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(54) Title: THREE-DIMENSIONAL MICROARRAY SYSTEM FOR PARALLEL GENOTYPING OF SINGLE NUCLEOTIDE POLYMORPHISMS

(57) Abstract: The present invention provides methods for detecting nucleic acid mutations and genetic polymorphisms by single base extension analysis. Specifically, the invention provides an apparatus and methods for the detection of single base extension of a particular oligonucleotide in an oligonucleotide microarray following hybridization between oligonucleotides bound to defined regions of a polymeric hydrogel-based microarray and nucleic acids in a biological sample.

THREE-DIMENSIONAL MICROARRAY SYSTEM FOR PARALLEL GENOTYPING OF SINGLE NUCLEOTIDE POLYMORPHISMS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to the detection of nucleic acid mutations and genetic polymorphisms by single base extension analysis. In particular, the invention relates to an apparatus and methods for the detection of single base extension of a particular oligonucleotide in an oligonucleotide microarray following hybridization between oligonucleotides bound to defined regions of a polymeric hydrogel-based microarray and nucleic acids in a biological sample.

2. Background of the Invention

The detection of single base mutations and genetic polymorphisms in nucleic acids is an important tool in modern diagnostic medicine and biological research. In addition, nucleic acid-based assays also play an important role in identifying infectious microorganisms such as bacteria and viruses, in assessing levels of both normal and defective gene expression, and in detecting and identifying mutant genes associated with disease such as oncogenes. Improvements in the speed, efficiency, economy, and specificity of such assays are thus significant needs in the medical arts.

Ideally, such assays should be sensitive, specific, and easily amenable to automation. Efforts to improve sensitivity in nucleic acid assays are known in the prior art. For example, the polymerase chain reaction (Mullis, U.S. Patent No. 4,683,195, issued July 28, 1987) provides the capacity to produce useful amounts (about 1 μg) of a specific nucleic acid in a sample in which the original amount of the specific nucleic acid is substantially smaller (about 1 *pg*). However, the prior art has been much less successful in improving specificity of nucleic acid hybridization assays.

The specificity of nucleic acid assays is determined by the extent of molecular complementarity of hybridization between probe and target sequences. Although it is theoretically possible to distinguish complementary targets from one or two mismatched targets under rigorously-defined conditions, the dependence of hybridization on target/probe concentration and hybridization conditions limits the extent to which

hybridization mismatch can be used to reliably detect, *inter alia,* mutations and genetic polymorphisms.

Detection of single base extension has been used for mutation and genetic polymorphism detection in the prior art.

U.S. Patent No. 5,925,520 disclosed a method for detecting genetic polymorphisms using single base extension and capture groups on oligonucleotide probes using at least two types of dideoxy, chain-terminating nucleotide triphosphates, each labeled with a detectable and distinguishable fluorescent labeling group.

U.S. Patent No. 5,710,028 disclosed a method of determining the identity of nucleotide bases at specific positions in nucleic acids of interest, using detectably-labeled chain-terminating nucleotides, each detectably and distinguishably labeled with a fluorescent labeling group.

U.S. Patent No. 5,547,839 disclosed a method for determining the identity of nucleotide bases at specific positions in a nucleic acid of interest, using chain-terminating nucleotides comprising a photoremovable protecting group.

U.S. Patent No. 5,534,424 disclosed a method for determining the identity of nucleotide bases at specific positions in a nucleic acid of interest, using each of four aliquots of a target nucleic acid annealed to an extension primer and extended with one of four chain-terminating species, and then further extended with all four chain-extending nucleotides, whereby the identity of the nucleotide at the position of interest is identified by failure of the primer to be extended more that a single base.

U.S. Patent No. 4,988,617 disclosed a method for determining the identity of nucleotide bases at specific positions in a nucleic acid of interest, by annealing two adjacent nucleotide primers to a target nucleic acid and providing a linking agent such as a ligase that covalently links the two oligonucleotides to produce a third, combined oligonucleotide only under circumstances wherein the two oligonucleotides are perfectly matched to the target nucleic acid at the 3' extent of the first oligonucleotide and at the 5' extent of the second oligonucleotide.

