLEXMAG™), etc. In an example, the application of the magnetic force involves placing an elastomeric strip embedded with magnetic particles on an exterior surface **60** of the flow cell **24** adjacent to the other of the two opposed sequencing surfaces (i.e., the sequencing surface **30** that does not have the target material **11** immobilized thereon). In some examples, the magnet may be applied manually. In other examples, the application of the magnetic force may be automated, e.g., when it is integrated into the sequencing system.

(195) The time frame for application of the magnet **58** (and thus the magnetic force) depends, in part, upon the strength of the magnet and the concentration of the complexes **10**A, **10**B in the fluid **56**. As an example, the magnet **58** may be applied for 5 seconds to about 2 minutes. Examples of the method then include ceasing the application of the magnetic force. This may be accomplished by removing the magnet **58**.

(196) It is to be understood that some target material **11** (e.g., complexes **10**A, **10**B, clustered solid supports **13**) in the fluid **56** may not become immobilized at either of the sequencing surface **30**, **30′** or **32**, **32′** or **31**, **31′**, and this target material **11** can be removed from the flow cell **24** before further processing. As such, this example method may include washing away the fluid **56** and non-trapped target material **11** from the flow cell **24**. Washing may involve introducing a washing fluid into the flow cell **24**. The flow may push any target material **11** that has not become immobilized at the sequencing surfaces **30**, **30′** or **32**, **32′** or **31**, **31′** out through an exit port of the flow cell **24**. The immobilization mechanism (e.g., binding pair, hybridization, covalent bonding, etc.) between the target material **11** and the capture sites **44**, **44′** of the sequencing surfaces **30**, **30′** or **32**, **32′** or **31**, **31′** may prevent any immobilized target material **11** from becoming part of the exit flow.

(197) While the example shown in FIG. **4**A and FIG. **4**B illustrates the flow cell **24** with sequencing surfaces **30** and **30′**, it is to be understood that this method may be performed with any example of the flow cell **24** disclosed herein, including those with the patterned sequencing surface **32**, **32′**. When clustered solid supports **13** including magnetically responsive solid supports **12′** are used, a flow cell **24** without amplification primers **42**, **42′** may be used, such as that shown and described in reference to FIG. **2**D. Moreover, any other magnetically responsive target material may be used in this example of the method.

(198) A kit to perform the method described in reference to FIGS. **4**A and **4**B may include a preparation fluid including a plurality of magnetic solid supports **12′** therein; and an introduction fluid (e.g., fluid **56**) having a density approximately equivalent to a density of the magnetic solid support **12′**. The kit may also include other library preparation components, such as a nucleic acid sample, partial Y-adapters, transposase enzymes, etc.; each of which may be contained in a separate fluid until it is desirable to form the target material **11**, such as the complex **10**A, **10**B, clustered solid support **13**, etc. Some examples of the kit may also include the flow cell **24**. Still other examples of the kit may include an amplification mix including a liquid form of a temperature responsive material.

(199) The methods shown in FIG. **5**A and FIG. **5**B, FIG. **6**A and FIG. **6**B, FIG. **7**A and FIG. **7**B, and FIG. **8**A and FIG. **8**B will now be described. Each of these methods uses a combination of target materials (e.g., **11**A and **11**B, or **11**C and **11**D, etc.), and different target material combinations are described in more detail with respect to each set of figures. Each set of figures depicts the method being performed with the flow cell **24** having non-patterned sequencing surfaces **30**, **30′**. However, it is to be understood that any of these methods may be performed with any example of the flow cell **24** disclosed herein, including those with the patterned surfaces **32**, **32′**. Additionally, when the clustered solid supports **13** are used as the target materials (e.g., **11**A and **11**B, etc.), a flow cell **24** without amplification primers **42**, **42′**, such as that shown and

described in reference to FIG. **2**D, may be used.

(200) One example of the method that utilizes a combination of target materials **11**A, **11**B is shown in FIG. **5**A and FIG. **5**B. In this example, the target materials **11**A, **11**B have densities that are different from each other and different from a carrier fluid.

(201) This example method generally includes simultaneously immobilizing a first target material **11**A at a first **30** or **32** or **31** of two opposed sequencing surfaces **30**, **30′**, or **32**, **32′**, or **31**, **31′** of a flow cell **24** and a second target material **11**B at a second **30′** or **32′** or **31′** of the two opposed sequencing surfaces **30**, **30′**, or **32**, **32′**, or **31**, **31′** by introducing, into the flow cell **24**, a target fluid **56′** including the first target material **11**A and the second target material **11**B, wherein the carrier fluid of the target fluid **56′** has a fluid density; the first target material **11**A has a first density less than the fluid density; and the second target material **11**B has a second density greater than the fluid density.

(202) The density of the carrier fluid of the target fluid **56′** may be measured at a capture temperature of the target materials **11**A, **11**B that are introduced into the flow cell **24**. In an example, the capture temperature ranges from about 18° C. to about 40° C.

(203) In one example, the density of one of the target materials **11**A is at least 0.1 g/cm.sup.3 less than the density of the carrier fluid at the capture temperature, and the density of the other of the target materials **11**B is at least 0.1 g/cm.sup.3 greater than the density of the carrier fluid at the capture temperature. In one specific example, when the density of the carrier fluid is X g/cm.sup.3 at the capture temperature, the density of one of the target materials **11**A or **11**B is X g/cm.sup.3−0.1 g/cm.sup.3, and the density of the other the target materials **11**B or **11**A is X g/cm.sup.3+0.1 g/cm.sup.3.

(204) The carrier fluid of the target fluid **56′** may be any of the aqueous buffer solutions or aqueous salt solutions set forth herein. The salt concentration in the aqueous buffer solution or aqueous salt solution may be adjusted so that the density of the carrier fluid at the capture temperature is between the respective densities of the target materials **11**A, **11**B. In another example, the carrier fluid of the target fluid **56′** is an ionic liquid.

(205) The target materials **11**A, **11**B may be complexes **10**A, **10**B or clustered solid supports **13**. The support **12** for the target materials **11**A, **11**B may be any of the examples set forth herein as long as the densities of the respective materials **11**A, **11**B are different with respect to the carrier fluid, as described in this example method. The density of the solid support **12** in each of the target materials **11**A, **11**B is at least approximately equal to the density of the respective target material **11**A, **11**B. As such, the solid support **12** of the target material **11**A is selected to have a density that is lower than the density of the carrier fluid of the target fluid **56′** at the capture temperature, and the solid support **12** of the target material **11**B is selected to have a density that is higher than the density of the carrier fluid of the target fluid **56′** at the capture temperature.

(206) As shown in FIG. **5**A, this method involves introducing the target fluid **56′** including the target materials **11**A, **11**B into the flow cell **24**. The target fluid **56′** may be allowed to incubate in the flow cell **24** for a predetermined time. In an example, the predetermined time may range from about 5 minutes to about 30 minutes in order to obtain a desirable number of immobilized target materials **11**A, **11**B on the sequencing surfaces **30**, **30′**. Longer incubation times may also be used.

(207) As mentioned, the solid support **12** of the target material **11**A has a lower density than the density of the carrier fluid at the capture temperature, and thus the target material **11**A migrates or floats to the upper sequencing surface **30**, as shown in FIG. **5**B. The capture sites **44** (not shown in FIG. **5**B) immobilize at least some of the target material **11**A at the upper sequencing surface **30**. Also as mentioned, the solid support **12** of the target material **11**B has a higher density than the density of the carrier fluid at the capture temperature, and thus the target material **11**B migrates to or settles on the bottom sequencing surface **30′**, as shown in FIG. **5**B. The capture sites **44′** (also not shown in FIG. **5**B) immobilize at least some of the target material **11**B at the lower/bottom sequencing surface **30′**.

