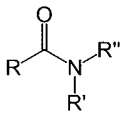
[00035] The alkyl may be substituted with a halide or halogen, which means any one of the radio-stable atoms of column 7 of the Periodic Table of the Elements, e.g., fluorine, chlorine, bromine, or iodine. This group is referred to as an “alkyl halide”.

[00036] As used herein, “alkenyl” or “alkene” refers to a straight or branched hydrocarbon chain containing one or more double bonds. The alkenyl group may have 2 to 20 carbon atoms. Example alkenyl groups include ethenyl, propenyl, butenyl, pentenyl, hexenyl, and the like. The alkenyl group may be designated as, for example, "C2-4 alkenyl," which indicates that there are two to four carbon atoms in the alkenyl chain.

[00037] As used herein, “alkynyl” or "alkyne" refers to a straight or branched hydrocarbon chain containing one or more triple bonds. The alkyne group may have 2 to 20 carbon atoms. The alkyne group may be designated, for example, as "C2-4 alkynyl," which indicates that there are two to four carbon atoms in the alkyne chain.

[00038] An “amido” functional group refers to 

, where R is any group that can attach to a fluorescent label, R’ is H, and R’’ is any group that can attached to a nucleotide sequence.

[00039] An “amino” functional group refers to an $-NR_aR_b$ group, where R_a and R_b are each independently selected from hydrogen, C1-6 alkyl, C2-6 alkenyl, C2-6 alkynyl, C3-7 carbocyclyl, C6-10 aryl, 5-10 membered heteroaryl, and 5-10 membered heterocyclyl, as defined herein).

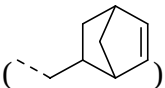
[00040] As used herein, "aryl" refers to an aromatic ring or ring system (i.e., two or more fused rings that share two adjacent carbon atoms) containing only carbon in the ring backbone. When the aryl is a ring system, every ring in the system is aromatic. The aryl group may have 6 to 18 carbon atoms, which may be designated as C6-18. Examples of aryl groups include phenyl, naphthyl, azulenyl, and anthracenyl.

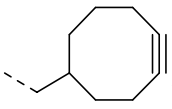
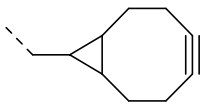
[00041] An “azide” or “azido” functional group refers to $-N_3$.

[00042] 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.

[00043] As used herein, "carbocyclyl" means a ring or ring system containing carbon atoms in the ring 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).

[00044] As used herein, “cycloalkenyl” or “cycloalkene” 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 norbornyl (). Also as used herein, “heterocycloalkenyl” or “heterocycloalkene” means a carbocyclyl ring or ring system with at least one heteroatom in ring backbone, having at least one double bond, wherein no ring in the ring system is aromatic.

[00045] As used herein, “cycloalkynyl” or “cycloalkyne” means a carbocyclyl ring or ring system having at least one triple bond, wherein no ring in the ring system is aromatic. An example is cyclooctyne (). Another example is bicyclononyne (i.e., a bicyclic ring system, such as ). Also as used herein, “heterocycloalkynyl” or “heterocycloalkyne” means a carbocyclyl ring or ring system with at least one heteroatom in ring backbone, having at least one triple bond, wherein no ring in the ring system is aromatic.

[00046] The term “chemical cleavage,” as used herein, refers to a chemical reaction that removes the quality control tracer or a portion thereof from a support.

[00047] As used herein, the term “cleavable nucleotide sequence” refers to a single stranded nucleic acid sequence that can be broken at an excision site or at a linker molecule.

[00048] The term “each,” when used in reference to a collection of items, is intended to identify 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.

including carbon atoms and heteroatoms). The heterocyclyl group may be designated as "3-6 membered heterocyclyl" or similar designations. In some examples, the heteroatom(s) are O, N, or S.

[00056] The term “hydrazine” or “hydrazinyl” as used herein refers to a -$NHNH_2$ group.

[00057] As used herein, the term “hydrazone” or “hydrazonyl” refers to a group $R_a(R_b)C=N-NH_2$, in which R_a and R_b are previously defined herein.

[00058] As used herein, “hydroxyl” is an –OH group.

