poly(propylene imine), poly(vinyl alcohol), poly(2-ethyl-2-oxazoline), polyacrylic acid, poly(trolox ester), and combinations thereof.

[0023] In the second aspect of the array ~~10~~, a molar ratio of the spacer to the sequencing primer ranges from about 0.25:1 to about 5:1.

[0024] One example of the second aspect of the array further comprises a triplet state quencher, an anti-oxidant, or a fluorescence resonance energy transfer (FRET) donor bound to the spacer. In some examples of this aspect, the triplet state quencher is selected from the group consisting of cyclo-octyltetraene (COT), Trolox, and nitrobenzyl alcohol (NBA). In some examples of this aspect, the anti-oxidant is selected from the group consisting of ascorbate, glutathione, gallic acid, catechin, Trolox, and vitamin E. In some examples of this aspect, the FRET donor is selected from the group consisting of a donor dye to FRET with a green-emitting dye and a donor dye to FRET with a red-emitting dye.

[0025] In one example of the second aspect of the array, the spacer is grafted to the gel material through a terminal functional group. In some examples, the functional group is selected from the group consisting of an alkyne, a norbornyl, a copper free click moiety, and a thiol.

[0026] It is to be understood that any features of the second aspect of the array 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 second aspect may be used together, and/or that any features from either or both of these aspects may be combined with any of the examples disclosed herein.

[0027] An aspect of the method comprises positioning a gel material in each of a plurality of discrete wells of a support, grafting a sequencing primer to the gel material, and grafting a non-sequencing entity to the gel material.

[0028] In one example of this aspect of the method, the sequencing primer is grafted to the gel material before or after the non-sequencing entity is grafted to the gel material.

[0029] In another example of this aspect of the method, the sequencing primer and the non-sequencing entity are co-grafted to the gel material. Co-grafting is accomplished by depositing a mixture of the sequencing primer and the non-sequencing entity onto the support having the gel material thereon; and incubating the support at a predetermined temperature.

[0030] In this aspect of the method, the non-sequencing entity is selected from the group consisting of a dendrimer, polydextran, methacryloyloxyethyl phosphorylcholine, poly(ethylene

examples disclosed herein, the non-sequencing entity in its as-grafted form is not capable of undergoing DNA or RNA sequencing. In other words, from the point at which the non-sequencing entity is grafted to the gel material, it is not able to be sequenced. As such, additional processing steps do not have to be taken in order to render the non-sequencing entity non-reactive during sequencing. Rather, when the non-sequencing entity is grafted, it is not able to be sequenced.

[0043] As used herein, the term "attached" refers to the state of two things being joined, fastened, adhered, connected, or bound to each other. For example, a nucleic acid can be attached to a material, such as the gel material, by a covalent or non-covalent bond. A covalent bond is characterized by the sharing of pairs of electrons between atoms. A non-covalent bond is a chemical bond that does not involve the sharing of pairs of electrons and can include, for example, hydrogen bonds, ionic bonds, van der Waals forces, hydrophilic interactions and hydrophobic interactions.

[0044] An "azide" or "azido" functional group refers to $-N_3$.

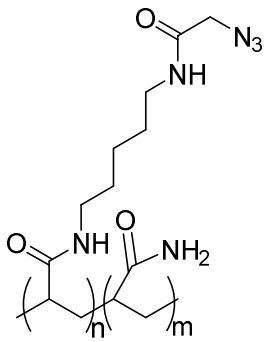
[0045] As used herein, "carbocyclyl" means a non-aromatic cyclic ring or ring system containing only carbon atoms in the ring system backbone. When the carbocyclyl is a ring system, two or more rings may be joined together in a fused, bridged or spiro-connected fashion. Carbocyclyls may have any degree of saturation, provided that at least one ring in a ring system is not aromatic. Thus, carbocyclyls include cycloalkyls, cycloalkenyls, and cycloalkynyls. The carbocyclyl group may have 3 to 20 carbon atoms (i.e., C3-20). Examples of carbocyclyl rings include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexenyl, 2,3-dihydro-indene, bicyclo[2.2.2]octanyl, adamantyl, and spiro[4.4]nonanyl.