(208) The immobilization of the target materials **11**A, **11**B occurs simultaneously upon introduction of the target fluid **56**′ to the flow cell **24** due to the different densities of the target materials **11**A, **11**B with respect to the carrier fluid. As such, in the method of FIG. **5**A and FIG. **5**B, at least some of the first target material **11**A becomes immobilized by respective capture sites **44** on the first of the two opposed sequencing surfaces **30**, and at least some of the second target material **11**B becomes immobilized by respective capture sites **44**′ on the second of the two opposed sequencing surfaces **30**′.
(209) It is to be understood that some target materials **11**A, **11**B may not become immobilized, and these target materials **11**A, **11**B will be removed from the flow cell **24** before further processing. As such, this example method then includes washing away the carrier fluid of the target fluid **56**′ and non-immobilized target materials **11**A, **11**B from the flow cell **24**. Washing may involve introducing a washing fluid into the flow cell **24**. The flow may push any target materials **11**A, **11**B that have not become immobilized at the sequencing surfaces **30**, **30**′ out through an exit port of the flow cell **24**. The immobilization mechanism (e.g., binding pair, hybridization, covalent bonding, etc.) between the respective target materials **11**A, **11**B and the capture sites **44**, **44**′ of the sequencing surfaces **30**, **30**′ may prevent any immobilized target materials **11**A, **11**B from becoming part of the exit flow.
(210) When complexes **10**A or **10**B are used as the target materials **11**A, **11**B, this washing step may be followed by library fragment release and amplification (e.g., an example of which is described in reference to FIG. **9**A through FIG. **9**C). When clustered solid supports **13** are used, this washing step may be followed by sequencing.
(211) A kit to perform the method described in reference to FIGS. **5**A and **5**B may include the target fluid **56**′, which includes the carrier fluid having a fluid density; a first target material **11**A having a first density less than the fluid density; and a second target material **11**B having a second density greater than the fluid density.
(212) In some examples, the first and second target materials **11**A, **11**B are complexes **10**A or **10**B. In these examples, the first target material **11**A includes a first solid support **12** having a first solid support density approximately equal to the first density (i.e., less than the fluid density), and sequencing-ready nucleic acid fragments **14**, **14**′, **14**″ attached to the first solid support **12**; and the second target material **11**B includes a second solid support **12** having a second solid support density approximately equal to the second density (i.e., greater than the fluid density), and sequencing-ready nucleic acid fragments **14**, **14**′, **14**″ attached to the second solid support **12**.
(213) In other examples, the first and second target materials **11**A, **11**B are clustered solid supports **13**. In these examples, the first target material **11**A includes a first solid support **12** having a first solid support density approximately equal to the first density (i.e., less than the fluid density), and a first cluster of template strands **64** attached to the first solid support **12**; and the second target material **11**B includes a second solid support **12** having a second solid support density approximately equal to the second density (i.e., greater than the fluid density), and second cluster of template strands **64** attached to the second solid support **12**.
(214) The kit may alternatively include the carrier fluid, reagents and materials to prepare the target material **11**A, and reagents and materials to prepare the target material **11**B. In this example, the respective target materials **11**A, **11**B may be prepared using the respective reagents and materials and as described herein, and then they may be added to the carrier fluid to form the target fluid **56**′.
(215) Other examples of the method utilize different target materials and different modalities to immobilize the target materials. These examples generally include introducing first and second target materials to a flow cell **24** including two opposed sequencing surfaces **30**, **30**′ or **32**, **32**′ or **31**, **31**′, wherein the first target material has at least one property that is different from the second target material, wherein the at least one property is selected from the group consisting of density, charge, magnetism, and combinations thereof; and exposing the first and second target materials to at least one condition, thereby causing the first target material to become immobilized by a capture

site **44** on a first of the two opposed sequencing surfaces **30**, **32**, or **31** and the second target material to become immobilized by a capture site **44′** on a second of the two opposed sequencing surfaces **30′**, **32′**, **31′**.

(216) One example method is shown in FIG. **6**A and FIG. **6**B. In this example, the target materials **11**C, **11**D have opposite charges.

(217) As depicted in FIG. **6**A, the first target material **11**C has a negative charge and the second target material **11**D has a positive charge. Any examples of the charged solid supports **12** described herein may be used in this example. In one example, the negatively charged first target material **11**C is selected from the group consisting a carboxylated solid support, a polyglutamic acid coated solid support, and a sulfate functionalized solid support; and the positively charged second target material **11**D is selected from the group consisting of an amine functionalized solid support, such as a chitosan functionalized solid support and a polylysine functionalized solid support.

(218) The target materials **11**C, **11**D may be part of a fluid **56″** that is introduced into the flow cell **24**. In this example, the fluid **56″** used to introduce the charged target materials **11**C, **11**D to the flow cell **24** may be an electrolyte. As one example, the fluid **56″** may be a combination of tris(hydroxymethyl amino-methane and boric acid present at the same molarity (e.g., 4.5 mM of each). When complexes **10**A, **10**B are used as the target materials **11**C, **11**D, a low salt buffer may be used, such as a saline-sodium citrate (SSC) buffer (e.g., about 45 mM) with about 4 mM of Mg.sup.2+). This type of fluid **56″** can maximize charges on the charged target materials **11**C, **11**D while also allowing hybridization when the library fragments **14**, **14′**, **14′** are released. When clustered solid supports **13** are used as the target materials **11**C, **11**D, water may be used as the fluid **56″**.

(219) Moreover, the density of the fluid **56″** and the target materials **11**C, **11**D may be approximately equal so that the density of the target materials **11**C, **11**D does not interfere with the electrostatically induced migration of the target materials **11**C, **11**D. In another example, the density of the fluid **56″** and the target materials **11**C, **11**D may not be equal. In this example, the strength of the force due to the applied electric field **62** is greater than any force due to the difference in density.

(220) In this example method, the condition to which the charged target materials **11**C, **11**D are exposed to initiate simultaneous migration and immobilization is an electric field **62** applied between the two opposed sequencing surfaces **30** and **30′**, **32** and **32′**, or **31** and **31′** to generate positive charges **66** at the first of the two opposed sequencing surfaces **30**, **32**, **31** and negative charges **68** at the second of the two opposed sequencing surface **30′**, **32′**, **31′**.

(221) To generate the electric field **62** across the flow cell **24**, each sequencing surface **30**, **30′** or **32**, **32′** or **31**, **31′** can be electrically coupled to a power source to produce the respective electric charges **66**, **68** that attract the respective target materials **11**C, **11**D. In the example shown in FIG. **6**A and FIG. **6**B, the electric field **62** is applied in the direction towards the lower/bottom sequencing surface **30′**, resulting in the upper sequencing surface **30** being positively charged and the lower/bottom sequencing surface **30′** being negatively charged.

(222) The immobilization of the target materials **11**C, **11**D occurs simultaneously upon exposure of fluid **56″** in the flow cell **24** to the electric field **62**. This is due to the positive and negative charges of the target materials **11**C, **11**D and their respective responses to the applied electric field **62**. The negatively charged target material **11**C migrates toward the now positively charged sequencing surface **30**, where it becomes immobilized by the capture sites **44** (not shown in FIG. **6**B) of the upper sequencing surface **30**. The positively charged target material **11**D migrates toward the now negatively charged sequencing surface **30′**, where it becomes immobilized by the capture sites **44** (not shown in FIG. **6**B) of the lower/bottom sequencing surface **30′**.

(223) The electric field **62** may be applied for a predetermined time. In an example, the predetermined time may range from about 1 minute to about 30 minutes in order to obtain a desirable number of immobilized target materials **11**C, **11**D on the respective sequencing surfaces

30, **30**′. In other examples, the electric field **62** may be applied for a time ranging from about 1 minute to about 2 minutes, or from about 1 minute to about 5 minutes, or from about 5 minutes of about 30 minutes, etc.

(224) It is to be understood that some target materials **11**C, **11**D may not become immobilized, and these target materials **11**C, **11**D will be removed from the flow cell **24** before further processing. The electric field **62** may be ceased prior to removal of non-immobilized target materials **11**C, **11**D. As such, this example method may include, removing the electric field **62**, and then washing away the fluid **56**″ and non-immobilized target material **11**C, **11**D from the flow cell **24**. Washing may involve introducing a washing fluid into the flow cell **24**. The flow may push any target materials **11**C, **11**D that have not become immobilized at the sequencing surfaces **30**, **30**′ out through an exit port of the flow cell **24**. The immobilization mechanism (e.g., binding pair, hybridization, covalent bonding, etc.) between the respective target materials **11**C, **11**D and the capture sites **44**, **44**′ of the sequencing surfaces **30**, **30**′ may prevent any immobilized target materials **11**C, **11**D from becoming part of the exit flow.

(225) When complexes **10**A or **10**B are used as the target materials **11**C, **11**D, this washing step may be followed by library fragment release and amplification (e.g., an example of which is described in reference to FIG. **9**A through FIG. **9**C). When clustered solid supports **13** are used, this washing step may be followed by sequencing.

(226) Another example method is shown in FIG. **7**A and FIG. **7**B. In this example, the target materials **11**E, **11**F are different in terms of magnetism and density.

(227) In this example (as shown in FIG. **7**A), the target materials **11**E, **11**F are introduced into the flow cell **24** in a fluid **56**‴ which has a first density. As is described in more detail below, the density of each of the target materials **11**E, **11**F is selected with respect to this first density, i.e., the density of the fluid **56**‴ at the capture temperature of the target materials **11**E, **11**F. The capture temperature ranges from about 18° C. to about 40° C.