[00059] As used herein, the term “interstitial region” refers to an area in a substrate/support or on a surface that separates other areas of the substrate or surface. For example, an interstitial region can separate one feature of an array from another feature of the array. The two features that are separated from each other can be discrete, i.e., lacking contact with each other. In another example, an interstitial region can separate a first portion of a feature from a second portion of a feature. In many examples, the interstitial region is continuous whereas the features are discrete, for example, as is the case for a plurality of wells defined in an otherwise continuous surface. The separation provided by an interstitial region can be partial or full separation. Interstitial regions may have a surface material that differs from the surface material of the features defined in the surface. For example, features of an array can have an amount or concentration of gel material and a quality control tracer that exceeds the amount or concentration present at the interstitial regions. In some examples, gel material and quality control tracer(s) may not be present at the interstitial regions.

[00060] The term “linker molecule,” as used herein, refers to a molecule that includes, at one end, a functional group that can attach to the detectable or fluorescent label and, at the other end, a functional group that can attach to a nucleotide sequence. The attachment points are covalent bonds. Similarly, the term “linked,” as used herein, refers to two entities that are connected via one or more covalent bonds, either directly or via a linker molecule.

[00061] “Nitrile oxide,” as used herein, means a “$R_aC\equiv N^+O^-$” group in which R_a is selected from hydrogen, C1-6 alkyl, C2-6 alkenyl, C2-6 alkynyl, C3-7 carbocyclyl, C6-10 aryl, 5-10 membered heteroaryl, and 5-10 membered heterocyclyl. Examples of preparing nitrile oxide include in situ generation from aldoximes by treatment with chloramide-T or through action of base on imidoyl chlorides [RC(Cl)=NOH].

[00074] As used herein, the term “well” refers to a discrete concave feature in a support having a surface opening that is completely surrounded by interstitial region(s) of the support surface. Wells 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 well taken orthogonally with the surface can be curved, square, polygonal, hyperbolic, conical, angular, etc.

[00075] In some embodiments, the cleavable nucleotide sequence is cleavable via enzymatic cleavage or chemical cleavage.

[00076] In some instances, the cleavable nucleotide sequence comprises a linker molecule attaching the fluorescent label, wherein the linker molecule comprises a moiety cleavable via enzymatic cleavage or chemical cleavage. In some embodiments, the linker molecule comprises a vicinal diol and the chemical cleavage is accomplished by oxidative conditions, such as, for example, sodium periodate ($NaIO_4$). In other embodiments, the linker molecule comprises a disulfide and the chemical cleavage is accomplished with a thiol or a tertiary phosphine; or the linker molecule is a silane and the chemical cleavage is accomplished with an acid or a fluoride ion; or the linker molecule is an azobenzene and the chemical cleavage is accomplished with a sodium dithionate ($Na_2S_2O_4$); or the linker molecule is a photocleavable group and the chemical cleavage is accomplished with light; or the linker molecule is an azido and the chemical cleavage is accomplished with a tertiary phosphine. In another example of the second aspect, the method further comprises cleaving the fluorescent label from the cleavable nucleobase of the non-reactive nucleotide sequence using an exonuclease.

[00077] In one example of this aspect, the quality control tracer comprises the cleavable nucleotide sequence, and the cleavable nucleotide sequence includes an excision site. In an example, the cleavable nucleotide sequence is a primer nucleotide sequence.

[00078] In another example of this aspect, the quality control tracer is the cleavable nucleotide sequence, and the cleavable nucleotide sequence includes a linker molecule attaching the fluorescent label. In some embodiments, the linker molecule comprises a functional group selected from the group consisting of a diol, a disulfide, a silane, an azobenzene, a photocleavable group, and an azido. In this other example of this aspect, the cleavable nucleotide sequence further includes a primer nucleotide sequence attached to the linker molecule.

material 24. The attachment technique that is used, and whether separate, primer nucleotide sequences 20, 20’ are included will depend, in part, upon the quality control tracer 22 that is utilized. Various examples of the quality control tracer 22 are shown in FIGS. 3A through 3D and in FIG. 4. Each example will now be described.

[000128] FIG. 3A depicts an example of the quality control tracer 22A that is included on the gel material 24/gel pad 24’ with separate primer nucleotide sequence(s) 20, 20’. In this example, the quality control tracer 22A is a cleavable nucleotide sequence 27 tagged, at its 3’ end, with a fluorescent label 28, and the primer nucleotide sequence(s) 20, 20’ are untagged primer nucleotide sequence(s).