[0046] As used herein, the term "carboxylic acid" or "carboxyl" as used herein refers to -C(O)OH.

[0047] As used herein, "cycloalkyl" means a fully saturated carbocyclyl ring or ring system. Examples include cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

[0048] As used herein, "cycloalkylene" means a fully saturated carbocyclyl ring or ring system that is attached to the rest of the molecule via two points of attachment.

[0049] As used herein, "cycloalkenyl" or "cycloalk~~a~~ene" means a carbocyclyl ring or ring system having at least one double bond, wherein no ring in the ring system is aromatic. Examples include cyclohexenyl or cyclohexene and norbornene or norbornenyl. Also as used



wherein n is an integer in the range of 1-20,000, and m is an integer in the range of 1-100,000. As with Formula (I), one of ordinary skill in the art will recognize that the "n" and "m" subunits are recurring units that are present in random order throughout the polymer structure.

[0096] The molecular weight of the Formula (I) polymer or PAZAM polymer may range from about 10 kDa to about 1500 kDa, or may be, in a specific example, about 312 kDa.

[0097] In some examples, the Formula (I) polymer or PAZAM polymer is a linear polymer. In some other embodiments, the Formula (I) or PAZAM polymer is a lightly cross-linked polymer. In other examples, the Formula (I) or PAZAM polymer comprises branching. Other suitable polymers are co-polymers of SFA and SFA derivatized with a bromo-acetamide group (e.g., BRAPA), or co-polymers of SFA and SFA derivatized with an azido-acetamide group.

[0098] Other examples of suitable gel materials 24 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, see, for example, U.S. Patent Publication No. 2011/0059865, which is incorporated herein by reference in its entirety), or an azidolyzed version of SFA. Examples of suitable polyacrylamide polymers may be formed from acrylamide and an acrylic acid or an acrylic acid containing a vinyl group as described, for example, in WO 2000/031148 (incorporated herein by reference in its entirety) or from monomers that form [2+2] photo-cycloaddition reactions, for example, as described in WO 2001/001143 or WO 2003/0014392 (each of which is incorporated herein by reference in its entirety).

[0099] The gel material 24 may be a preformed gel material. Preformed gel materials may be coated using spin coating, or dipping, or flow of the gel under positive or negative pressure, or techniques set forth in U.S. Patent No. 9,012,022, which is incorporated herein by reference in its entirety. Dipping or dip coating may be a selective deposition technique, depending upon the support 12 and the gel material 24 that are used. As an example, the patterned support 12 is

dipped into a preformed gel material 24, and the gel material 24 may fill the sites 16 selectively (i.e., the gel material 24 does not deposit on the interstitial regions 18), and polishing (or another removal process) may not be necessary.

[00100] Preformed polymer or PAZAM may be coated on the patterned support 12 using spin coating, or dipping, or flow of the gel under positive or negative pressure, or techniques set forth in U.S. Patent No. 9,012,022. The attachment of the polymer or PAZAM may also take place via a surface initiated atom transfer radical polymerization (SI-ATRP) to a silanized surface. In this example, the support 12 surface may be pre-treated with APTS (methoxy or ethyoxy silane) to covalently link silicon to one or more oxygen atoms on the surface (without intending to be held by mechanism, each silicon may bond to one, two or three oxygen atoms). This chemically treated surface is baked to form an amine group monolayer. The amine groups are then reacted with Sulfo-HSAB to form an azido derivative. UV activation at 21°C with 1 J/cm^2 to 30 J/cm^2 of energy generates an active nitrene species, which can readily undergo a variety of insertion reactions with the PAZAM.

[00101] Other examples for coating PAZAM on the support 12 are described in U.S. Patent Publication No. 2014/0200158, which is incorporated herein by reference in its entirety), and include ultraviolet (UV) mediated linking of PAZAM monomers to an amine-functionalized surface, or a thermal linkage reaction involving an active group (acryloyl chloride or other alkene or alkyne-containing molecule) with subsequent deposition of PAZAM and application of heat. In some examples, the surface ~~30~~ is modified with alkenyl or cycloalkenyl groups, which can then react with azido-functionalized polymers such as PAZAM or those comprising azido-derivatized SFA, under conditions such as click chemistry, to form covalent bonds between the modified surface and the polymer.