(228) In the example shown in FIG. **7**A and FIG. **7**B, the first target material **11**E is magnetic, and the second target material **11**F is non-magnetic and has a density greater than the first density (i.e., the density of the fluid **56**‴ at the capture temperature).

(229) In this example, the first target material **11**E includes any of the magnetically responsive solid supports **12**′ disclosed herein. Additionally, the density of the fluid **56**‴ and the target material **11**E may be approximately equal so that the density of the target material **11**E does not interfere with the magnetically induced migration of the target material **11**E. In another example, the density of the fluid **56**‴ and the target material **11**E may not be equal. In this example, the strength of the force due to the applied magnetic field **70** is greater than any force due to the difference in density.

(230) Also in this example, the second target material **11**F includes any of the solid supports **12** disclosed herein that are not magnetically responsive. The density of the solid support **12**, and thus the target material **11**F, is greater than the density of the fluid **56**‴ at the capture temperature. As such, the target material **11**F is non-responsive to the applied magnetic field and is able to migrate to or settle on the bottom sequencing surface **30**′ due to it being heavier than the fluid **56**‴.

(231) In this example method, the fluid **56**‴ including the target materials **11**E, **11**F is introduced into the flow cell **24** (FIG. **7**A), and the condition to which the target materials **11**E, **11**F are exposed to initiate simultaneous migration and immobilization is the application of a magnetic force **70** (FIG. **7**B). The density of the fluid **56**‴ may also be considered a condition that affects the migration and immobilization.

(232) The magnetic force (or magnetic field **70** as shown in FIG. **7**B) may be applied as described in reference to FIG. **4**A and FIG. **4**B. In the example shown in FIG. **7**B, the magnetic force/field **70** is applied in the direction of the upper sequencing surface **30** so that the magnetically responsive (first) target material **11**E migrates in that same direction toward the upper sequencing surface **30**. The capture sites **44** (not shown in FIG. **7**A or FIG. **7**B) immobilize at least some of the target material **11**E at the upper sequencing surface **30**. At the same time, the solid support **12** of the target

material **11**F is not magnetically responsive and is heavier than fluid **56**‴ at the capture temperature. As such, the target material **11**F migrates to or settles on the bottom sequencing surface **30**′, as shown in FIG. **7**B. The capture sites **44**′ (also not shown in FIG. **7**A or FIG. **7**B) immobilize at least some of the target material **11**F at the lower/bottom sequencing surface **30**′.
(233) The magnetic force/field **70** may be applied for a predetermined time. In an example, the predetermined time may range from about 5 minutes to about 30 minutes in order to obtain a desirable number of immobilized target materials **11**E on the sequencing surfaces **30**.
(234) The immobilization of the target materials **11**E, **11**F occurs simultaneously upon introduction of the target fluid **56**‴ to the flow cell **24** and upon exposure to the magnetic field **70** due to the properties (both density and magnetism) of the target materials **11**E, **11**F. In the method of FIG. **7**A and FIG. **7**B, at least some of the first target material **11**E becomes immobilized by respective capture sites **44** on the first of the two opposed sequencing surfaces **30**, and at least some of the second target material **11**F becomes immobilized by respective capture sites **44**′ on the second of the two opposed sequencing surfaces **30**′.
(235) It is to be understood that some target materials **11**E, **11**F may not become immobilized, and these target materials **11**E, **11**F will be removed from the flow cell **24** before further processing. The magnetic force/field **70** may be ceased prior to removal of non-immobilized target materials **11**E, **11**F. As such, this example method may include, removing the magnetic force/field **70**, and then washing away the fluid **56**‴ and non-immobilized target material **11**E, **11**F from the flow cell **24**. Washing may involve introducing a washing fluid into the flow cell **24**. The flow may push any target materials **11**E, **11**F that have not become immobilized at the sequencing surfaces **30**, **30**′ out through an exit port of the flow cell **24**. The immobilization mechanism (e.g., binding pair, hybridization, covalent bonding, etc.) between the respective target materials **11**E, **11**F and the capture sites **44**, **44**′ of the sequencing surfaces **30**, **30**′ may prevent any immobilized target materials **11**E, **11**F from becoming part of the exit flow.
(236) When complexes **10**A or **10**B are used as the target materials **11**E, **11**F, this washing step may be followed by library fragment release and amplification (e.g., an example of which is described in reference to FIG. **9**A through FIG. **9**C). When clustered solid supports **13** are used as the target materials **11**E, **11**F, this washing step may be followed by sequencing.
(237) The example method shown in FIG. **7**A and FIG. **7**B may also be performed so that the target material **11**E that is magnetically responsive is immobilized on the lower/bottom sequencing surface **30**′ and the target material **11**F that is non-magnetically responsive is immobilized on the upper sequencing surface **30**. In this example, the non-magnetically responsive target material **11**F includes the solid support **12** that is selected to have a density less than the density of the fluid **56**‴ at the capture temperature. In this example, the target material **11**E is responsive to the magnetic force/field (applied in the direction of the bottom sequencing surface **30**′) and is attracted to the bottom sequencing surface **30**′, while the target material **11**F is non-responsive to the applied magnetic field and is able to float or migrate to the upper sequencing surface **30** due to it being lighter than the fluid **56**‴.
(238) Another example method is shown in FIG. **8**A and FIG. **8**B. In this example, the target materials **11**G, **11**H are different in terms of charge and density.
(239) In this example, the target materials **11**G, **11**H are introduced into the flow cell **24** in a fluid **56**⁗ which has a first density. As is described in more detail below, the density of each of the target materials **11**G, **11**H is selected with respect to this first density, i.e., the density of the fluid **56**⁗ at the capture temperature of the target materials **11**G, **11**H. The capture temperature ranges from about 18° C. to about 40° C.
(240) In these examples, the fluid **56**⁗ is an electrolyte.
(241) In the example shown in FIG. **8**A and FIG. **8**B, the first target material **11**G is negatively charged, and the second target material **11**H is neutral (not charged) and has a density greater than the first density (i.e., the density of the fluid **56**⁗ at the capture temperature). In this example, the

first target material **11**G includes any of the negatively charged solid supports disclosed herein, such as a carboxylated solid support, a polyglutamic acid coated solid support, or a sulfate functionalized solid support. Additionally, the density of the fluid **56**″″ and the density of the target material **11**G may be approximately equal so that the density of the target material **11**G does not interfere with the electrostatically induced migration of the negatively charged target material **11**G. Alternatively, the density of the target material **11**G may be less than the density of the fluid **56**″″, and the density and charge can both aid in migration of the target material **11**G.

(242) In other examples of the method represented by FIG. **8**A and FIG. **8**B, the first target material **11**G is positively charged, and the second target material **11**H is neutral (not charged) and has a density greater than the first density (i.e., the density of the fluid **56**″ at the capture temperature). In this example, the first target material **11**G includes any of the positively charged solid supports disclosed herein, such as an amine functionalized solid support (e.g., a chitosan or a polylysine functionalized solid support). Additionally, the density of the fluid **56**″″ and the density of the target material **11**G may be approximately equal so that the density of the target material **11**G does not interfere with the electrostatically induced migration of the positively charged target material **11**G. Alternatively, the density of the target material **11**G may be less than the density of the fluid **56**″″, and the density and charge can both aid in migration of the target material **11**G.

(243) In the example methods represented in FIG. **8**A and FIG. **8**B, the second target material **11**H includes any of the solid supports **12** disclosed herein that are not charged. The density of the solid support **12**, and thus the target material **11**H, is greater than the density of the fluid **56**″″ at the capture temperature. As such, the target material **11**H is non-responsive to the applied electric field **62** and is able to migrate to or settle on the bottom sequencing surface **30**′ due to it being heavier than the fluid **56**″″.

(244) The fluid **56**″″ including the target materials **11**G, **11**H is introduced into the flow cell **24**, and the condition to which the target materials **11**G, **11**H are exposed to initiate simultaneous migration and immobilization is the application of an electric field **62**. The density of the fluid **56**″″ may also be considered a condition that affects the migration and immobilization.