[000129] The cleavable nucleotide sequence 27 of FIG. 3A may include a functional group at its 5’ end that is capable of attaching to the gel material 24. Examples of this functional group include an alkyne, a norbornyl (or other cycloalkenyls), a copper free click moiety (e.g., dibenzocyclooctyne (DIBO), other cycloalkynes, or others), and a thiol. This functional group may be selected based upon the gel material 24 that is used. For example, alkynes, norbornyls, and copper free click moieties may react with azides of PAZAM via click reactions, and thiols may react with SFA. In some embodiments, the functional group is an alkyne.

[000130] The cleavable nucleotide sequence 27 of FIG. 3A may also include a functional group at its 3’ end that is capable of attaching to the fluorescent label 28. An example of this functional group, which can be used during oligonucleotide synthesis, is 5'-Dimethoxytrityl-5-[N-(trifluoroacetylaminohexyl)-3-acrylimido]-2'-deoxyUridine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (i.e., amino modifier C6 dT). Other examples include 5'-Dimethoxytrityl-N-dimethylformamidine-5-[N-(trifluoroacetylaminohexyl)-3-acrylimido]-2'-deoxyCytidine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (amino modifier C6 dC) and the use of solid supports such as (2-Dimethoxytrityloxymethyl-6-fluorenylmethoxycarbonylamino-hexane-1-succinoyl)-long chain alkylamino-CPG. The functional group at the 3’ end may be selected based upon the fluorescent label 28 that is used.

[000131] The detectable or fluorescent label 28 may be any suitable fluorophore that can attach to the cleavable nucleotide sequence 27, e.g., at the 3’ end (or at or near the 3’ end). Examples of suitable fluorescent labels 28 include Texas Red® (a sulfonyl chloride dye, ThermoFisher Scientific), Cy7®, Cy7.5® or sulfo-Cyanine7 NHS ester (cyanine dyes from Lumiprobe), a red wavelength dye, such as TEX™ 615 (Exiqon), fluorescent dyes , such as those in the Alexa

[000146] To form the example shown in FIG. 3A, sequential grafting or co-grafting may be used.

[000147] Sequential grafting may be accomplished by exposing the support 12 (having the gel material 24 in the sites 16) to a solution or mixture containing the quality control tracer 22A and incubating, and then to a solution or mixture containing the primer nucleotide sequence(s) 20, 20' and incubating. Alternatively, sequential grafting may be accomplished by exposing the support 12 (having the gel material 24 in the sites 16) to a solution or mixture containing the primer nucleotide sequence(s) 20, 20' and incubating, and then to a solution or mixture containing the quality control tracer 22A and incubating.

[000148] Co-grafting may be accomplished by exposing the support 12 (having the gel material 24 in the sites 16) to a solution or mixture containing the quality control tracer 22A and the primer nucleotide sequence(s) 20, 20', and then incubating. Exposure of the support 12 to this solution or mixture may be accomplished by depositing a mixture of the quality control tracer 22A and the primer nucleotide sequence(s) 20, 20' onto the support 12. In an example, the solution or mixture may be drawn across the gel material 24 coated support 12 (shown in FIG. 2C).

[000149] In any of the grafting examples used to form the example shown in FIG. 3A, incubation takes place at a predetermined temperature which depends, in part, upon the quality control tracer 22A and the primer nucleotide sequence(s) 20, 20' used. As examples, incubation may be accomplished at a temperature ranging from about 50 °C to about 70 °C.

[000150] Also in any of the grafting examples used to form the example shown in FIG. 3A, the solution may include the quality control tracer 22A and/or the primer nucleotide sequence(s) 20, 20', water, a buffer, and a catalyst. The quality control tracer 22A and/or the primer nucleotide sequence(s) 20, 20', whether present in the same solution/mixture or separate solutions/mixtures, may be present at any desired predetermined ratio.

[000151] FIG. 3B depicts another example of the quality control tracer 22B that is included on the gel material 24/gel pad 24' with separate primer nucleotide sequence(s) 20, 20'. In this example, the quality control tracer 22B is a non-reactive nucleotide sequence 32 with the fluorescent label 28 attached to a cleavable nucleobase, and the primer nucleotide sequence(s) 20, 20' are untagged primer nucleotide sequence(s).

quality control tracer 22A. In any of the grafting examples used to form the example shown in FIG. 3B, incubation takes place at a predetermined temperature which depends, in part, upon the quality control tracer 22B and the primer nucleotide sequence(s) 20, 20' used. Also in any of the grafting examples used to form the example shown in FIG. 3B, the solution may include the quality control tracer 22B and/or the primer nucleotide sequence(s) 20, 20', water, a buffer, and a catalyst. The quality control tracer 22B and/or the primer nucleotide sequence(s) 20, 20', whether present in the same solution/mixture or separate solutions/mixtures, may be present at any desired predetermined ratio.