[00102] In still other examples, the PAZAM may be deposited on the support 12, which includes, at its surface, a silane or silane derivative that can attach to a functional group of the PAZAM. For example, the silane or silane derivative may contain an unsaturated moiety (e.g., cycloalkenes, cycloalkynes, heterocycloalkenes, heterocycloalkynes, substituted variants thereof, and combinations thereof) that can covalently attach to a functional group of the PAZAM. Examples of cycloalkene unsaturated moieties include norbornene, a norbornene derivative (e.g., a (hetero)norbornene including an oxygen or nitrogen in place of one of the carbon atoms), transcyclooctene, transcyclooctene derivatives, transcyclopentene, transcycloheptene, trans-

cyclononene, bicyclo[3.3.1]non-1-ene, bicyclo[4.3.1]dec-1 (9)-ene, bicyclo [4.2.1]non-1(8)-ene, and bicyclo[4.2.1]non-1-ene. Any of these cycloalkenes can be substituted as described in U.S. Patent Publication No. 2015/0005447, which is incorporated herein by reference in its entirety. An example of the norbornene derivative includes [(5-bicyclo[2.2.1]hept-2-enyl)ethyl]trimethoxysilane. Examples of cycloalkyne unsaturated moieties include cyclooctyne, a cyclooctyne derivative, or bicyclononynes (e.g., bicyclo[6.1.0]non-4-yne or derivatives thereof, bicyclo[6.1.0]non-2-yne, or bicyclo[6.1.0]non-3-yne). These cycloalkynes can also be substituted as described in U.S. Patent Publication No. 2015/0005447.

[00103] In these examples, the PAZAM may be deposited on the surface of the silanized support 12 using spin coating, or dipping or dip coating, or flow of the functionalized molecule under positive or negative pressure, or techniques set forth in U.S. Patent No. 9,012,022. For deposition, the PAZAM may be present in a solution (e.g., an ethanol and water mixture). After being deposited, the PAZAM solution may also be exposed to a curing process to form the gel material 24.

[00104] The gel material 24 may be a liquid that subsequently forms the gel material 24. An example of applying liquid that subsequently forms the gel material 24 is the coating of an array of sites 16 with silane free acrylamide and N-[5-(2-bromoacetyl) aminopentyl]acrylamide (BRAPA) in liquid form and allowing the reagents to form a gel by polymerization on the surface. Another example involves coating of an array of sites 16 with PAZAM monomers in liquid form and allowing the reagents to form a gel by polymerization on the surface. Coating of an array in this way can use chemical reagents and procedures as set forth in U.S. Patent Publication No. 2011/0059865.

[00105] The gel material 24 may be covalently linked to the support 12 (at the sites 16) or may not be covalently linked to the support 12. The covalent linking of the gel material ~~12~~24 to the sites 16 is helpful for maintaining the gel in the structured sites 16 throughout the lifetime of the array 10 during a variety of uses. The following are some examples of reactions that can take place between PAZAM and a silane or silane derivative on some examples of the support 12, which lead to covalent linkages.

[00106] When the silane or silane derivative on the support 12 includes norbornene or a norbornene derivative as the unsaturated moiety, the norbornene or a norbornene derivative can: i) undergo a 1,3-dipolar cycloaddition reaction with an azide/azido group of PAZAM; ii)

[00128] Examples of the polymer strand include a dendrimer (e.g., poly(amido) amine or PAMAM), polydextran, methacryloyloxyethyl phosphorylcholine (PCA), poly(ethylene glycol) (PEG, i.e., poly(ethylene oxide) or PEO), poly(ethylene imine) (PEI), poly-L-lysine (PLL), propargyl methacrylate (PMA), poly(methyl methacrylate) (PMMA), poly(N-isopropylacrylamide) (PNIPAM), poly(ethylene glycol) acrylate (POEGA), poly(propylene imine) (PPI, which is a dendrimer core), poly(vinyl alcohol) (PVA), poly(2-ethyl-2-oxazoline), polyacrylic acid (PAA), and poly(trolox ester).