U.S. Patent No. 4,656,127 disclosed a method for determining the identity of nucleotide bases at specific positions in a nucleic acid of interest, using primer extension with a chain-terminating or other nucleotide comprising an exonuclease-resistant

linkage, followed by exonuclease treatment of the plurality of extension products to detect the resistant species therein.

Biochip microarrays are generally comprised of a solid supporting substrate, an attachment matrix, and a plurality of biomolecular probes immobilized to the attachment matrix. Polymeric hydrogel pads, especially polyacrylamide gel pads, have been used as an attachment matrix in a variety of biochip microarrays (*see* U.S. Patent Nos. 5,552,270; 5,616,478; 5,736,257; and 5,741,700). Microarrays fabricated from polymeric hydrogel pads offer distinct advantages over other types of microarrays in that microarrays fabricated from polymeric hydrogel pads are more economical and permit a wider diversity of functional groups to be used for biomolecular immobilization. Furthermore, the polymeric hydrogel provides a three-dimensional structure thereby permitting for the attachment of a larger number of biomolecular probes, which in turn increases the likelihood of interactions between the immobilized probes and target molecules in a biological sample.

There remains a need in this art for simple, economical, and efficient ways to detect single base extension products of nucleic acid assays for detecting mutation and genetic polymorphisms in biological samples containing a nucleic acid of interest.

SUMMARY OF THE INVENTION

The present invention provides for the detection of nucleic acid mutations and genetic polymorphisms by single base extension analysis. Specifically, the invention provides an apparatus and methods for the detection of single base extension of a particular oligonucleotide in an oligonucleotide microarray following hybridization between oligonucleotides bound to defined regions of a polymeric hydrogel-based microarray and nucleic acids in a biological sample.

This invention provides methods and apparatus for detecting mutations and genetic polymorphisms in a biological sample containing a nucleic acid of interest. Detection of single base extension using the methods and apparatus of the invention is achieved by sequence-specific incorporation of labeled chain-terminating nucleotide species. In preferred embodiments, single base extension is performed using hybridization to an oligonucleotide array, most preferably an addressable array wherein

the sequence of each oligonucleotide in the array is known and associated with a particular address in the array.

In the practice of the methods of the invention, the invention provides an array of oligonucleotide probes immobilized via linker moieties to porous, polymeric pads. Preferably, the sequence of each oligonucleotide at each particular identified test site (or "address") in the array is known and at least one of said oligonucleotides is complementary to a sequence in a nucleic acid contained in the biological sample to be assayed (termed the "target" or "target nucleic acid").

In one embodiment of the methods of the present invention, the sequence of at least one oligonucleotide is selected to hybridize to a position immediately adjacent to the nucleotide position in the sample nucleic acid that is to be interrogated, *i.e.*, for the purpose of determining whether there is a mutation or genetic polymorphism at that nucleotide position. The term "adjacent" in this context is intended to encompass positions that are one nucleotide base upstream of base to be interrogated, *i.e.* in the 3' direction with respect to the template strand of the target DNA. Hybridization of oligonucleotide probes in the array to nucleic acid in the sample is performed at test sites comprising said oligonucleotide probes, and in a hybridization buffer, for a time and at a temperature that permits hybridization to occur between nucleic acid in the sample and oligonucleotide probes in the array that are complementary to said nucleic acid. Single base extension is performed using a polymerase, most preferably a thermally stable polymerase, in the presence of labeled chain-terminating primer extension units. In a preferred embodiment, each chain-terminating nucleotide species (for example, dideoxy(dd)ATP, ddGTP, ddCTP and ddTTP) is conjugated to a different label, most preferably a radioactive label, an electrochemical label or a fluorescent dye. Single base extension is detected by determining the identity of the label incorporated into the oligonucleotide using, *e.g.* conventional optical detection methods.