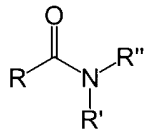
[000159] FIG. 3C depicts still another example of the quality control tracer 22C that is included on the gel material 24/gel pad 24' with separate primer nucleotide sequence(s) 20, 20'. In this example, the quality control tracer 22C is a non-reactive nucleotide sequence 32' with the fluorescent label 28 attached to a cleavable nucleobase (near the 3' end) and with an excision site 30, and the primer nucleotide sequence(s) 20, 20' are untagged primer nucleotide sequence(s).

[000160] Since this example of the quality control tracer 22C includes the fluorescent label 28 attached to a cleavable nucleobase, an exonuclease may be used to remove the fluorescent label 28 after the quality control method has been performed. In this example, the non-reactive nucleotide sequence 32' remains at least substantially intact after the quality control method has been performed, and thus the non-reactive nucleotide sequence 32' may be orthogonal to the primer nucleotide sequence(s) 20, 20' also attached to the gel material 24.

[000161] Moreover, since this example of the quality control tracer 22C also includes the excision site 30, enzymatic cleavage may be used to remove a cleavable portion 31' of the non-reactive sequence 32' after the quality control method has been performed. In this example, the cleavable portion 31' includes any sequence of oligonucleotides, the fluorescent label 28 attached to a nucleobase along the sequence, and the excision site 30. The portion 29' of the non-reactive nucleotide sequence 32' that remains attached to the gel material 24 after enzymatic cleavage is also a non-reactive nucleotide sequence, and thus will not participate in or interfere with a sequencing operation that is to be performed or is being performed. The remaining portion 29' may be a short poly T or poly A sequence, or may be a sequence that is orthogonal to the primer nucleotide sequence(s) 20, 20' also attached to the gel material 24.

[000162] The non-reactive nucleotide sequence 32' of FIG. 3C may include a functional group at its 5' end that is capable of attaching to the gel material 24. Examples of this functional group

available phosphoramidite modification at the 3’ end of the nucleotide sequence X to a functional group attached to the fluorescent label 28. An example of a suitable photocleavable group, an ortho-nitrobenzyl group, is shown as 34E in FIG. 4. In this example, the photocleavable group includes amine groups at either end. In this example, the amine group at one end of the photocleavable group is attached to a linker such as an alkyl chain which is attached to the nucleotide sequence X, and the amine group at the other end of the photocleavable group forms an amido linkage attached to the fluorescent label 28 through –$(CH_2)_4$-O-. The fluorescent label 28 is attached via any suitable attachment site (e.g. alcohol, amine, carboxylic acid). In an example, at least the fluorescent label 28 is cleaved from the quality control tracer 22E at the photocleavable site by exposing the quality control tracer 22E to light of a predetermined wavelength for a predetermined time.

[000183] As mentioned herein, an azido is any molecule including the group N_3. An example of a suitable azido is shown as the O-azidoalkyl group 34F in FIG. 4. In this example, the amine group at one end of the azido is attached to a linker such as an alkyl chain which is attached to the nucleotide sequence X at the 3’ end, and the amine group at the other end of the azido forms an amido linkage () where (in this example) R is –$(CH_2)_4$-O-fluorescent label 28, R’ is H, and R’’ is –$(CH_2)_3$-O-(CN_3)–(CH_2)-$(O(CH_2)_2)_2$-(CH_2)–(C=O)-NH–$(CH_2)_3$-X. The fluorescent label 28 is attached via any suitable attachment site (e.g. alcohol, amine, carboxylic acid). In an example, the azido may be cleaved by exposing the quality control tracer 22E to a tertiary phosphine ($R^6{}_3P$, where R^6 is an appropriately substituted alkyl or aryl group). The tertiary phosphine and the azido undergo the Staudinger reduction reaction.