(245) The electric field **62** may be applied as described in reference to FIG. **6**A and FIG. **6**B. In the example shown in FIG. **8**A (when the target material **11**G is negatively charged), the electric field **62** is applied in the direction towards the lower/bottom sequencing surface **30**′. This results in the upper sequencing surface **30** being positively charged and the lower/bottom sequencing surface **30**′ being negatively charged. In this example, the negatively charged target material **11**G migrates toward the now positively charged sequencing surface **30**, where it becomes immobilized by the capture sites **44** (not shown in FIG. **8**A or FIG. **8**B) of the upper sequencing surface **30**. At the same time, the solid support **12** of the target material **11**H is not charged and is heavier than fluid **56**″″ at the capture temperature. As such, the target material **11**H migrates to or settles on the bottom sequencing surface **30**′, as shown in FIG. **8**B. The capture sites **44**′ (also not shown in FIG. **8**A or FIG. **8**B) immobilize at least some of the target material **11**H at the lower/bottom sequencing surface **30**′.

(246) As mentioned above, in other examples of the method represented by FIG. **8**A and FIG. **8**B, the target material **11**G is positively charged. In this example, the electric field **62** is applied in the direction towards the upper sequencing surface **30** (i.e., in the opposite direction from that shown in FIG. **8**A and FIG. **8**B). This results in the lower sequencing surface **30**′ being positively charged and the upper sequencing surface **30** being negatively charged. In this example, the positively charged target material **11**G migrates toward the now negatively charged upper sequencing surface **30**, where it becomes immobilized by the capture sites **44** of the upper sequencing surface **30**. At the same time, the solid support **12** of the target material **11**H is not charged and is heavier than fluid **56**″″ at the capture temperature. As such, the target material **11**H migrates to or settles on the bottom sequencing surface **30**′, similar to FIG. **8**B. The capture sites **44**′ (also not shown in FIG. **8**B) immobilize at least some of the target material **11**H at the lower/bottom sequencing surface **30**′.

(247) In any of the examples represented by FIG. **8**A and FIG. **8**B, the electric field **62** may be applied for a predetermined time. In an example, the predetermined time may range from about 1 minute to about 30 minutes in order to obtain a desirable number of immobilized charged target materials **11**G on the oppositely charged sequencing surface **30** or **30**′.
(248) The immobilization of the target materials **11**G, **11**H occurs simultaneously upon introduction of the target fluid **56**′′′′ to the flow cell **24** and upon exposure to the electric field **62** due to the properties (both density and charge) of the target materials **11**G, **11**H. In the method of FIG. **8**A and FIG. **8**B, at least some of the first target material **11**G becomes immobilized by respective capture sites **44** on the first of the two opposed sequencing surfaces **30**, and at least some of the second target material **11**H becomes immobilized by respective capture sites **44**′ on the second of the two opposed sequencing surfaces **30**′.
(249) It is to be understood that some target materials **11**G, **11**H may not become immobilized, and these target materials **11**G, **11**H will be removed from the flow cell **24** before further processing. The electric field **62** may be ceased prior to removal of non-immobilized target materials **11**G, **11**H. As such, this example method may include, removing the electric field **62**, and then washing away the fluid **56**′′′′ and non-immobilized target material **11**G, **11**H from the flow cell **24**. Washing may involve introducing a washing fluid into the flow cell **24**. The flow may push any target materials **11**G, **11**H that have not become immobilized at the sequencing surfaces **30**, **30**′ out through an exit port of the flow cell **24**. The immobilization mechanism (e.g., binding pair, hybridization, covalent bonding, etc.) between the respective target materials **11**G, **11**H and the capture sites **44**, **44**′ of the sequencing surfaces **30**, **30**′ may prevent any immobilized target materials **11**G, **11**H from becoming part of the exit flow.
(250) When complexes **10**A or **10**B are used as the target materials **11**G, **11**H, this washing step may be followed by library fragment release and amplification (e.g., an example of which is described in reference to FIG. **9**A through FIG. **9**C). When clustered solid supports **13** are used as the target materials **11**G, **11**H, this washing step may be followed by sequencing.
(251) The example method shown in FIG. **8**A and FIG. **8**B may also be performed so that the target material **11**G is not charged and has a density that is less than the density of the target fluid **56**′′′′. In this example, the target material **11**H is positively charged. In this example, the positively charged target material **11**H is responsive to the electric field **62** (applied in the direction of the bottom sequencing surface **30**′) and is attracted to the bottom sequencing surface **30**′. Also in this example, the target material **11**G is non-responsive to the applied magnetic field and is able to float or migrate to the upper sequencing surface **30** due to it being lighter than the fluid **56**′′′.
(252) It is to be understood that other orthogonal modalities may be combined in order to immobilize two different target materials **11**. Each target material **11** may be responsive to one of the orthogonal modalities but not the other, which allows the modalities to independently affect one of the target materials **11**. For example, a non-charged magnetically responsive target material **11** may be combined with a charged, non-magnetic target material **11**. In this example, a magnetic field **70** may be applied in one direction to guide the migration of the non-charged magnetically responsive target material **11** to one **30**, **32**, **31**, of the opposed sequences surfaces **30**, **30**′ or **32**, **32**′, or **31**, **31**′, and an electric field **62** may be applied in the opposite direction to guide the migration of the charged, non-magnetic target material to the other **30**′, **32**′, **31**′ of the opposed sequences surfaces **30**, **30**′ or **32**, **32**′, or **31**, **31**′. While several examples have been provided, it is contemplated that other target material combinations and modalities may be utilized.
(253) Library Fragment Release from Complexes and Sequencing
(254) With the target material **11** immobilized on both of the opposed surfaces **30** and **30**′ or **32** and **32**′ or **31** and **31**′ of the flow cell **24**, the flow cell **24** is ready for downstream analysis.
(255) In the examples utilizing the complexes **10**A, **10**B immobilized on both of the opposed sequences surfaces **30** and **30**′ or **32** and **32**′, the flow cell **24** is ready for library fragment release, amplification, and sequencing.

(256) After immobilization and removal of non-immobilized target material (e.g., complexes **10**A, **10**B) examples of the method include initiating release of the sequencing-ready nucleic acid fragments **14**, **14′**, **14″** from the solid support **12** or **12′** of immobilized complexes **10**A, **10**B, thereby seeding at least some the sequencing-ready nucleic acid fragments **14**, **14′**, **14″** to respective primers **42**, **42′** of the two opposed sequencing surfaces **30**, **30′** or **32**, **32′**; and removing the solid support **12** or **12′** and non-seeded sequencing-ready nucleic acid fragments **14**, **14′**, **14″**. These steps may be followed by any of the amplification techniques described herein, including that described in reference to FIG. **9**A through FIG. **9**C.
(257) Prior to fragment **14**, **14′**, **14″** release, an external immobilization agent may be introduced to the flow cell **24**. In an example, the external immobilization agent is air, or a liquid medium or a viscous medium that is not miscible with the target material **11** (specifically, the complexes **10**A, **10**B) that have been introduced to the flow cell **24**. Air may be used to aspirate the washing fluid out of the flow cell **24**, which can create a liquid droplet that surrounds the complexes **10**A, **10**B and forms a diffusion barrier around each of the complexes **10**A, **10**B. The liquid or viscous external immobilization agent at least partially surrounds the complexes **10**A, **10**B that are immobilized within the flow cell **24**. The external immobilization agent can help to minimize diffusion of the sequencing-ready nucleic acid fragments **14**, **14′**, **14″** when the fragments **14**, **14′**, **14″** are released from the solid supports **12** or **12′**. When the external immobilization agent is a temperature responsive material, raising the temperature to the seeding temperature may render the agent more viscous and in a form that can further minimize library diffusion.
(258) The release of the sequencing-ready nucleic acid fragments **14**, **14′**, **14″** from the solid support **12** or **12′** may then be initiated. In one example, a cleaving agent may be introduced into the flow cell **24**, and a stimulus may be applied to trigger the cleaving agent to release the sequencing-ready nucleic acid fragments **14**, **14′**, **14″** from the solid support **12** or **12′**. In other examples, the release of the sequencing-ready nucleic acid fragments **14**, **14′**, **14″** may involve heating the flow cell **24** above a melting temperature of a primer that is hybridized to the fragments **14**, **14′**, **14″**.
(259) Upon release, transport and seeding of the sequencing-ready nucleic acid fragments **14**, **14′**, or **14″** may be restricted by the external immobilization agent. As such, the fragments **14**, **14′**, or **14″** of any particular complex **10**A, **10**B, may be confined to an area of the sequencing surface **30**, **30′** or **32**, **32′** near the particular complex **10**A, **10**B from which the fragments **14**, **14′**, or **14″** are released.
(260) The primers **42**, **42′** of the respective sequencing surfaces **30**, **30′** or **32**, **32′** of the flow cell **24** can seed the released sequencing-ready nucleic acid fragments **14**, **14′**, or **14″**. Seeding is accomplished through hybridization between the first or second sequence of the fragment **14**, **14′**, or **14″** and a complementary one of the primers **42**, **42′** of the respective sequencing surfaces **30**, **30′** or **32**, **32′**. Seeding may be performed at a suitable hybridization temperature for the fragment **14**, **14′**, or **14″** and the primer(s) **42**, **42′**. In one example, seeding takes place at about 80° C., which is followed by a temperature reduction down to room temperature (e.g., 25° C.).
(261) The location at which the sequencing-ready nucleic acid fragments **14**, **14′**, or **14″** seed within the flow cell **24** depends, in part, upon how the primers **42**, **42′** are attached. In examples of the flow cell **24** having the non-patterned sequencing surfaces **30**, **30′**, the released sequencing-ready nucleic acid fragments **14**, **14′**, or **14″** will seed across polymeric hydrogels **40**, **40′** in the concave regions **38**, **38′**. In examples of the flow cell **24** having the patterned sequencing surfaces **32**, **32′**, the released sequencing-ready nucleic acid fragments **14**, **14′**, or **14″** will seed across polymeric hydrogels **40**, **40′** within each of the depressions **48**, **48′**.
(262) An example of the seeded sequencing-ready nucleic acid fragments **14**, **14′**, or **14″** in different depressions **48**, **48′** along the patterned sequencing surfaces **32**, **32′** of the flow cell **24** is shown in FIG. **9**A.
(263) The solid supports **12**, **12′** may then be removed from the flow cell **24**. Removal of the solid