In another embodiments of the methods of the present invention, the sequence of at least one oligonucleotide probe is selected to hybridize to a target nucleic acid so that the 3' residue of the oligonucleotide corresponds to the nucleotide position in the target nucleic acid that is to be interrogated for mutation or genetic polymorphism. In the array, oligonucleotide probes having sequence identify to target nucleic acids hybridize

at the 3' residue and are capable of being extended in the polymerase-mediated single base extension step as described below. Hybridization of the oligonucleotides to target nucleic acids polymorphic at the position corresponding to the 3' residue of the oligonucleotide also hybridize (because hybridization conditions are chosen to permit such hybridization), but there will be a "mismatch" at the 3' residue of the oligonucleotide that does not hybridize at the mismatch. In this embodiment, only oligonucleotide hybridized to target nucleic acid species that are properly-hybridized at the oligonucleotides' 3' residue will be extended, using a polymerase that will not recognize the mismatch. Thus, in this method of the invention, single base extension is directed at a site adjacent to the polymorphic site, and most preferably comprises a non-polymorphic site that can be addressed using only a single, labeled chain-terminating species.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides methods for the detection of nucleic acid mutations and genetic polymorphisms, most preferably single nucleotide polymorphism (SNP), by single base extension analysis. Specifically, the invention provides an apparatus and methods for the detection of single base extension of a particular oligonucleotide in an oligonucleotide microarray following hybridization between oligonucleotides bound to defined regions of a polymeric hydrogel-based microarray and nucleic acids in a biological sample.

As used herein, the term "array" refers to an ordered spatial arrangement, particularly an arrangement of immobilized biomolecules at a plurality of test sites.

As used herein, the term "addressable array" refers to an array wherein the individual test sites have precisely defined x- and y-coordinates, so that a given test site at a particular position in the array can be identified.

As used herein, the terms "probe" and "biomolecular probe" refer to a biomolecule, specifically an oligonucleotide, used to detect a complementary biomolecule (referred to herein as a target molecule), preferably a nucleic acid.

As used herein, the terms "microarray," "biochip" and "biochip array" refer to an ordered spatial arrangement of immobilized biomolecular probes arrayed at test sites on a solid supporting substrate. Biochips, as used in the art, encompass substrates containing arrays or microarrays, preferably ordered arrays and most preferably ordered, addressable arrays, of biological molecules that comprise one member of a biological binding pair. The microarrays of the present invention are preferably oligonucleotide arrays comprising a nucleotide sequence that is complementary to at least one sequence that may be or is expected to be present in a biological sample.

As used herein, the term "test site" refers to a predefined region on a substrate to which a plurality probe molecules are immobilized. The test site may have any convenient shape, *e.g.*, circular, rectangular, elliptical, or wedge-shaped. In preferred embodiments of the apparatus of the present invention, the test sites have an area of about 1 cm^2. In more preferred embodiments, the test sites have an area of less than 1 mm^2, less than 0.5 mm^2, less than about 10,000 μm^2, or less than 100 μm^2.

The devices of the invention are particularly useful for analyzing target nucleic acids for the diagnosis of infectious and genetic disease. The target nucleic acid is generally a portion of a gene having a known nucleotide sequence that is associated with an infectious agent or genetic disease; more specifically, the disease is caused by a single nucleotide (or point) mutation. The device incorporates a nucleic acid oligonucleotide array specific for the target gene, and means for detecting and determining the identity of a specific single base in the target sequence adjacent to the hybridization site of at least one probe in the oligonucleotide array (termed the "3' offset method") or encompassing the 3' residue of at least one oligonucleotide probe in the array (termed the "3' inclusive method").

The present invention provides an array of oligonucleotide probes immobilized to a polymeric hydrogel-based attachment matrix. Preferably, the sequence of each oligonucleotide at each address in the array is known and at least one oligonucleotide in said oligonucleotide array is complementary to part of a sequence in a nucleic acid in the

sample to be assayed. The sequence of at least one oligonucleotide is most preferably selected to extend to a position immediately adjacent to the nucleotide position in the sample nucleic acid that is to be interrogated, *i.e.*, for mutation or genetic polymorphism. Alternatively, the oligonucleotide is selected to encompass the site of mutation or genetic polymorphism; in these latter embodiments, it is generally preferred to provide a single oligonucleotide, most preferably having the sequence of the wildtype allele or species, or alternatively a multiplicity of oligonucleotides having one of each possible nucleotide at the polymorphic position to ensure hybridization of at least one of the oligonucleotides in the array to nucleic acid in the sample. Hybridization and extension reactions are performed in a reaction chamber and in a hybridization buffer for a time and at temperature that permits hybridization and single base extension to occur between nucleic acid in the sample and the oligonucleotides in the array complementary thereto.