[000184] Each of the linker molecules 34 disclosed herein can undergo a chemical cleavage reaction that removes at least the fluorescent label 28 from the quality control tracer 22E after an example of the quality control method has been performed. The remaining portion may be a non-reactive nucleotide sequence (that will not participate in sequencing) or a primer nucleotide sequence (that will participate in sequencing).

[000185] When the nucleotide sequence X of the quality control tracer 22E is non-reactive, the quality control tracer 22E may be sequentially grafted or co-grafted with separate primer nucleotide sequence(s) 20, 20’ as previously described herein. When the nucleotide sequence X

of the quality control tracer 22E is a primer nucleotide sequence(s) 20, 20', one type of quality control tracer 22E (each of which includes the same primer nucleotide sequence(s) 20 or 20') may be grafted, or different types of quality control tracers 22E (e.g., some of which include the primer nucleotide sequence 20 and others of which include a different primer nucleotide sequence 20') may be co-grafted. Grafting or co-grafting may be performed as previously described, except that an example of the quality control tracer 22E is used instead of the quality control tracer 22A.

[000186] Referring back to FIG. 2D, an example of the quality control tracer(s) 22 with separate primer nucleotide sequence(s) 20, 20' is depicted. In examples of the tracer 22 that include the primer nucleotide sequence(s) 20, 20' (e.g., tracer 22D and some examples of tracer 22E), separate primer nucleotide sequence(s) 20, 20' will not be present with the tracer(s) 22.

[000187] The array 10 disclosed herein may be used in a quality control method. An example of the quality control method is depicted in FIG. 5. The method 50 generally includes grafting a quality control tracer 22 to a gel material 24 in a well 16' on a support 12, the quality control tracer being selected from the group consisting of a cleavable nucleotide sequence 27, 27' tagged, at its 3' end, with a fluorescent label 28 and a non-reactive nucleotide sequence 32, 32' with the fluorescent label 28 attached to a cleavable nucleobase (as shown at reference numeral 52); detecting the quality control tracer 22 using fluorescence (as shown at reference numeral 54); and based at least in part on the fluorescence, determining a density, or a distribution, or the density and the distribution, of a primer nucleotide sequence 20, 20' grafted to the gel material 24 (as shown at reference numeral 56).

[000188] The quality control method may be performed after the quality control tracer 22, alone or in combination with separate primer nucleotide sequence(s) 20, 20', is/are grafted to the gel material 24 and prior to completion of flow cell manufacturing (e.g., prior to the bonding of the array 10 to a top substrate flow cell). The quality control method may also or alternatively be performed by an end-user of the fully manufactured flow cell. When the quality control method is performed by the end-user of the flow cell, it is to be understood that the method may be performed prior to loading any sequencing workflow reagents and DNA sample onto the flow cell. Because each quality control tracer 22A, 22B, 22C, 22D, 22E includes the detectable fluorescent label 28, hybridization of fluorescently-labeled complementary nucleotides does not need to be performed as part of the quality control method disclosed herein.

sequencing workflow. Examples of suitable enzymes include exonucleases (which remove successive nucleotides from an end of the sequence), endonucleases (which cleave phosphodiester bonds within a sequence), base excision repair enzymes (which remove specific bases to form an apurinic/apyrimidinic (AP) site, which can then be cleaved by an AP endonuclease), restriction enzymes (which scan the quality control tracer 22A for a particular sequence of 4 to 6 nucleotides at which to cut the single-stranded sequence), etc. Some specific examples are shown in Table 1 below.

TABLE 1

Enzyme	**QC Tracer**	**Cleavage**
USER enzyme	5′-NNNN**U**NNNN-*- 3′	Before read 1 of sequencing workflow
FPG (formamido pyrimidine DNA glycosylase)	5′-NNNN(**oxo-G**)NNNN-*- 3′	Before read 1 or read 2 of sequencing workflow

[000195] In Table 1, * represents the fluorescent label 28, N represents nucleotides, and the excision site is in bold.

[000196] In one example in Table 1, the excision site 30 is a uracil (dU) that is targeted by the USER enzyme. The USER enzyme is a mixture of uracil DNA glycosylase (UDG) and the DNA glycosylase-lyase endonuclease VIII. UDG catalyzes the excision of the uracil base, forming an abasic (apyrimidinic) site while leaving the phosphodiester backbone intact. The lyase activity of endonuclease VIII breaks the phosphodiester backbone at the 3´ and 5´ sides of the abasic site so that base-free deoxyribose is released. As such, cleavage of tracer 22A at excision site 30 removes a portion 31 of the sequence 27 and the fluorescent label 28 from the support 12. In the other example in Table 1, the excision site 30 is an oxidized purine (e.g., oxoG) that is targeted by FPG. FPG recognizes and removes the oxidized guanine. The FPG acts as both an N-glycosylase and an AP-lyase.