supports **12**, **12′** may involve any suitable technique, which depends upon the mechanism attaching the solid support **12**, **12′** to the capture site **44**, **44′**. As examples, denaturing, bond cleaving, etc. may be used. Removal of the solid supports **12**, **12′** may also remove non-seeded sequencing-ready nucleic acid fragments **14**, **14′**, **14″**. Removal of the solid supports **12**, **12′** may also remove liquid or viscous forms of the external immobilization agent.

(264) The seeded sequencing library fragments **14**, **14′**, **14″** can then be amplified using cluster generation.

(265) In one example of cluster generation, the sequencing-ready nucleic acid fragments **14**, **14′**, or **14″** are copied from the hybridized primers **42**, **42′** by 3′ extension using a high-fidelity DNA polymerase. The high-fidelity DNA polymerase may be part of an amplification mix that is introduced into the flow cell **24**. The amplification mix may also include other suitable polymerase chain reaction reagents. The original sequencing-ready nucleic acid fragments **14**, **14′**, or **14″** are denatured, leaving the copies immobilized to the sequencing surfaces **30**, **30′** or **32**, **32′**. Isothermal bridge amplification or some other form of amplification may be used to amplify the immobilized copies. For example, the copied templates loop over to hybridize to an adjacent, complementary primer **42**, **42′**, and a polymerase copies the copied templates to form double stranded bridges, which are denatured to form two single stranded strands. These two strands loop over and hybridize to adjacent, complementary primers **42**, **42′** and are extended again to form two new double stranded loops. The process is repeated on each template copy by cycles of isothermal denaturation and amplification to create dense clonal clusters. Each cluster of double stranded bridges is denatured. In an example, the reverse strand is removed by specific base cleavage, leaving forward template polynucleotide strands. Clustering results in the formation of several template polynucleotide strands along the sequencing surfaces **30**, **30′** or **32**, **32′**. This example of clustering is bridge amplification, and is one example of the amplification that may be performed. It is to be understood that other amplification techniques may be used, such as the exclusion amplification (Examp) workflow (Illumina Inc.).

(266) Another example of amplification, and thus cluster generation, involves the use of a temperature responsive material. This example is shown schematically in FIG. **9**A through FIG. **9**C. This example method involves introducing an amplification mix, including a liquid form **63** of a temperature responsive material, to the flow cell **24**; causing the liquid form **63** of the temperature responsive material to gel (which generates the gel form **63′** of the temperature responsive material); initiating amplification of the seeded sequencing-ready nucleic acid fragments **14**, **14′**, **14″** to generate template strands **64**, whereby the gel form **63′** of the temperature responsive material reduces diffusion of the template strands **64**; causing the gel form **63′** of the temperature responsive material to liquefy (which generates the liquid form **63** of the temperature responsive material); and removing the liquid form **63** of the temperature responsive material from the flow cell **24**.

(267) As shown in FIG. **9**A, the amplification mix, including the liquid form **63** of the temperature responsive material, has been introduced into the flow channel **28**, e.g., via an inlet. In addition to the liquid form **63** of the temperature responsive material, this example of the amplification mix also includes the high-fidelity DNA polymerase and any other suitable polymerase chain reaction reagents.

(268) The temperature responsive material is able to transition from the liquid form **63** to the gel form **63′** by changing the temperature conditions to which the material is exposed. In the liquid form **63**, the molecules of the temperature responsive material are unlinked and thus are able to flow. In the gel form **63′**, the molecules of the temperature responsive material are crosslinked, and thus are unable to flow. The gel form **63′** includes pores, channels or other openings that can i) facilitate the diffusive exchange of small molecules, proteins and reagents to access the seeded sequencing-ready nucleic acid fragments **14**, **14′**, **14″** for amplification, and also ii) impede or prevent the movement of the seeded sequencing-ready nucleic acid fragments **14**, **14′**, **14″** or

template strands **64** due to diffusion or convection. As such, any temperature sensitive material that i) facilitates in-gel amplification, ii) limits the diffusion, convection, or other movement of the seeded sequencing-ready nucleic acid fragments **14**, **14′**, **14″** and template strands **64**, iii) can be pumped or otherwise flowed as a liquid before crosslinking, iv) can be controllably crosslinked and gelled, and v) can be controllably unlinked and liquefied.

(269) Examples of the temperature responsive material include disulfide crosslinked polyacrylamide, agarose, alginate, and a copolymer of poly(N-isopropylacrylamide) (PNIPAAm) and polyethylene glycol (PEG). For each of these materials, amplification may be performed at temperatures that will not melt the gel form **63′**.

(270) The copolymer of PNIPAAm and PEG is a liquid at lower temperatures and a gel at higher temperatures. One example of the copolymer of PNIPAAm and PEG is a liquid at temperatures less than 29° C. and a gel at temperatures higher than 32° C. The gelling temperature of the copolymer of PNIPAAm and PEG may be tuned by altering the ratio of the poly(N-isopropylacrylamide) and polyethylene glycol in the copolymer.

(271) The amplification mix is loaded into the flow cell **24** at conditions where the amplification reaction does not occur. For example, amplification does not occur at 4° C., and thus the amplification mix (including the liquid form **63** of the temperature responsive material) may be introduced at this temperature.

(272) Causing the liquid form **63** of the temperature responsive material to gel, and thus generating the gel form **63′**, may be performed by adjusting the temperature of the flow cell **24**, and the temperature responsive material contained therein, to a gelation temperature of the temperature responsive material. The gel form **63′** is shown in FIG. **9**B. The temperature to which the flow cell **24** is adjusted will depend upon the temperature responsive material being used.

(273) Initiating amplification of the seeded sequencing-ready nucleic acid fragments **14**, **14′**, **14″** generates template strands **64**, as shown in FIG. **9**B. Amplification may be initiated by adjusting the temperature of the flow cell **24**, and the amplification mix contained therein, to a temperature where the PCR reagents are active. During amplification, the gel form **63′** of the temperature responsive material reduces movement of the seeded sequencing-ready nucleic acid fragments **14**, **14′**, **14″** and the template strands **64**.

(274) Causing the gel form **63′** of the temperature responsive material to liquefy, and thus generating the liquid form **63**, may be performed by again adjusting the temperature of the flow cell **24** and the temperature responsive material contained therein, to a liquefaction temperature of the temperature responsive material. Again, the temperature to which the flow cell **24** is adjusted will depend upon the temperature responsive material being used.

(275) The liquid form **63** may then be pumped out of the flow cell **24**, readying the flow cell **24** for subsequent sequencing. The flow cell **24** after the liquid form **63** of the temperature responsive material is removed is shown in FIG. **9**C.

(276) In one specific example, the copolymer of PNIPAAm and PEG is used in the amplification mix and used in conjunction with recombinase-mediated polymerase chain reaction (PCR). A temperature program may be used to control the amplification as a typical recombinase-mediated isothermal PCR is inactive at 4° C. and active at 37° C. or other high temperatures, and the copolymer of PNIPAAm and PEG is a liquid at temperatures less than 29° C. and a gel at temperatures higher than 32° C. In this example, the amplification mix may be introduced into the flow cell **24** at about 4° C. as a liquid mixture. The temperature may then be raised to about 37° C. to both gel the copolymer and start the PCR amplification. Upon completion, the gel form **63′** of the copolymer may be liquefied by lowering the temperature to less than 29° C., e.g., to about 8° C. (which is a suitable sequencing temperature). The liquid form **63** may then be pumped out of the flow cell **24**, readying the flow cell **24** for subsequent sequencing.