In one embodiment of the invention, the apparatus comprises a supporting substrate comprising an array of test sites; a plurality of porous, polymeric pads in contact with the supporting substrate at the test sites; a plurality of linker moieties in contact with the porous, polymeric pads at the test sites; a plurality of oligonucleotide probes immobilized to the linker moieties, wherein said oligonucleotide probes specifically bind to or interact with nucleic acids in a biological sample; a reaction solution in contact with the porous, polymeric pads; linker moieties; and oligonucleotide probes, wherein the reaction solution comprises a hybridization buffer, a polymerase, a plurality of primer extension units further comprising chain-terminating nucleotide species, wherein each different chain-terminating nucleotide species is conjugated to a distinguishable label; and a means for detecting the labeled chain-terminating nucleotide species.

The supporting substrate of the apparatus of the invention is advantageously made from any solid material, including but not limited to glass, silicon, silicon nitride, plastic, rubber, fabric, ceramics, printed circuit board, compound semiconductors (*e.g.*, GaAs), or combinations thereof. In preferred embodiments, the supporting substrate of the apparatus of the present invention is composed of silicon or glass. The supporting substrate has a surface area between about 0.01 μm^2 and about 5 cm^2 containing from 1

to about 10^8 test sites. In a preferred embodiment, the supporting substrate has a surface area of about 10,000 μm^2 and contains about 10^4 test sites. In preferred embodiments, the test sites are arranged on the supporting substrate so that they are separated by a distance of from about 0.05 μm to 0.5 mm. In more preferred embodiments, the test sites are regularly spaced on the solid supporting substrate with a uniform spacing there between. Preferably, the oligonucleotide probes at any particular test site are identical to each other, while each test site comprises oligonucleotide probes that are unique to that test site.

The porous, polymeric pads of the apparatus of the invention are composed of materials including, but not limited to, polyacrylamide gel, agarose gel, polyethylene glycol, cellulose gel, sol gel, polypyrrole, carbon, carbides, oxides, nitrides, or other porous, polymeric materials known to those with skill in the art. In a preferred embodiment, the porous, polymeric pads comprise polyacrylamide gel. The porous, polymeric hydrogel matrix of the apparatus can be fabricated, and biomolecular probes immobilized thereto, using methods as described in co-owned and co-pending U.S. Patent Application Serial Nos. 09/344,217; 09/344,620; and 09/438,209, each of which is incorporated herein by reference.

The probe used in the present invention is preferably an oligonucleotide, having upper and lower limits of length that are empirically determined. The lower limit of oligonucleotide length is the length required for stable hybridization, since it is known in the art that probes that are too short do not form thermodynamically-stable duplexes sufficient for single base extension under the hybridization conditions of the assay. The upper limit of oligonucleotide length is the length required to produce a duplex in a region other than that of the predetermined interrogation target, leading to artifactual incorporation of labeled primer extension unit(s). Preferred oligonucleotide probes used in the present invention have a length of from about 8 to about 50, more preferably from about 10 to about 40, even more preferably from about 12 to about 30, and most preferably from about 15-25 nucleotides. However, longer probes, *i.e.* longer than 40 nucleotides, may also be used. The oligonucleotides are immobilized to the porous, polymeric pads at their 5' ends, leaving the 3' end of the oligonucleotide available for single base extension using a polymerase as described herein.

In some embodiments of the present invention, the linker moieties comprise a conjugated polymer or copolymer film. Such conjugated polymer or copolymer film is composed of materials including, but not limited to, polypyrrole, polythiophene, polyaniline, polyfuran, polypyridine, polycarbazole, polyphenylene, poly(phenylenvinylene), polyfluorene, or polyindole, or their derivatives, copolymers, or combinations thereof. In another preferred embodiment, the linker moieties comprise a neutral pyrrole matrix. In still other embodiments, the linker moieties comprise thiol linkers. In still other embodiments, the linker moieties further comprise streptavidin (and the probe molecules are biotinylated).