[000197] The portion 29 of the quality control tracer 22A that remains attached to the gel material 24 after enzymatic cleavage is a non-reactive nucleotide sequence, and thus will not

templates anchored to the gel material 24, and several million dense clusters of double-stranded DNA are generated (i.e., cluster generation). The sequencing reactions are then carried out. The data are aligned and compared to a reference, and sequencing differences are identified.

[000208] To further illustrate the present disclosure, an example is given herein. It is to be understood that this example is provided for illustrative purposes and is not to be construed as limiting the scope of the present disclosure.

EXAMPLE

[000209] The following quality control tracer was used:

5′-Hexyne-poly T tail-CATCTAGGCATCTAAGCATCAAUCTTACA[Amino C6 dT-Texas Red]-3’ (SEQ. ID NO. 25)

[000210] To evaluate an example of the QC tracer for quality control of P5/P7 primer grafting onto a substrate surface, P5/P7 primer grafting mixes were prepared with a 10% spike of a QC tracer and without the QC tracer spike (control). Substrate surfaces grafted with the P5/P7 primer mix that included the 10% spike of QC tracer were evaluated by measuring tracer fluorescence. The control surfaces (without QC tracer spike) were evaluated by hybridization-based TET QC. TET is a dye labeled oligonucleotide having complementary sequence to the P5/P7 primers. TET is hybridized to the P5/P7 primers on a surface, the excess TET is washed away, and the attached dye concentration is measured by fluorescence detection.

[000211] FIGS. 6A and 6B respectively show a fluorescence image of grafted control substrate surfaces hybridized with complementary dye-containing oligonucleotides (TET QC) and a fluorescence image of grafted substrate surfaces that include QC tracers. FIG. 6C shows a plot of QC tracer fluorescence versus TET fluorescence for the grafted surfaces of FIGS. 6A and 6B. The data in FIGS. 6A-6C show that fluorescence intensity from the QC tracer correlates with P5/P7 density on the substrate surface.

[000212] To evaluate cleavage of grafted QC tracers from the surface of a flow cell during a standard cluster generation process, flow cells grafted with different QC tracers were used. FIG. 7A shows an initial fluorescence image, which shows flow cells grafted with QC tracers before cluster generation. FIG. 7B shows a post-clustering fluorescence image, which shows flow cells grafted with QC tracers after cluster generation. The QC tracers (at 200 nM) were:

Claims

1. An array, comprising:
a support comprising a plurality of discrete wells;
a gel material positioned in each of the plurality of discrete wells; and
a quality control tracer grafted to the gel material in each of the plurality of discrete wells;
wherein the quality control tracer comprises (a) a cleavable nucleotide sequence comprising a cleavage site and (b) a detectable label;
the detectable label is attached to the cleavable nucleotide sequence at the cleavage site or at a position in the cleavable region that is distal to the cleavage site;
the quality control tracer comprising a grafted region having a first end grafted to the gel material and a second end linked to a cleavable region that is linked to the detectable label and comprises the cleavage site;
wherein the grafted region comprises a primer nucleotide sequence; and
further wherein the detectable label is a fluorescent label, and the quality control tracer comprises a non-reactive nucleotide sequence with the fluorescent label attached to the cleavable nucleotide sequence.

2. The array of claim 1, wherein the cleavable nucleotide sequence comprises a non-reactive nucleotide sequence.

3. The array of claim 1, wherein the grafted region comprises a non-reactive nucleotide sequence.

4. The array of any of the preceding claims, wherein the cleavage site comprises an enzymatically cleavable nucleobase.

5. The array of claim 4, wherein the enzymatically cleavable nucleobase comprises an excision site.

6. The array of claim 4, wherein the enzymatically cleavable nucleobase is cleavable by reaction with a glycosylase and an endonuclease or with an exonuclease.

7. The array of claim 6, wherein the cleavable nucleotide sequence is grafted to the gel material at its 5’ end.

8. The array of claim 7, wherein the detectable label is attached at or near the 3’ end of the cleavable nucleotide sequence.