(277) The use of the temperature responsive material **63**, **63′** can minimize the diffusion of seeded sequencing-ready nucleic acid fragments **14**, **14′**, or **14″** and the amplified template strands **64** from

moving (e.g., as a result of diffusion or free convection) to a nearby depression **48**, **48′** of the patterned sequencing surfaces **32**, **32′** or away from an initial seeding location on the non-patterned sequencing surfaces **30**, **30′**. By limiting or preventing this movement, the clusters remain in relatively isolated areas of the flow cell **24**, which enables each cluster to be read individually, without redundancy. Movement can also generate hybrid molecules that are not present in the original sequencing library fragments **14**, **14′**, **14″**, which results in inaccurate sequencing data. By limiting or preventing this movement, these hybrid molecules are not generated, thus improving the accuracy of the resulting sequencing data.

(278) While FIG. **9**A through FIG. **9**C depicts the flow cell **24** with the patterned sequencing surfaces **32**, **32′**, it is to be understood that the method may be performed using the non-patterned sequencing surfaces **30**, **30′** as well.

(279) Moreover, the method shown in FIG. **9**A through FIG. **9**C may be performed with any sequencing-ready nucleic acid fragments **14**, **14′**, **14″**, including those that are not tethered to a solid support **12**, **12′**. In this example, any suitable library preparation technique may be used that adds the desired adapters to the fragmented DNA sample. The sequencing-ready nucleic acid fragments **14**, **14′**, **14″** may be introduced and seeded on the flow cell sequencing surface(s) **30**, **30′** or **32**, **32′**. Once the library fragments are seeded, the method described in FIG. **9**A through FIG. **9**C may be performed.

(280) It is to be further understood that the method shown in FIG. **9**A through FIG. **9**C may not be performed with the clustered solid supports **13**, as these target materials **11** are not exposed to amplification on the flow cell **24**.

(281) A sequencing primer may then be introduced that hybridizes to a complementary sequence on the template polynucleotide strand. This sequencing primer renders the template polynucleotide strand **64** ready for sequencing. The 3′-ends of the templates **64** and any flow cell-bound primers **42**, **42′** (not attached to the copy) may be blocked to prevent interference with the sequencing reaction, and in particular, to prevent undesirable priming.

(282) To initiate sequencing, an incorporation mix may be added to the flow cell **24**. In one example, the incorporation mix includes a liquid carrier, a polymerase, and fluorescently labeled nucleotides. The fluorescently labeled nucleotides may include a 3′ OH blocking group. When the incorporation mix is introduced into the flow cell **24**, the fluid enters the flow channel **28**, and in some examples, into the depressions **48**, **48′** (where the template polynucleotide strands are present).

(283) The fluorescently labeled nucleotides are added to the sequencing primer (thereby extending the sequencing primer) in a template dependent fashion such that detection of the order and type of nucleotides added to the sequencing primer can be used to determine the sequence of the template. More particularly, one of the nucleotides is incorporated, by a respective polymerase, into a nascent strand that extends the sequencing primer and that is complementary to the template polynucleotide strand. In other words, in at least some of the template polynucleotide strands across the flow cell **24**, respective polymerases extend the hybridized sequencing primer by one of the nucleotides in the incorporation mix.

(284) The incorporation of the nucleotides can be detected through an imaging event. During an imaging event, an illumination system (not shown) may provide an excitation light to the respective sequencing surfaces **30**, **30′** or **32**, **32′**.

(285) In some examples, the nucleotides can further include a reversible termination property (e.g., the 3′ OH blocking group) that terminates further primer extension once a nucleotide has been added to the sequencing primer. For example, a nucleotide analog having a reversible terminator moiety can be added to the sequencing primer such that subsequent extension cannot occur until a deblocking agent is delivered to remove the moiety. Thus, for examples that use reversible termination, a deblocking reagent can be delivered to the flow cell **24** after detection occurs.

(286) Wash(es) may take place between the various fluid delivery steps. The SBS cycle can then be

repeated n times to extend the sequencing primer by n nucleotides, thereby detecting a sequence of length n.
(287) In some examples, the forward strands may be sequenced and removed, and then reverse strands are constructed and sequenced as described herein.
(288) While SBS has been described in detail, it is to be understood that the flow cells **24** described herein may be utilized with other sequencing protocol, for genotyping, or in other chemical and/or biological applications. In some instances, the primers **42**, **42′** of the flow cell **24** may be selected to enable simultaneous paired-end sequencing, where both forward and reverse strands are present on the polymeric hydrogel **40**, **40′**, allowing for simultaneous base calling of each read. Sequential and simultaneously paired-end sequencing facilitates detection of genomic rearrangements and repetitive sequence elements, as well as gene fusions and novel transcripts.
(289) Clustered Solid Supports and Sequencing
(290) As noted above, with the target material **11** immobilized on both of the opposed surfaces **30** and **30′** or **32** and **32′** or **31** and **31′** of the flow cell **24**, the flow cell **24** is ready for downstream analysis. When the clustered solid supports **13** are immobilized on both of the opposed surfaces **31** and **31′** of the flow cell **24**, the flow cell **24** is ready for sequencing. In these examples, the flow cell **24** is ready for sequencing because amplification and cluster generation have taken place on the solid support **12** or **12′** off of the flow cell **24**.
(291) Sequencing may be performed as described herein by introducing the sequencing primer and incorporation mix, and performing sequential sequencing cycles.
(292) To further illustrate the present disclosure, examples are given herein. It is to be understood that these examples are provided for illustrative purposes and are not to be construed as limiting the scope of the present disclosure.
NON-LIMITING WORKING EXAMPLES
Example 1
(293) Complexes similar to those shown in FIG. **1**A were prepared having an average diameter of 3 μm. The solid supports of the complexes were DYNABEADS™ M-280 Streptavidin beads from ThermoFisher Scientific. The solid supports each a density of about 1.18 g/cm.sup.3. The fragments on a particular bead were from the same long DNA molecule (from the PhiX genome). The library fragments were attached to the solid support via a desthiobiotin oligo, which has weaker affinity than biotin to streptavidin on the bead surface. The library fragments included P5′ and P7 sequences, along with index sequences, and read 1 and read 2 sequences.
(294) The complexes were loaded into a flow cell including opposed patterned sequencing surfaces (including P5 and P7 primers) using an example of the method similar to that described in FIG. **3**A and FIG. **3**B.
(295) More specifically, the complexes were first divided between two fluids, the first of which had a density of about 2 g/cm.sup.3 and the second of which had a density of about 1 g/cm.sup.3. The first fluid was a 1 g/ml sodium polytungstate solution (500 mg sodium polytungstate per 500 μL of the saline sodium citrate buffer with sodium dodecyl sulfate), and included the complexes at a concentration of 600,000 complexes per 1 μL. The second fluid was a saline sodium citrate buffer with sodium dodecyl sulfate, and included the complexes at a concentration of 600,000 complexes per 1 μL.
(296) The first fluid was introduced into the flow cell and the complexes were allowed to immobilize to the top surface of the flow cell. The flow cell was then washed with a washing solution. The second fluid was introduced into the flow cell and the complexes were allowed to immobilize to the bottom surface of the flow cell. Attachment of the complexes to the respective surfaces was accomplished with an anchor (e.g., complementary primers with biotin were hybridized to the P5 primers attached to the gel material or alkyne-PEG-biotin linkers were covalently attached to free azides on the gel material using click chemistry).
(297) FIG. **10**A illustrates a bright field image of the top surface after complex immobilization and