Preferred polymerases for performing single base extensions using the methods and apparatus of the invention are polymerases having little or no exonuclease activity. More preferred are polymerases that tolerate and are biosynthetically-active at temperatures greater than physiological temperatures, for example, at 50°C or 60°C or 70°C or are tolerant of temperatures of at least 90°C to about 95°C. Preferred polymerases include *Taq* polymerase from *T. aquaticus* (commercially available from Perkin-Elmer Cetus, Foster City, CA), Sequenase® and ThermoSequenase® (commercially available from U.S. Biochemical, Cleveland, OH), and Exo(-)Pfu polymerase (commercially available from New England Biolabs, Beverley, MA).

In still other embodiments of the present invention, the apparatus further comprises a plurality of wells wherein each well encompasses a porous, polymeric pad, wherein a plurality of oligonucleotide probes is immobilized to linker moieties that are in contact with the porous, polymeric pad. The term "wells" is used herein in its conventional sense, to describe a portion of the supporting substrate in which the porous, polymeric pad is contained in a defined volume; said wells can protrude from the surface of the supporting substrate, or be embedded therein. Preferably, the oligonucleotide probes in any particular well are identical to each other, while each well comprises oligonucleotide probes unique to that well.

The inventive methods for SNP detection provided by the invention generally comprise: (1) preparing a sample containing the target nucleic acid(s) of interest to obtain single-stranded nucleic acid that spans the specific position (typically by denaturing the sample); (2) contacting the single- stranded target nucleic acid with an

oligonucleotide probe of known sequence that hybridizes with a portion of the nucleotide sequence in the target nucleic acid immediately adjacent to the nucleotide base to be interrogated (thereby forming a duplex between the primer and the target such that the nucleotide base to be interrogated is the first unpaired base in the target immediately 5' of the nucleotide base annealed with the 3'-end of the primer in the duplex; this oligonucleotide is preferably a specific oligonucleotide occupying a particular address in an addressable array); (3) contacting the duplex with a reagent which includes a buffer solution, a polymerase, and at least one primer extension unit, wherein the primer extension unit comprises an extension moiety, most preferably a chain-terminating nucleotide, an optional linker, and a detectable label. The primer extension reaction catalyzed by the polymerase results in the incorporation of the extension moiety of the primer extension unit at the 3'-end of the primer, and the extension of the primer by a single base; (4) removing the unincorporated primer extension unit(s); and (5) determining the identity of the incorporated primer extension unit in the extended duplex by its unique label or tag. Preferred labels or tags include, for example, radioactive labels, electrochemical labels and fluorescent dyes.

In alternative embodiments, the oligonucleotide is constructed so that the 3' residue is complementary only to one of a plurality of polymorphic species of target nucleic acids. In these embodiments, target nucleic acids that are complementary to the full extent of the oligonucleotide, including the 3' residue, support single base extension of the oligonucleotide and incorporation of a labeled primer extension unit therein, whereas target nucleic acids that are not complementary to the 3' residue of the oligonucleotide do not support single base extension of the oligonucleotide and labeled primer extension units are not incorporated. In these embodiments, the incorporated primer extension units are preferably complementary to a site immediately adjacent to the polymorphic site, which site is preferably non-polymorphic.

The extension moiety in the primer extension unit is preferably a chain-terminating moiety, most preferably dideoxynucleoside triphosphates (ddNTPs), such as ddATP, ddCTP, ddGTP, and ddTTP; however other terminators known to those skilled in the art, such as acyclonucleotide analogs or arabinoside triphosphates, are also within the scope of the present invention. These ddNTPs differ from conventional

deoxynucleoside triphosphates (dNTPs) in that they lack a hydroxyl group at the 3' position of the sugar component. This prevents chain extension of incorporated ddNTPs, and thus terminates the chain. The present invention provides primer extension units labeled with, for example, electrochemical labels, fluorescent dyes or radioactive labels, which can be detected using electrochemical, optical or radioactivity detectors. However, any label that does not interfere with the incorporation of the ddNTP into the nucleotide chain may be used.