[00129] Each of these polymer strand materials may function as spacers to create additional space between the sequencing primers 20 that are attached to the gel material 24. Some of these polymer strand materials also have properties that can add hydrophilicity to the gel material 24 and limit non-specific binding on the gel material 24. An example of this polymer strand material includes PEG. In the examples disclosed herein, PEG has a molecular weight ranging from about 0.5 KDa to about 10 KDa. Other polymer strand materials are also multi-functional, for example, poly(trolox ester) may be a spacer and may be an anti-oxidant.

[00130] The polymer strand either includes a functional group that can react with group(s) of the gel material 24, or is modified to include a functional group that can react with group(s) of the gel material 24. Examples of such functional groups include an alkyne, a norbornyl, a copper free click moiety (e.g., dibenzocyclooctyne (DIBO) or others), and a thiol. Alkynes, norbornyls, and copper free click moieties may react with azides of PAZAM via click reactions. Thiols may react with SFA. An example of a polymer strand material that includes one of the listed functional groups is PMA, which includes an alkyne. Examples of modified polymer strands include alkyne terminated PEG, alkyne terminated PMMA, norbornyl terminated PMMA, thiol terminated PNIPAM, alkyne terminated PVA, thiol terminated PVA, alkyne terminated PAA, and thiol terminated PAA.

[00131] In some examples, the polymer strands as described may be grafted to the gel material 24 as the non-sequencing entity 22.

[00132] In other examples, the polymer strands as described may be a linker molecule that attaches a fluorescence enhancer, such as a triplet state quencher, an anti-oxidant, or a FRET donor, to the gel material 24. For example, the polymer strand may be terminated with an alkyne on one end to attach to the gel material 24, and a functional group (e.g., thiol, amine, aldehyde,

[00141] 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.

EXAMPLES

[00142] ***Example 1***

[00143] Sequencing primers (e.g., P5/P7 with poly T tails) were grafted with a non-sequencing primer (i.e., 5'-hexyne-TTT, referred to as NSP) in wells of a flow cell available from Illumina, Inc. The molar ratio of the non-sequencing primer to the sequencing primer was varied among the samples. The sequencing primer was used without a non-sequencing primer for two control samples.

[00144] A mixture was prepared for each sample. The mixture included ultrapure water, a buffer, and an excess of catalysts (e.g., $CuSO_4$ (20 mM – 200 mM), ascorbate (20 mM - 200 mM), and pentamethyldiethylenetriamine (PMDTA) (105 mM – 1050 mM). The concentration of the sequencing primer(s) was about 1 μM. The total amount of catalyst was the same in each sample. The molar ratio of the non-sequencing primer (NSP) to the sequencing primer (P5/P7) is shown in Table 1.

TABLE 1

Flow Cell Lane	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Sample	**Control 1**	**2**	**3**	**4**	**Control 5**	**6**	**7**
NSP:P5/P7	0:1	2:1	5:1	10:1	0:1	20:1	50:1

[00145] Each mixture was applied to a respective flow cell lane and incubated at about 60°C. The flow cell was washed to remove unbound primers.

[00146] A tetrachloro-fluorescein (TET) mixture including a buffer and TET oligos (i.e., dye labeled oligonucleotides having complementary sequences to the ~~T10~~ P5/P7 primers) was applied to the flow cell lanes to stain the surface sequencing primers. TET can be hybridized to the ~~P10~~ P5/P7 primers on a surface; the excess TET can be washed away, and the attached dye concentration (in terms of intensity) can be measured by fluorescence detection using a scanning instrument, such as a Typhoon Scanner (General Electric). From the intensity data, spatial distribution and

~~What is claimed is:~~Claims

1. An array, comprising:

a support including a plurality of discrete wells, each of the plurality of discrete wells having a surface opening that is completely surrounded by an interstitial region of the support;

a gel material positioned in each of the discrete wells;

~~a~~ sequencing primers grafted to the gel material, the sequencing primers consisting of forward amplification primers and reverse amplification primers; and

a non-sequencing entity grafted to the gel material~~;~~

~~each of the sequencing primer and the non-sequencing entity being in its as-grafted form.~~