FIG. **10**B illustrates a bright field image of the bottom surface after complex immobilization. The darker areas of each image depict the immobilized complexes.
(298) Free biotin in saline sodium citrate buffer with sodium dodecyl sulfate was introduced and the flow cell was heated to about 80° C. to release the libraries from the respective complexes. Clustering was performed using isothermal amplification. The clusters were stained with Sytox green and the resulting images (not reproduced herein) confirmed clusters of template strands were formed on each of the sequencing surfaces of the flow cell.
(299) Sequencing was then performed on the flow cell. Some of the sequencing data collected for the top and bottom surfaces of the flow cell is shown in FIG. **11**A and FIG. **11**B.
(300) FIG. **11**A depicts a molecular coverage histogram for one lane of the flow cell on the top and bottom surfaces. This data shows the range and uniformity of sequencing coverage for the lane.
(301) FIG. **11**B depicts the percentage of Qscores greater than Q30 for various sequencing cycles in one lane of the flow cell on the top and bottom surfaces. A Qscore of 30 (Q30) is equivalent to the probability of an incorrect base call 1 in 1000 times. This means that the base call accuracy (i.e., the probability of a correct base call) is 99.9%. A lower base call accuracy of 99% (Q20) will have an incorrect base call probability of 1 in 100, meaning that every 100 base pair sequencing read will likely contain an error. When sequencing quality reaches Q30, virtually all of the reads will be perfect, having zero errors and ambiguities. As depicted in FIG. **11**B, the percentage of Qscores higher than Q30 generally ranged from 60% to 99% for all sequencing cycles.
(302) All of the collected data confirmed that the more dense fluid (in this example the first fluid) was compatible with the sequencing surface of the flow cell.
Example 2
(303) Complexes similar to those shown in FIG. **1**A were prepared having an average diameter of 3 μm. The solid supports of the complexes were DYNABEADS™ M-280 Streptavidin beads from ThermoFisher Scientific. The solid supports each a density of about 1.18 g/cm.sup.3. The fragments on a particular bead were from the same long DNA molecule (from the PhiX genome).
(304) In this example, flow cell lanes (having opposed surfaces coated with a gel material) were prepared with different concentrations of capture sites (namely alkyne-PEG-biotin linkers). These linkers were covalently attached to free azides on the gel material in the flow cell lanes using click chemistry. The flow cell lanes were washed and respectively exposed to an alkyne-PEG-biotin solution having concentrations of about 0.5 μM, about 5 μM, or about 25 μM. The solutions were allowed to incubate for about 30 minutes at about 60° C. The flow cell lanes were then washed again.
(305) The complexes were first divided between two fluids, the first of which had a density of about 1 g/cm.sup.3 and the second of which had a density of about 2 g/cm.sup.3. The first fluid was a saline sodium citrate buffer with sodium chloride, and included the complexes at a concentration of 25,000 per μL. The second fluid was a 2 g/ml sodium polytungstate solution, and included the complexes at a concentration of 25,000 per μL.
(306) The first fluid was introduced into the respective flow cell lanes and the complexes were allowed to immobilize to the bottom surfaces of the flow cell lanes. The aspiration rate was 100 μL/min, and the first fluid remained in the flow cells for 180 seconds. The flow cells were then washed with a washing solution. The second fluid was introduced into the respective flow cell lanes and the complexes were allowed to immobilize to the top surfaces of the flow cell lanes. The aspiration rate was 100 μL/ms, and the second fluid remained in the flow cell lanes for 450 seconds. The flow cell lanes were then washed with a washing solution.
(307) The bottom and top surfaces of each of the flow cell lanes were imaged and the immobilized complexes (beads) on each surface were counted using microscope images.
(308) The number of beads per mm.sup.2 for the bottom surfaces are shown in FIG. **12**A and the number of beads per mm.sup.2 for the top surfaces are shown in FIG. **12**B. The concentrations for each bar in FIG. **12**A and FIG. **12**B represent the alkyne-PEG-biotin concentration (about 0.5 μM,

about 5 μM, or about 25 μM) used to prep the flow cells prior to complex immobilization. As depicted, the alkyne-PEG-biotin concentration did not impact the immobilization on the bottom surfaces, as each of these had from about 2,100 beads/mm.sup.2 to about 2,300 beads/mm.sup.2. The number of complexes immobilized on the top surfaces was not quite as high as the bottom surfaces, as they ranged from about 550 beads/mm.sup.2 to about 1,150 beads/mm.sup.2. For the top surfaces, the lanes treated with higher concentrations of alkyne-PEG-biotin linkers had a higher number of complexes/beads immobilized thereon.

(309) These results illustrate that the heavier fluid does help to immobilize complexes on the top surfaces, and that increasing the capture size concentration on the top surface can also help with immobilization.

Example 3

(310) Complexes similar to those shown in FIG. **1**A were prepared having an average diameter of 3 μm. The solid supports of the complexes were DYNABEADS™ M-280 Streptavidin beads from ThermoFisher Scientific. The solid supports each a density of about 1.18 g/cm.sup.3. The fragments on a particular bead were from the same long DNA molecule (from the PhiX genome).

(311) In this example, eight flow cell lanes (having opposed surfaces coated with a gel material) were prepared with capture sites (namely alkyne-PEG-biotin linkers). These linkers were covalently attached to free azides on the gel material in the flow cell lanes using click chemistry. The flow cell lanes were washed and respectively exposed to an alkyne-PEG-biotin solution having concentrations of about 5 μM. The solution was allowed to incubate for about 30 minutes at about 60° C. The flow cell lanes were then washed again.

(312) The complexes were first divided between two fluids, the first of which had a density of about 1 g/cm.sup.3 and the second of which had a density of about 2 g/cm.sup.3. The first fluid was a sodium citrate buffer, and included the complexes at a concentration of 40,000 per μL. The second fluid was a 2 g/ml sodium polytungstate solution, and included the complexes at a concentration of 40,000 per μL.

(313) The first fluid was introduced into seven of the flow cell lanes and the complexes were allowed to immobilize to the bottom surfaces. The aspiration rate was 100 μL/min, and the first fluid remained in each of the lanes for 240 seconds. The flow cell lanes were then washed with a washing solution. The second fluid was introduced into each of the seven flow cell lanes and the complexes were allowed to immobilize to the top surfaces. The aspiration rate ranged from 80 μL/ms to 100 μL/ms, and the second fluid remained in the flow cells for 300 seconds. The flow cell lanes were then washed with a washing solution.

(314) In the eighth lane, the fluids were diluted to 100 μL each, and the introduction of the respective fluid was performed twice. Thus, lane 8 had a double loading.

(315) The bottom and top surfaces of each of the flow cell lanes were imaged and the immobilized complexes (beads) on each surface were counted. Table 1 provides the average number of beads per mm.sup.2 for each of the flow cell lanes.

(316) TABLE-US-00001 TABLE 1 Top Surface Bottom Surface Lane ID (# Complexes/mm.sup.2) (# Complexes/mm.sup.2) 1 3393 ± 343 3335 ± 151 2 2291 ± 583 3044 ± 556 3 3576 ± 290 3395 ± 281 4 3657 ± 606 3148 ± 95 5 3577 ± 467 3243 ± 229 6 3877 ± 770 3194 ± 245 7 3672 ± 594 3629 ± 94 8 6272 ± 2000 4389 ± 950

(317) The target number of complexes (beads) for each of the surfaces was 4,000 beads/mm.sup.2. While lanes 1-7 were slightly under target, the number of complexes on the top and bottom surfaces for these lanes was relatively consistent. Lane 8 (exposed to a double loading) exceeded the target number of complexes on both surfaces.

(318) FIG. **13**A illustrates the target number of beads and the number of beads per mm.sup.2 as measured along the length of the flow cell lane 1 from the inlet (1) to the outlet (5). FIG. **13**B illustrates the target number of beads, and the number of beads per mm.sup.2 as measured along the length of the flow cell lane 7 from the inlet (1) to the outlet (5). The measurements were taken

at equal distances along the length. These results illustrate that the immobilization is relatively consistent along the length of the flow channel lanes on both the top and bottom surfaces.

Example 4

(319) Complexes similar to those shown in FIG. **1**A were prepared having an average diameter of 3 μm. The solid supports of the complexes were DYNABEADS™ M-280 Streptavidin beads from ThermoFisher Scientific. The solid supports each a density of about 1.18 g/cm.sup.3. The fragments on a particular bead were from the same long DNA molecule (from the PhiX genome).

(320) In this example, ten flow cell lanes (having opposed surfaces coated with a gel material) were prepared with capture sites (namely alkyne-PEG-biotin linkers). These linkers were covalently attached to free azides on the gel material in the flow cell lanes using click chemistry. The flow cell lanes were washed and respectively exposed to an alkyne-PEG-biotin solution having concentrations of about 5 μM. The solution was allowed to incubate for about 30 minutes at about 60° C. The flow cell lanes were then washed again.

(321) The complexes were first divided between two fluids, the first of which had a density of about 1 g/cm.sup.3 and the second of which had a density of about 2 g/cm.sup.3. The first fluid was a sodium citrate buffer, and included the complexes at a concentration of 10 μg per 50 μL. The second fluid was a 2 g/ml sodium polytungstate solution, and included the complexes at a concentration of 12.5 μg per 50 μL.