Optionally, the target DNA in the sample to be investigated can be amplified by means of *in vitro* amplification reactions, such as the polymerase chain reaction (PCR) technique well known to those skilled in the art. Enriching the target DNA in a biological sample to be used in the methods of the invention provides more rapid and more accurate template-directed synthesis by the polymerase.

Single base extension is performed using a polymerase in the presence of at least one primer extension unit in a buffer solution appropriate for the biochemical activity of the polymerase. A general formula of a preferred embodiment of the primer extension unit is:

ddNTP-L-*

where ddNTP represents a dideoxyribonucleotide triphosphate including ddATP, ddGTP, ddCTP, ddTTP, L represents an optional linker moiety, and * represents an appropriate label or tag. In preferred embodiments, each chain-terminating nucleotide species (for example, dideoxy(dd)ATP, ddGTP, ddCTP and ddTTP) is labeled with a different label.

The label comprising the chain-terminating nucleotides of the invention is optionally linked to the extension nucleotide through a linker (L), preferably having a length of from about 10 to about 20 Angstroms. The linker can be an organic moiety such as a hydrocarbon chain $(CH_2)_n$, or can comprise an ether, ester, carboxyamide, or thioether moiety, or a combination thereof. The linker can also be an inorganic moiety such as siloxane (O-Si-O). The length of the linker is selected so that the label does not interfere with either nucleic acid hybridization between the bound oligonucleotide probe and target nucleic acid, or with polymerase-mediated chain extension.

In the use of the apparatus of the invention to perform a single base extension

reaction, a reaction mixture is prepared containing at least one labeled chain-terminating nucleotide, a hybridization buffer compatible with the polymerase and having a salt concentration sufficient to permit hybridization between the target nucleic acid and oligonucleotide probes under the conditions of the assay, and a DNA polymerase such as *Taq* DNA polymerase or ThermoSequenase. Single stranded target nucleic acid, *for example*, having been denatured by incubation at a temperature >90°C, is diluted to a concentration appropriate for hybridization in deionized water and added to the reaction mixture. The resulting hybridization mixture is exposed to the polymeric hydrogel pads of the apparatus of the invention, the pads having a plurality of probes possessing known sequences attached thereto. At least one of the probes has a nucleotide sequence capable of hybridizing with a portion of the nucleotide sequence of the target comprising the nucleotide base to be interrogated under the hybridization conditions employed in the assay.

In one embodiment, a duplex between the probe and the target is formed wherein the nucleotide base to be interrogated is the first unpaired base in the target nucleic acid immediately 5' of the nucleotide base that is annealed with the 3'-end of the primer in the duplex. Single base extension of the 3' end of the annealed probe is achieved by incorporation of a labeled chain-terminating nucleotide into the probe. The probe sequence and labeled chain-terminating nucleotide are chosen so that incorporation of the nucleotide is informative of the identity (*i.e.*, mutant, wildtype or polymorphism) of the interrogated nucleotide in the target.

Alternatively, the probe comprises a 3' terminal residue that corresponds to and hybridizes with the interrogated base. In these embodiments, oligonucleotides having a "mismatch" at the 3' terminal residue will hybridize but will not be extended by the polymerase. Detection of incorporation of the primer extension unit by interrogating the label is then informative of the identity of the interrogated nucleotide base, provided that the sequence of the oligonucleotide probe is known at each position in the array.

After the single base extension reaction is performed, the test site is washed at high stringency (*i.e.*, in a low-salt solution, such as 0.1 X SSC, 0.015 M NaCl, 15 mM sodium citrate, pH 7.0), optionally including a detergent such as sodium dodecyl sulfate at temperature of between about10-65°C for a time and at a temperature wherein the

target nucleic acid is removed. Wash conditions vary depending on factors such as probe length and probe complexity. Detection of single base extension is then carried out using conventional detection means for detecting electrochemical, fluorescent or radioactive labels.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

WHAT WE CLAIM IS:

1. An apparatus for detecting single base extension of an oligonucleotide probe comprising a microarray, wherein extension is effected by a polymerase and directed by a nucleotide sequence of a nucleic acid in a biological sample, the apparatus omprising:

(a) a supporting substrate comprising an array of test sites;

(b) a plurality of porous, polymeric pads in contact with the supporting substrate at the test sites;