~~2. The array as defined in claim 1, wherein,~~ the non-sequencing entity ~~is selected from the group~~ consisting of a dendrimer, polydextran, methacryloyloxyethyl phosphorylcholine, poly(ethylene glycol), poly(ethylene imine), poly-L-lysine, propargyl methacrylate, poly(methyl methacrylate), poly(N-isopropylacrylamide), poly(ethylene glycol) acrylate, poly(propylene imine), poly(vinyl alcohol), poly(2-ethyl-2-oxazoline), polyacrylic acid, poly(trolox ester), a peptide, ~~and~~ or a non-functional primer;

each of the sequencing primers and the non-sequencing entity being in its as-grafted form, and wherein the gel material, the sequencing primers, the sequencing primer, and the non-sequencing entity are not present at the interstitial region.

~~3~~2. The array as defined in claim ~~2~~1, wherein the non-sequencing entity ~~is~~ consists of the non-functional primer, and wherein the non-functional primer is polyT or polyA.

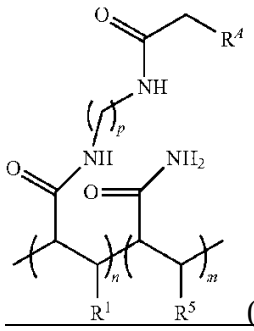
~~4~~3. The array as defined in claim ~~2~~1, wherein the non-sequencing entity ~~is~~ consists of poly(ethylene glycol) having a molecular weight ranging from about 0.5 KDa to about 10 KDa.

~~5~~4. The array as defined in ~~any of~~ claim 1 ~~or claim 2~~, wherein the non-sequencing entity is grafted to the gel material through a terminal functional group selected from the group consisting of an alkyne, a norbornyl, a copper free click moiety, and a thiol.

5. The array as defined in claim 1, wherein a molar ratio of the non-sequencing entity to the sequencing primers ranges from about 0.25:1 to about 5:1.

6. The array as defined in claim 1, wherein:

the gel material includes a recurring unit of Formula (I):



(I)

wherein:

R^1 is H or optionally substituted alkyl;

R^A is selected from the group consisting of azido, optionally substituted amino, optionally substituted alkenyl, optionally substituted hydrazone, optionally substituted hydrazine, carboxyl, hydroxy, optionally substituted tetrazole, optionally substituted tetrazine, nitrile oxide, nitrone, and thiol;

R^5 is selected from the group consisting of H and optionally substituted alkyl;

each of the $-(CH_2)_p-$ 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.

~~6~~7. ~~The array as defined in any of claim 1 or claim 5,~~ An array, comprising:

a support including a plurality of discrete wells;

a gel material positioned in each of the discrete wells;

a sequencing primer grafted to the gel material; and

a non-sequencing entity grafted to the gel material;

each of the sequencing primer and the non-sequencing entity being in its as-grafted form and wherein the non-sequencing entity includes a linker and a triplet state quencher or an anti-oxidant bound to the linker.

~~7~~8. The array as defined in claim ~~6~~7, wherein:

the linker is selected from the group consisting of a dendrimer, polydextran, methacryloyloxyethyl phosphorylcholine, poly(ethylene glycol), poly(ethylene imine), poly-L-lysine, propargyl methacrylate, poly(methyl methacrylate), poly(N-isopropylacrylamide), poly(ethylene glycol) acrylate, poly(propylene imine), poly(vinyl alcohol), poly(2-ethyl-2-oxazoline), polyacrylic acid, and poly(trolox ester); and

one of:

the triplet state quencher is selected from the group consisting of cyclo-octyltetraene (COT), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and nitrobenzyl alcohol (NBA); ~~or~~and

the anti-oxidant is selected from the group consisting of ascorbate, glutathione, gallic acid, catechin, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and vitamin E.

~~8~~9. ~~The array as defined in any of claim 1 or claim 5,~~ An array, comprising:

a support including a plurality of discrete wells;

a gel material positioned in each of the discrete wells;

a sequencing primer grafted to the gel material; and

a non-sequencing entity grafted to the gel material;

each of the sequencing primer and the non-sequencing entity being in its as-grafted form;

wherein the non-sequencing entity includes a linker and a fluorescence resonance energy transfer (FRET) donor bound to the linker.