9. The array of claim 8, wherein the detectable label is cleavable by reaction of the quality control tracer with the exonuclease.

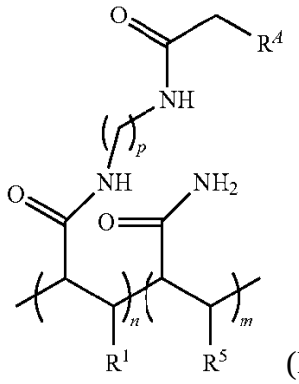
10. The array of claim 8, wherein the detectable label is cleavable by reaction of the quality control tracer with the glycosylase and the endonuclease.

11. The array of any one of claims 1-3, wherein the cleavage site comprises a chemically cleavable linker.

12. The array of claim 11, wherein the chemically cleavable linker comprises a vicinal diol, a disulfide, a silane, an azobenzene, a photocleavable group, or an azido.

13. The array of any one of claims 1-12, further comprising an unlabeled primer comprising a primer nucleotide sequence grafted to the gel material in each of the plurality of discrete wells, wherein the quality control tracer and the unlabeled primer are present in a predetermined ratio.

14. The array of any one of the preceding claims, wherein the gel material comprises a polymer 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.

15. The array of claim 14, wherein the gel material comprises PAZAM.

16. The array of claim 1, wherein the fluorescent label is selected from the group consisting of: a sulfonyl chloride dye, cyanine dyes, a red wavelength dye, a fluorescent dye, derivatives of fluorescein, xanthene fluorophores, and an indocarbocyanine dye.

17. A method of determining the density, the distribution, or the density and the distribution of a primer nucleotide sequence grafted to a support comprising:

providing a support comprising a plurality of discrete wells and a gel material positioned in each of the plurality of discrete wells and a quality control tracer and the primer nucleotide sequence grafted to the gel material in each of the plurality of discrete wells;

wherein the quality control tracer comprises (a) a cleavable nucleotide sequence comprising a cleavage site and (b) a detectable label; and wherein the detectable label is attached to the cleavable nucleotide sequence at the cleavage site or at a position in the cleavable region that is distal to the cleavage site;

detecting a signal from the detectable label;

determining the density and/or distribution of the quality control tracer based at least in part on the signal from the detectable label; and

determining the density and/or distribution of the grafted primer nucleotide sequence based at least in part on the determined density and/or distribution of the quality control tracer;

said method further comprising grafting the quality control tracer to the gel material wherein the quality control tracer comprises a first end granted to the gel material and the second end linked to a cleavable region that is linked to the detectable label and comprises the cleavage site; and the primer nucleotide sequence is grafted to the gel material, either before or at the same time as the grafting of the quality control tracer, and prior to the detecting step, and further wherein the quality control tracer and the primer nucleotide sequence are grafted to the gel material in a predetermined ratio and the determining of the density and/or distribution of the primer nucleotide sequence is based on the detected signal and predetermined ratio.

18. The method of claim 17, wherein the quality control tracer comprises a non-reactive nucleotide sequence.

19. The method of claims 17 or 18, wherein the quality control tracer comprises the primer nucleotide sequence and the grafting of the quality control tracer also serves to graft the primer nucleotide sequence to the gel material.

20. The method of any one of claims 17-19, wherein the cleavable nucleotide sequence comprises a grafted region with a first end and a second end, where the first end is grafted to the gel material and the second end is linked to a cleavable region comprising the cleavage site, wherein the second end of the grafted region is linked to the cleavage site of the cleavable region.

21. The method of claim 20, wherein the grafted region comprises a non-reactive nucleotide sequence.

22. The method of any one of claims 17-21, further comprising removing the detectable label from the quality control tracer by a cleavage reaction at the cleavage site.

23. The method of claim 22, wherein the removing is accomplished by enzymatic cleavage or chemical cleavage.

24. The method of any one of claims 17-23, wherein the detectable label is a fluorescent label and the detected signal is fluorescence.