(c) a plurality of linker moieties in contact with the porous, polymeric pads at the test sites;

(d) a plurality of oligonucleotide probes having a 5' and a 3' residue wherein the oligonucleotide is immobilized to the linker moieties at the 5'residue, wherein said oligonucleotide probes specifically bind to or interact with target nucleic acids in a biological sample;

(e) a reaction solution in contact with the porous, polymeric pads, linker moieties, and oligonucleotide probes, wherein the reaction solution comprises a hybridization buffer, a polymerase, a plurality of primer extension units further comprising chain-terminating nucleotide species, wherein each different chain-terminating nucleotide species is conjugated to a detectable label, wherein each chain-terminating nucleotide species is conjugated to a different detectable label that can be distinguished from other detectable labels; and

(f) a detector for detecting the labeled chain-terminating nucleotide species;

wherein, when a biological sample comprises a nucleic acid that hybridizes to an oligonucleotide probe and is exposed to the test sites under moderate to high stringency hybridization conditions, the nucleotide sequence of said hybridized oligonucleotide is extended by the incorporation of at least one of the labeled chain-terminating nucleotides.

2. The apparatus of Claim 1, wherein the chain-terminating nucleotide species are dideoxyribonucleotide species.

3. The apparatus of Claim 2, wherein the chain terminating nucleotide species is dideoxyadenosine, dideoxyguanine, dideoxyinosine, dideoxyxanthine, dideoxycytosine, dideocythymidine or dideoxyuracil.

4. The apparatus of Claim 1, wherein the chain-terminating nucleotide species are acyclonucleotide species.

5. The apparatus of Claim 4, wherein the chain-terminating species is acycloguanosine, acycloadenosine, acycloinosine, acycloxanthine, acyclocytosine, acyclothymidine or acyclouracil.

6. The apparatus of Claim 1, wherein the polymerase is a thermostable polymerase.

7. The apparatus of Claim 1, wherein the chain-terminating nucleotide species are labeled with a radioactive isotope, an electrochemical label or a fluorescent dye.

8. The apparatus of Claim 1, wherein the 3' residue of at least one oligonucleotide probe is complementary to a polymorphic residue in the target nucleic acid.

9. A method for detecting single base extension of an oligonucleotide probe comprising a microarray, wherein extension is effected by a polymerase and directed by a nucleotide sequence of a nucleic acid in a biological sample, using the apparatus of Claim 1, the method comprising the steps of:

(a) exposing oligonucleotide probes contained in the microarray to a biological sample containing a target nucleic acid that hybridizes to an oligonucleotide probe contained on the microarray under moderate to high stringency hybridization conditions in a reaction solution comprising a hybridization buffer, a polymerase, and a

plurality of labeled chain-terminating nucleotide species;

(b) incubating the microarray for a time and at a temperature sufficient to permit hybridization of at least one oligonucleotide in the microarray to a target nucleic acid in the biological sample and for single base extension of said hybridized oligonucleotide probe comprising the microarray with at least one of the labeled chain-terminating nucleotide species to occur; and

(c) detecting the labeled chain-terminating nucleotide species incorporated into the oligonucleotide.

10. The method of Claim 9, wherein the chain-terminating nucleotide species are dideoxyribonucleotide species.

11. The method of Claim 10, wherein the chain terminating nucleotide species is dideoxyadenosine, dideoxyguanine, dideoxyinosine, dideoxyxanthine, dideoxycytosine, dideocythymidine or dideoxyuracil.

12. The method of Claim 9, wherein the chain-terminating nucleotide species are acyclonucleotide species.

13. The method of Claim 12, wherein the chain-terminating species is acycloguanosine, acycloadenosine, acycloinosine, acyclocytosine, acyclothymidine or acyclouracil.

14. The method of Claim 9, wherein the polymerase is a thermostable polymerase.

15. The method of Claim 9, wherein the chain-terminating nucleotide species are labeled with a radioactive isotope, an electrochemical label or a fluorescent dye.

16. The method of Claim 9, wherein the 3' residue of at least one oligonucleotide probe that hybridizes to the target nucleic acid is complementary to a polymorphic residue in the target nucleic acid.