(322) The first fluid was introduced into the ten flow cell lanes and the complexes were allowed to immobilize to the bottom surfaces. The aspiration rate was 100 μL/min, and the first fluid remained in each of the lanes for 300 seconds. The flow cell lanes were then washed with a washing solution. The second fluid was introduced into each of the ten flow cell lanes and the complexes were allowed to immobilize to the top surfaces. The aspiration rate was 80 μL/ms, and the second fluid remained in the flow cells for 360 seconds. The flow cell lanes were then washed with a washing solution.

(323) The bottom and top surfaces of each of the flow cell lanes were imaged and the immobilized complexes (beads) on each surface were counted.

(324) FIG. **14** illustrates the target number of beads, and the number of beads per mm.sup.2 as measured along the length of one lane of the flow cell from the inlet (1) to the outlet (10). FIG. **14** also illustrates the linear fit for the top surface and bottom surface data. These results illustrate that the immobilization is relatively consistent along the lengths of the top and bottom surfaces of the flow channel when the complexes are introduced in accordance with an example of the method disclosed herein.

Example 5

(325) Complexes similar to those shown in FIG. **1**A were prepared having an average diameter of 3 μm. The solid supports of the complexes were DYNABEADS™ M-280 Streptavidin beads from ThermoFisher Scientific. The solid supports each a density of about 1.18 g/cm.sup.3. The fragments on a particular bead were from the same long DNA molecule (from the PhiX genome). The library fragments were attached to the solid support via a desthiobiotin oligo, which has weaker affinity than biotin to streptavidin on the bead surface.

(326) In this example, eight flow cell lanes (having opposed surfaces coated with a gel material) were prepared with capture sites (namely alkyne-PEG-biotin linkers). These linkers were covalently attached to free azides on the gel material in the flow cell lanes using click chemistry. The flow cell lanes were washed and respectively exposed to an alkyne-PEG-biotin solution having concentrations of about 5 μM. The solution was allowed to incubate for about 30 minutes at about 60° C. The flow cell lanes were then washed again.

(327) The complexes were first divided between two fluids, the first of which had a density of about 1 g/cm.sup.3 and the second of which had a density of about 2 g/cm.sup.3. The first fluid was a sodium citrate buffer, and included the complexes at a concentration of 10 μg per 50 μL. The second fluid was a 2 g/ml sodium polytungstate solution, and included the complexes at a

concentration of 12.5 μg per 50 μL.
(328) The first fluid was introduced into eight of the flow cell lanes and the complexes were allowed to immobilize to the bottom surfaces. The aspiration rate was 100 μL/min, and the first fluid remained in each of the lanes for 240 seconds. The flow cell lanes were then washed with a washing solution. The second fluid was introduced into each of the eight flow cell lanes and the complexes were allowed to immobilize to the top surfaces. The aspiration rate ranged from 80 μL/ms to 100 μL/ms, and the second fluid remained in the flow cells for 300 seconds. The flow cell lanes were then washed with a washing solution.
(329) The bottom and top surfaces of each of the flow cell lanes were imaged and the immobilized complexes (beads) on each surface were counted.
(330) Free biotin in sodium citrate buffer was introduced and the flow cell was heated to about 80° C. to release the libraries from the respective complexes. Clustering was performed using bridge amplification. Sequencing was then performed on the flow cell. The sequencing data collected included passing filter (% PF) (percentage). Passing filter (PF) is the metric used to describe clusters which pass a chastity threshold and are used for further processing and analysis of sequencing data. A higher % passing filter result indicates an increased yield of unique clusters used for sequencing data.
(331) Table 2 provides the average number of beads per mm.sup.2 for each of the flow cell lanes, as well as the PF data for each lane.
(332) TABLE-US-00002 TABLE 2 Total Top + Bottom Surfaces Lane ID (# Complexes/mm.sup.2) % PF 1 6728 58.16 ± 6.4 2 5335 64.62 ± 4.74 3 6971 65.97 ± 4.18 4 6805 66.48 ± 3.59 5 6820 65.23 ± 5.29 6 7072 65.64 ± 6.76 7 7302 71.25 ± 4.33 8 10334 66.83 ± 6.85
(333) The target number of complexes (beads) for each of the surfaces of lanes 1-7 was 4,000 beads/mm.sup.2 (8,000 beads/mm.sup.2 total). The target number of complexes (beads) for each of the surfaces of lane 8 was 5,500 beads/mm.sup.2 (11,000 beads/mm.sup.2 total). While lanes 1-8 were slightly under target, the total number of complexes on the top and bottom surfaces for these lanes was relatively consistent. The passing filter data indicated that a majority of the nanowells were occupied by monoclonal clusters.
ADDITIONAL NOTES
(334) Furthermore, it is to be understood that the ranges provided herein include the stated range and any value or sub-range within the stated range, as if they were explicitly recited. For example, a range represented by from about 2 mm to about 300 mm, should be interpreted to include not only the explicitly recited limits of from about 2 mm to about 300 mm, but also to include individual values, such as about 15 mm, 22.5 mm, 245 mm, etc., and sub-ranges, such as from about 20 mm to about 225 mm, etc.
(335) It should be appreciated that all combinations of the foregoing concepts and additional concepts discussed in greater detail below (provided such concepts are not mutually inconsistent) are contemplated as being part of the inventive subject matter disclosed herein. In particular, all combinations of claimed subject matter appearing at the end of this disclosure are contemplated as being part of the inventive subject matter disclosed herein. It should also be appreciated that terminology explicitly employed herein that also may appear in any disclosure incorporated by reference should be accorded a meaning most consistent with the particular concepts disclosed herein.
(336) While several examples have been described in detail, it is to be understood that the disclosed examples may be modified. Therefore, the foregoing description is to be considered non-limiting.

Claims

1. A method, comprising: immobilizing a target material at each of two opposed sequencing surfaces of a flow cell, wherein the immobilizing involves: introducing a first fluid, including a

first portion of the target material therein, into the flow cell, whereby at least some of the target material becomes immobilized by capture sites on one of the two opposed sequencing surfaces; removing the first fluid and any non-immobilized target material from the flow cell; and introducing a second fluid, including a second portion of the target material therein, into the flow cell, whereby at least some of the target material becomes immobilized by capture sites on an other of the two opposed sequencing surfaces; wherein one of: the first fluid has a density less than a density of the target material and the second fluid has a density greater than the density of the target material; or the second fluid has the density less than the density of the target material and the first fluid has the density greater than the density of the target material.

2. The method as defined in claim 1, wherein the first or second fluid having the density less than the density of the target material is an aqueous buffer solution, and wherein the second or first fluid having the density greater than the density of the target material is a sodium polytungstate solution or a sodium chloride solution.

3. The method as defined in claim 1, wherein the density of the first or second fluid at a capture temperature is at least 0.1 g/cm.sup.3 less than the density of the target material at the capture temperature, and wherein the density of the second or first fluid at the capture temperature is at least 0.1 g/cm.sup.3 greater than the density of the target material at the capture temperature.

4. The method as defined in claim 1, wherein the density of the first or second fluid that is less than the density of the target material is about 1 g/cm.sup.3 at a capture temperature, and wherein the density of the second or first fluid that is greater than the density of the target material is about 2 g/cm.sup.3 at the capture temperature.

5. The method as defined in claim 1, further comprising allowing a predetermined time to pass before removing the first fluid and any non-immobilized target material from the flow cell.

6. The method as defined in claim 1, wherein the target material immobilized on the one of the two opposed sequencing surfaces remains immobilized on the one of the two opposed sequencing surfaces when the second fluid is introduced.

7. The method as defined in claim 1, wherein the target material is a complex including: a solid support; and sequencing-ready nucleic acid fragments attached to the solid support.

8. The method as defined in claim 7, further comprising: removing the second liquid and non-immobilized complexes from the flow cell; initiating release of the sequencing-ready nucleic acid fragments from the solid support of immobilized complexes, thereby seeding at least some the sequencing-ready nucleic acid fragments to respective primers of the two opposed sequencing surfaces; removing the solid support and non-seeded sequencing-ready nucleic acid fragments; introducing an amplification mix including a liquid form of a temperature responsive material to the flow cell; causing the liquid form of the temperature responsive material to gel; initiating amplification of the seeded sequencing-ready nucleic acid fragments to generate template strands, whereby the gel form of the temperature responsive material reduces diffusion of the template strands; causing the gel form of the temperature responsive material to liquify; and removing the liquid form of the temperature responsive material from the flow cell.

9. The method as defined in claim 8, wherein the temperature responsive material is a copolymer of poly(N-isopropylacrylamide) and polyethylene glycol.

10. The method as defined in claim 1, wherein the target material is a clustered solid support including: a solid support; and a cluster of template strands attached to the solid support.