25. A method, comprising:

grafting a quality control tracer to a gel material in a well on a support wherein the quality control tracer comprises a first end grafted to the gel material and a second end linked to a cleavable region that is linked to a fluorescent label and comprises the cleavage site, wherein the detectable label is attached to the cleavable nucleotide sequence at the cleavage site or at a position in the cleavable region that is distal to the cleavage site;

the quality control tracer is

a cleavable nucleotide sequence tagged, at its 3' end, with a fluorescent label;

detecting the quality control tracer using fluorescence; and

based at least in part on the fluorescence, determining a density, or a distribution, or the density and the distribution, of a primer nucleotide sequence grafted to the gel material;

and wherein the quality control tracer is the cleavable nucleotide sequence, and wherein the cleavable nucleotide sequence is the primer nucleotide sequence,

said method further comprising cleaving the fluorescent label from the primer nucleotide sequence after the density, or the distribution, or the density and the distribution, of the primer nucleotide sequence is determined;

prior to the detecting, the method further comprises grafting the primer nucleotide sequence to the gel material, wherein the cleavable nucleotide sequence and the primer nucleotide sequence are present in a predetermined ratio; and

the determining of the density, or the distribution, or the density and the distribution, of the primer nucleotide sequence is based on the fluorescence and the predetermined ratio.

26. The method of claim 25, wherein the cleaving is accomplished by enzymatic cleavage or chemical cleavage.

27. The method of claim 25, wherein cleaving the fluorescent label from the cleavable nucleotide sequence, is accomplished using an exonuclease.

28. A method of co-grafting a quality control tracer and a primer nucleotide sequence to an array, comprising:

combining the quality control tracer and the primer nucleotide sequence at a predetermined ratio to form a grafting mix;

wherein the quality control tracer comprises (a) a cleavable nucleotide sequence comprising a cleavage site and (b) a detectable label, wherein the detectable label is attached to the cleavable nucleotide sequence at the cleavage site or at a position in the cleavable region that is distal to the cleavage site;

and

exposing the grafting mix to a gel material in a well on a support; and

incubating the grafting mix and the gel material, thereby co-grafting the quality control tracer and the primer nucleotide sequence to the gel material.

29. The method of claim 28, wherein the quality control tracer is:

a cleavable nucleotide sequence tagged, at its 3’ end, with a fluorescent label; or

a non-reactive nucleotide sequence with the fluorescent label attached to a cleavable nucleobase.

30. The method of claim 28 or claim 29, further comprising:

detecting the grafted quality control tracer by detecting a signal from the detectable label; and

based at least in part on the detected signal and the predetermined ratio, determining a density, or a distribution, or the density and the distribution, of the primer nucleotide sequence grafted to the gel material.

31. The method of any one of claims 28-30, further comprising removing the detectable label from the cleavable nucleotide sequence via enzymatic cleavage or chemical cleavage at the cleavage site.

32. The method of any one of claims 28-31, wherein the cleavable nucleotide sequence includes a linker molecule attaching the detectable label.

33. The method of claim 32, wherein the linker molecule comprises a diol, a disulfide, a silane, an azobenzene, a photocleavable group, or an azido.

34. The method of any one of claims 28-31, wherein the cleavage site comprises a cleavable nucleobase.

35. The method of claim 34, further comprising removing the detectable label by reaction of the cleavable nucleobase with a glycosylase and an endonuclease or with an exonuclease.

36. The method of any one of claims 28 or 30-35, wherein the detectable label is a fluorescent label.

37. A method of grafting a quality control tracer to an array, comprising grafting a quality control tracer to a gel material in a well on a support;

wherein the quality control tracer comprises (a) a cleavable nucleotide sequence comprising a cleavage site and (b) a detectable label;

wherein the detectable label is attached to the cleavable nucleotide sequence at the cleavage site or at a position in the cleavable region that is distal to the cleavage site;

wherein the cleavable nucleotide sequence comprises a grafted region with a first end and a second end, where the first end is grafted to the gel material and the second end is linked to a cleavable region that is linked to the detectable label and comprises the cleavage site, and wherein the grafted region comprises a primer nucleotide sequence.

38. The method of claim 37, further comprising cleaving the quality control tracer at the cleavage site, thereby removing the detectable label and providing an unlabeled primer nucleotide sequence grafted to the gel material.

39. The method of claim 37, wherein the grafted region comprises a non-reactive nucleotide sequence.

40. The method of claim 39, further comprising grafting a primer comprising a primer nucleotide sequence to the gel material in a pre-determined ration with the quality control tracer.

41. The method of claim 40, further comprising removing the detectable label from the quality control tracer by cleavage reaction at the cleavage site.