~~9~~10. The array as defined in claim ~~8~~9, wherein:

the linker is selected from the group consisting of a dendrimer, polydextran, methacryloyloxyethyl phosphorylcholine, poly(ethylene glycol), poly(ethylene imine), poly-L-lysine, propargyl methacrylate, poly(methyl methacrylate), poly(N-

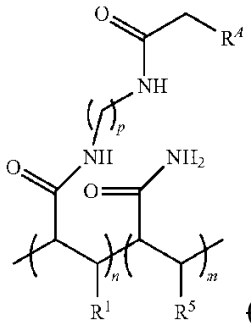
isopropylacrylamide), poly(ethylene glycol) acrylate, poly(propylene imine), poly(vinyl alcohol), poly(2-ethyl-2-oxazoline), polyacrylic acid, and poly(trolox ester); and

the FRET donor is selected from the group consisting of a donor dye to FRET with a green-emitting dye and a donor dye to FRET with a red-emitting dye.

~~10. The array as defined in any of the preceding claims, wherein a molar ratio of the non-sequencing entity to the sequencing primer ranges from about 0.25:1 to about 5:1.~~

~~11. The array as defined in any of the preceding claims wherein:~~

~~gel material includes a recurring unit of Formula (I):~~



~~(I)~~

~~wherein:~~

~~R^1 is H or optionally substituted alkyl;~~

~~R^A is selected from the group consisting of azido, optionally substituted amino, optionally substituted alkenyl, optionally substituted hydrazone, optionally substituted hydrazine, carboxyl, hydroxy, optionally substituted tetrazole, optionally substituted tetrazine, nitrile oxide, nitrone, and thiol;~~

~~R^5 is selected from the group consisting of H and optionally substituted alkyl;~~

~~each of the $-(CH_2)_p-$ 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.~~

~~12~~11. An array, comprising:

a support including a plurality of discrete wells, each of the plurality of discrete wells having a surface opening that is completely surrounded by an interstitial region of the support;

a gel material positioned in each of the discrete wells;

~~a~~ sequencing primers grafted to the gel material, the sequencing primers consisting of forward amplification primers and reverse amplification primers; and

a spacer grafted to the gel material, the spacer ~~being selected from the group~~ consisting of a dendrimer, polydextran, methacryloyloxyethyl phosphorylcholine, poly(ethylene glycol), poly(ethylene imine), poly-L-lysine, propargyl methacrylate, poly(methyl methacrylate), poly(N-isopropylacrylamide), poly(ethylene glycol) acrylate, poly(propylene imine), poly(vinyl alcohol), poly(~~2~~-ethyl-2-oxazoline), polyacrylic acid, poly(trolox ester), ~~and~~ or combinations thereof, and wherein the gel material, the sequencing primer, and the non-sequencing entities are not present at the interstitial region.

12. The array as defined in claim 11, wherein a molar ratio of the spacer to the sequencing primers ranges from about 0.25:1 to about 5:1.

13. The array as defined in claim 11, wherein the spacer is grafted to the gel material through a terminal functional group selected from the group consisting of an alkyne, a norbornyl, a copper free click moiety, and a thiol.

~~13~~14. ~~The array as defined in claim 12, further comprising~~ An array, comprising:

a support including a plurality of discrete wells;

a gel material positioned in each of the discrete wells;

a sequencing primer grafted to the gel material;

a spacer grafted to the gel material, the spacer being selected from the group consisting of a dendrimer, polydextran, methacryloyloxyethyl phosphorylcholine, poly(ethylene glycol), poly(ethylene imine), poly-L-lysine, propargyl methacrylate, poly(methyl methacrylate), poly(N-isopropylacrylamide), poly(ethylene glycol) acrylate, poly(propylene imine), poly(vinyl alcohol), poly(-ethyl-2-oxazoline), polyacrylic acid, poly(trolox ester), and combinations thereof; and

a triplet state quencher, an anti-oxidant, or a fluorescence resonance energy transfer (FRET) donor bound to the spacer.

~~14~~15. The array as defined in claim ~~13~~14, wherein:

the triplet state quencher is bound to the spacer; and

the triplet state quencher is selected from the group consisting of cyclo-octyltetraene (COT), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and nitrobenzyl alcohol (NBA).

~~15~~16. The array as defined in claim ~~13~~14, wherein:

the anti-oxidant is bound to the spacer; and

the anti-oxidant is selected from the group consisting of ascorbate, glutathione, gallic acid, catechin, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and vitamin E.

~~16~~17. The array as defined in claim ~~13~~14, wherein:

the FRET donor is bound to the spacer; and

the FRET donor is selected from the group consisting of a donor dye to FRET with a green-emitting dye and a donor dye to FRET with a red-emitting dye.

~~17. The array as defined in any of claims 12-16 wherein a molar ratio of the spacer to the sequencing primer ranges from about 0.25:1 to about 5:1.~~

~~18. The array as defined in any of claims 12-17, wherein the spacer is grafted to the gel material through a terminal functional group selected from the group consisting of an alkyne, a norbornyl, a copper free click moiety, and a thiol.~~

~~19~~18. A method, comprising:

positioning a gel material in each of a plurality of discrete wells of a support, each of the plurality of discrete wells having a surface opening that is completely surrounded by an interstitial region of the support;

grafting ~~a~~ sequencing primers to the gel material, the sequencing primers consisting of forward amplification primers and reverse amplification primers; and

grafting a non-sequencing entity to the gel material, the non-sequencing entity consisting of a dendrimer, polydextran, methacryloyloxyethyl phosphorylcholine, poly(ethylene glycol), poly(ethylene imine), poly-L-lysine, propargyl methacrylate, poly(methyl methacrylate), poly(N-

isopropylacrylamide), poly(ethylene glycol) acrylate, polypropylene imine), poly(vinyl alcohol), poly(2-ethyl-2-oxazoline), polyacrylic acid, poly(trolox ester), a peptide, or a non-functional primer;

wherein each of the sequencing primers and the non-sequencing entity is in its as-grafted form, and wherein the gel material, the sequencing primers, and the non-sequencing entity are not present at the interstitial region.

~~20~~19. The method as defined in claim ~~19~~18, wherein the sequencing primers ~~is~~ are grafted to the gel material before or after the non-sequencing entity is grafted to the gel material.

~~21~~20. The method as defined in claim ~~19~~18, wherein the sequencing primer and the non-sequencing entity are co-grafted to the gel material.

~~22~~21. The method as defined in claim ~~21~~20, wherein co-grafting is accomplished by:

depositing a mixture of the sequencing primers and the non-sequencing entity onto the support having the gel material thereon; and

incubating the support at a predetermined temperature.

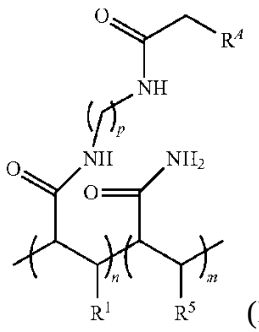
~~23~~22. The method as defined in ~~any of claims 19-22~~ claim 18, wherein:

~~the non-sequencing entity is selected from the group consisting of a dendrimer, polydextran, methacryloyloxyethyl phosphorylcholine, poly(ethylene glycol), poly(ethylene imine), poly-L-lysine, propargyl methacrylate, poly(methyl methacrylate), poly(ethylene glycol) acrylate, poly(propylene imine), poly(vinyl alcohol), poly(2-ethyl-2-oxazoline), polyacrylic acid, poly(trolox ester), a peptide, and a non-functional primer; and~~

the non-sequencing entity is grafted to the gel material through a terminal functional group selected from the group consisting of an alkyne, a norbornyl, a copper free click moiety, and a thiol.

~~24~~23. The method defined in ~~any of claims 19-23~~ claim 18, wherein:

the gel material includes a recurring unit of Formula (I):



(I)

wherein:

R^1 is H or optionally substituted alkyl;

R^A is selected from the group consisting of azido, optionally substituted amino, optionally substituted alkenyl, optionally substituted hydrazone, optionally substituted hydrazine, carboxyl, hydroxy, optionally substituted tetrazole, optionally substituted tetrazine, nitrile oxide, nitrone, and thiol;

R^5 is selected from the group consisting of H and optionally substituted alkyl;

each of the $-(CH_2)_p-$ 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.